

The Canadian Association
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8th Annual Canadian Neuroscience Meeting 2014



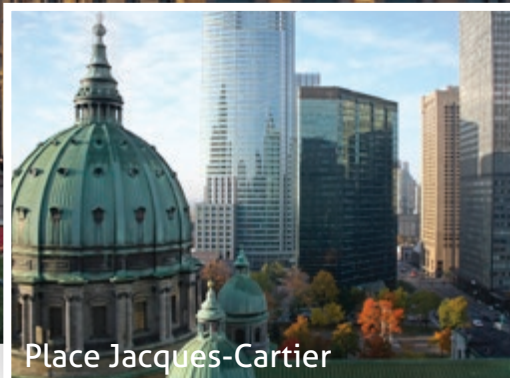
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CAN-ACN Abstract Booklet

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Invited Speakers**Presidential Lecture:**

Lynn Raymond, University of British Columbia

Mechanisms and neuroprotective strategies in neurodegeneration: Huntington disease can lead the way

Evidence indicates that NMDA-type glutamate receptor (NMDAR)-induced synaptic loss and neuronal dysfunction/death contributes to mechanisms underlying certain neurodegenerative diseases and acute neurological insults. Yet, cell signaling downstream of NMDARs can promote cell survival and plasticity as well as excitotoxicity, which may help explain why general NMDAR inhibitors have failed in clinical trials. A new paradigm developed over the past decade suggests that over-stimulation of extrasynaptic NMDARs triggers stress/death pathways whereas physiological activation of those inside the synapse contributes to cell survival, raising the possibility of neuroprotection based on subcellular localization. This idea has been tested in the inherited, predominantly adult onset, neurodegenerative disorder Huntington disease (HD), which manifests as progressive motor, mood and cognitive impairment. Caused by a polymorphic CAG repeat expansion in the *HD* gene that encodes an enlarged polyglutamine tract in the protein huntingtin, HD is associated with selective neurodegeneration, principally of striatal GABAergic spiny projection neurons (SPN) and cortical pyramidal neurons. Genetically accurate mouse models have facilitated understanding of HD pathogenesis. In one HD mouse model (YAC128), we have shown an increase in number, activity, and downstream signaling of extrasynaptic NMDARs on SPN beginning in the early postnatal period; selective inhibition of these receptors from an early age ameliorates later stage cell death signaling and also improves motor learning and coordination. Moreover, we and others have identified additional synaptic alterations that occur prior to overt motor manifestations. In particular, we have characterized morphological and electrophysiological changes in cortical-striatal co-cultures from HD mice, a simple model system that can serve as a platform for testing therapeutics. Since HD gene mutation carriers can be identified decades before clinical diagnosis, targeting early changes in cortical-striatal synaptic transmission may significantly delay onset of manifest disease. *Supported by the CIHR, Huntington Society of Canada, Cure*

Huntington Disease Initiative, and Michael Smith Foundation for Health Research.

Featured Plenary Speaker:

Edward S. Boyden, MIT

Tools for Mapping Brain Computations

The brain is a densely and precisely wired circuit made of heterogeneous cells, which themselves are complex computational devices made of an incredible repertoire of molecules. Our group develops tools for mapping, recording from, controlling, and building brain circuits, in order to reveal how they work, as well as to open up new therapeutic avenues. We have developed genetically-encoded reagents that, when expressed in specific neurons, enable their electrical activities to be precisely driven or silenced in response to millisecond timescale pulses of light. I will give an overview of these optogenetic tools, adapted from natural photosensory and photosynthetic proteins, and discuss new tools we are developing, including molecules that enable multiplexed, noninvasive, and ultraprecise optical neural control. We are also developing optogenetic tools that enable activation of endogenous protein and signaling pathways (e.g., lumitoxins). Often working in interdisciplinary collaborations, we are developing microfabricated hardware to enable complex and distributed neural circuits to be controlled and recorded in a fully 3-D fashion, new kinds of microscopes capable of whole-nervous system neural activity imaging, robots that can automatically record neurons intracellularly and integratively in live brain, and strategies for building 3-D brain circuits in vitro. We aim to provide these tools to the neuroscience community in order to open up new fundamental as well as clinically relevant explorations of how to observe and repair brain circuits.

Plenary Symposium: Development and application of optogenetic tools

Timothy H Murphy, University of British Columbia

In vivo optogenetic assessment and control of mouse cortical circuits

Optogenetics employs light to measure brain activity by assessing the effect of membrane voltage, intracellular calcium, or even extracellular neurotransmitter concentration on recombinant protein sensors. A second class of recombinant proteins-light-activated actuators, alter circuit function by activating excitatory or inhibitory ion channels or pumps. Relatively non-invasive through-skull in vivo imaging and

optical manipulation of cortex will be discussed. To assess changes in functional connectivity after stroke, we have developed an automated approach to monitor intrahemispheric and interhemispheric relationships by the activation of ChR2-expressing cortical neurons. To monitor regional cortical activity we employ organic voltage sensitive dyes or genetically encoded sensors. In vivo imaging of functional connectivity is extended genetically-encoded indicators of intracellular calcium using GCaMPs, glutamate iGluSNFR, and voltage sensitive fluorescent protein butterfly (VSPF-butterfly). We apply network analysis to connection matrices derived from functional maps to elucidate reciprocal connections between primary and secondary sensory areas, identify network hubs, and determine symmetries within intracortical connectivity. Comparisons of functional connectivity maps to the cortical structural connectome (Allen Institute) indicate that intracortical monosynaptic structural connections predict hemisphere-wide patterns of spontaneous and sensory-evoked depolarization. A new approach to stroke damage is to treat it as a disorder of connectivity and loss of function. The talk will provide an introduction into circuit-level optogenetic actuators and sensors, circuitry database such as the Allen Institute Mouse Brain Connectivity Atlas, and how these approaches can be applied to stroke damage.

Andrew Woolley, University of Toronto
Optogenetic control using photoactive yellow protein

Numerous processes, in addition to the firing of action potentials, that are of interest to neuroscientists exhibit complex spatiotemporal patterns of activity. Optogenetic tools for manipulation of these processes could offer new ways to probe the function in vivo. In addition to channelrhodopsins, a number of other photoswitchable domains exist that may be harnessed to control function. Strategies for achieving this have thus far relied mainly on control of protein localization. Photoactive yellow protein, a small, cytosolic domain, undergoes a particularly large change in conformation and dynamics upon exposure to blue light. This feature has allowed us to develop strategies to couple to PYP isomerization to changes in target protein function with affecting protein localization. In particular we have developed an approach to the optogenetic control of CREB activity by linking PYP to a dominant negative CREB inhibitor. Approaches to the photo-control

of this and other targets of interest to neuroscientists will be discussed.

Featured Plenary Speaker:
Eric Neslter, Mt Sinai, NYC
Transcriptional and Epigenetic Mechanisms of Drug Addiction

Drug addiction can be viewed as a stable form of drug-induced neural plasticity, whereby long-lasting changes in gene expression mediate some of the stable behavioral abnormalities that define an addicted state. Our laboratory has focused on two main transcriptional pathways in addiction. Chronic exposure to cocaine or opiates causes the prolonged activation of the transcription factor CREB within the brain's reward circuits and several other brain regions, and this adaptation mediates aspects of drug tolerance and dependence. In contrast, induction of another transcription factor, DeltaFosB, in brain reward regions by virtually all drugs of abuse exerts the opposite effect and contributes to sensitized responses to drug exposure. Studies are underway to explore the detailed molecular mechanisms by which CREB and DeltaFosB regulate target genes and thereby contribute to the complex state of addiction. One way to approach such molecular mechanisms of drug action in vivo is through the study of chromatin remodeling, that is, changes in the acetylation or methylation of histones that bind to certain drug-regulated gene promoters, or changes in methylation of the genes themselves, as revealed by chromatin immunoprecipitation (ChIP). We are utilizing ChIP to examine chromatin changes at specific candidate genes for CREB and DeltaFosB, as well as genome-wide measures to gain a more global view of target genes for these transcription factors. Prominent among these targets are those that regulate synaptic function and plasticity as well as the morphology of drug-regulated neurons. We have also demonstrated drug regulation of some of the enzymes that catalyze chromatin modifications, which indicates that chromatin remodeling mechanisms are themselves important targets of drug action. These findings establish chromatin remodeling as an important regulatory mechanism underlying drug-induced neural and behavioral plasticity, and provide fundamentally new insight into how CREB and DeltaFosB, and several other drug-regulated transcription factors, contribute to addiction by regulating the expression of specific target genes in the brain's reward circuitry. These advances can now be mined to develop improved diagnostic tests and treatments for addictive disorders.

Plenary Symposium: *Mechanisms in learning reward value*

Jonathan Britt, McGill University
Dissecting the Neural Circuits Underlying Motivated Behaviours Relevant to Reward Learning and Drug Addiction

The nucleus accumbens plays a major role in the generation of motivated behaviour. It integrates dopaminergic reinforcement signals with glutamate-encoded environmental stimuli. Prominent glutamate afferents to the nucleus accumbens come from the hippocampus, amygdala, thalamus, and prefrontal cortex. Pathway-specific activation of these inputs is known to produce distinct behavioral responses, but mechanistic explanations for these pathway-specific effects are lacking. This talk examines the pathway-specific differences in synaptic properties and innervation patterns between these glutamatergic inputs to the nucleus accumbens. While there are important distinctions between these afferent connections, optogenetic stimulations targeted to any of them can reinforce instrumental behaviour. This finding challenges the idea that these inputs encode motivationally-neutral information. Mice will also work to obtain optical manipulations to projections neurons throughout the striatum as well as downstream structures, but, regardless of which basal ganglia nuclei are targeted for self-stimulation, the behaviour is always sensitive to dopamine receptor blockade. This work characterizes some of the fundamental organizing principles of basal ganglia information processing.

Stan Floresco, University of British Columbia
Dopaminergic circuits mediating risk/reward decision biases

Choosing between smaller, assured rewards or larger, uncertain ones requires reconciliation of competing biases towards more certain or riskier options. These conflicting urges reflect an interplay between distributed neural circuits linking the frontal lobes to subcortical regions processing emotional and reward-related information that in turn influence response selection. Each of these regions is interconnected with the dopamine system. Our studies have used a probabilistic discounting task to probe the interactions between these systems in regulating risk/reward decision making. Data will be reviewed showing that subcortical circuitry linking the amygdala and the ventral striatum appears to promote a more

visceral bias towards larger, uncertain rewards, whereas prefrontal regions serve to temper these urges when riskier options become less profitable via top-down control over the amygdala. Dopamine D₁/D₂ transmission within these regions also makes dissociable, yet complementary, contributions to risk/reward judgments, promoting either exploitation of current favorable circumstances or exploration of more profitable ones when conditions change. Dynamic fluctuations in prefrontal and accumbens tonic dopamine transmission appear to encode distinct types of information related to decision making related to changes in reward availability, uncertainty and choice biases. On the other hand, phasic increases and decreases in dopamine activity, regulated in part by the lateral habenula, appear to play a key role in providing short-term information about recent outcomes that bias subsequent choice. These findings provide insight into the dynamic competition between cortical/subcortical circuits that shape decision biases and underlie conflicting urges when evaluating options that vary in terms of potential risks and rewards.

Keynote Lecture:
Michael E. Greenberg, Harvard Medical School

Signaling Networks that Regulate Synapse Development and Cognitive Function

Our interactions with the outside world trigger changes at synapses that are critical for proper brain development and higher cognitive function. Research in the Greenberg laboratory has focused on the identification of a genetic program that is activated by neuronal activity, the mechanisms of signal transduction that carry the neuronal activity-dependent signal from the membrane to the nucleus, and the identification of regulators of this experience-dependent process that affect synapse development and plasticity. Our recent studies using global screening techniques have identified activity-dependent genes that control 1) the complexity of the dendritic arbor, 2) the formation and maturation of excitatory and inhibitory synapses, 3) the composition of protein complexes at the pre- and post-synaptic sites, and 4) the production and secretion of neuropeptides that control neural circuit development. These activity-regulated processes are critical for normal brain development and function, and defects in the activity-dependent gene program contribute to disorders of human cognition such as Rett Syndrome (RTT) and Angelman Syndrome (AS), two neurological disorders associated with syndromic autism.

Understanding how the neuronal activity-dependent gene program functions may provide insight into how the dysregulation of this process leads to neurological diseases and, ultimately, may suggest therapies for treatment of disorders of cognitive function.

Featured Plenary Speaker:

Jay Gottfried, Northwestern University
All Roads Lead to Smell: What Odors Can Teach Us About Brain Function

An essential function of the brain is to encode and interpret the behavioral salience of stimuli encountered in the environment. Throughout much of the animal kingdom, odors are essential for directing animals toward a wide array of salient stimuli, including foods, friends, and friends with benefits (mates) -- it follows that the olfactory system should share intimate anatomical overlap with limbic brain regions involved in the control of emotion, decision making, and goal-directed behavior. Research in our lab combines sensory psychophysics with functional MRI, multivariate pattern-based analysis, and intracranial EEG recordings to investigate olfactory functional organization in the human animal, whose ability to talk and provide ratings of their experiences offers a highly tractable way to relate brain activity patterns directly to perception. This presentation will include an overview of recent studies examining the impact of attentional states on olfactory predictive coding, and the interactions of smells and sleep in modulating fear memory. I will also focus on new data that address the mechanisms by which olfactory perceptual experience and associative learning drive the de novo formation of object categories in entorhinal cortex and orbitofrontal cortex. Finally, I will discuss how the mere act of odor sampling, i.e., sniffing, can profoundly shape network dynamics and oscillations in the human brain, with relevance for memory and behavior.

Plenary symposium: *The Cognitive Neuroscience of the Senses*

Morris Moscovitch, University of Toronto:
Spatial (and event) memory in humans and rodents

Since the discovery of place cells, it has been believed the hippocampus in both rodents and humans is needed for representing spatial layouts (environments) allocentrically. That representation, in turn, is needed for navigation, regardless of whether the environment was encountered and learned recently or long ago. Evidence from studies on spatial memory and

navigation in humans and rats will be presented to examine this proposal. The results will show that the hippocampus is needed to retain and retrieve detailed memories of spatial layouts (scenes) for as long as the memories exist. With time, however, some of these memories are transformed, shedding contextual details, but retaining schematic cognitive maps that can represent space allocentrically. In the process, these transformed memories lose their hippocampal signature, and are represented in extra-hippocampal structures where they can be retained and from where they can be retrieved without hippocampal involvement. A multiple trace theory of hippocampal-neocortical interaction, and a transformation hypothesis, are proposed to account for the data.

Ingrid Johnsrude, Queen's University: *The role of prediction and attention in speech perception*

Abstract: When speech is heard in the presence of background sound, or when hearing is impaired, the sensory information at the ear is often too ambiguous to support speech recognition by itself. In order to disambiguate and interpret the incoming sounds, the brain must integrate the auditory information with other sensory information and with prior knowledge to facilitate understanding. Prior information can enhance speech perception in different ways. For example, intelligibility and segregability of familiar voices is greater than for unfamiliar voices in the presence of competing speech; and coherent, predictive, semantic context appears to reduce processing load. A recent series of experiments exploit behavioural and imaging methods to explore the mechanisms underlying the integrative processes that permit knowledge and experience to enhance understanding of degraded speech, and to examine how recruitment of such mechanisms is gated by attentional state. The field of visual perception has long recognized the important role played by feedback connections to early visual cortices in shaping perception; an emerging literature in the auditory domain is consistent with the idea that early auditory processing (in primary auditory cortex) is modulated by higher-level (linguistic) knowledge. This work adds to a growing literature indicating that primarily feedforward accounts of perceptual processing are incomplete, and that frontally mediated control processes are essential to accurate speech comprehension in the noisy and variable listening conditions that are characteristic of everyday life.

Parallel Symposia Abstracts

Symposium 1: Functional and dysfunctional regulation of brain blood flow

New roles for astrocyte Ca²⁺ in relation to brain blood flow

Grant Gordon, University of Calgary, Hotchkiss Brain Institute

Neurovascular coupling is an essential process ensuring blood flow is matched to the metabolic needs of the brain. The current model stipulates that increases in neuronal activity lead to a rapid elevation in astrocyte endfoot Ca²⁺, which triggers the release of diffusible messengers that initiate changes to arteriole diameter to control blood flow. While much in vitro data demonstrates the sufficiency of astrocyte Ca²⁺ in controlling arteriole diameter, recent in vivo studies call the current model into question. Using two-photon imaging in acute brain slices, we tested the causal role of synaptically evoked astrocyte Ca²⁺ transients in neurovascular coupling under conditions in which astrocytes were functionally silenced by the intracellular delivery of BAPTA. Surprisingly, we found that synaptically triggered astrocyte Ca²⁺ transients were not necessary for vasodilation. However, we found that Ca²⁺ chelation itself in astrocytes caused a prominent vasoconstriction of arterioles, suggesting that astrocytes provide steady-state vasodilation. Next, using two-photon imaging in awake mice, we found that endfoot Ca²⁺ transients followed, rather than preceded, sensory evoked vasodilation of arterioles. Furthermore, similar astrocyte endfoot Ca²⁺ transients could be evoked by intraluminal acetylcholine delivered IV, suggesting arteriole diameter/blood flow changes per se can drive endfoot Ca²⁺ transients. Our data redefine the potential role of astrocyte Ca²⁺ in relation to brain blood flow, in which basal astrocyte Ca²⁺ controls resting arteriole tone, and in which endfeet Ca²⁺ transients are mostly observed in response to increases in blood flow, rather than to initiate rapid changes caused by neural activity.

Pial Collaterals in humans: Imaging, hemodynamics, determinants and effect on clinical outcomes in patients with acute ischemic stroke

Bijoy Menon, University of Calgary

Pial collaterals are native (pre-existing) anastomoses that cross-connect a small number of distal-most arterioles within the crowns of the

cerebral artery trees. These collaterals represent a potential, under-recognized, emergency “backdoor” to maintain blood flow to brain that would otherwise die during an acute stroke. There is wide variability in the presence of these collaterals in humans. There are, however, very few studies that have sought to understand why this variability exists in the first place, although experimental studies in animals have identified large contributions from environmental factors and genes for differences in the density and diameter of these vessels. Knowledge of this nature in humans can potentially be used to identify therapeutic targets capable of modulating native collateral status, thereby achieving better clinical outcomes in patients with ischemic stroke. This presentation describes the challenges we face when trying to image pial collaterals in humans. It then goes on to describe what we have learned until now about pial collaterals, their physiology and their determinants from such studies. It also describes the vital role that measuring pial collaterals plays in determining treatment decisions and prognosis in patients with acute ischemic stroke. Finally, the presentation seeks to give an overview of exciting new research in this field in humans that is informed by current research in animal labs.

Imaging and augmenting collateral blood flow in the brain during acute ischemic stroke

Ian Winship, University of Alberta

Blockage of a cerebral artery induces focal ischemia downstream of the occlusion. While severe blood flow reduction in the core of the ischemic territory results in rapid cell death, surrounding “penumbral” regions have partially maintained circulation and delayed cell death. Maintained blood flow is primarily due to “collateral circulation.” Collateral circulation refers to alternative circulatory pathways for blood that allow blood to reach ischemic territories when primary routes are blocked, and is a critical predictor of clinical prognosis after stroke and response to recanalization. Here, I will discuss my lab’s work imaging and augmenting collateral blood flow in clinically relevant models of ischemic stroke. Our studies use high-resolution, wide field laser speckle contrast imaging to create maps of collateral circulation in the ischemic cortex, and two photon microscopy to quantitatively assess blood flow velocity and direction in ischemic tissue. Our data shows that anastomatic connections between the distal branches of the

arterial cerebral artery (ACA) and the middle cerebral artery (MCA) can partially compensate for ischemia induced by proximal or distal MCA occlusion. Additionally, we demonstrate that transiently diverting blood flow from peripheral circulation towards the brain via intra-aortic catheter and balloon induces persistent increases in blood flow through these anastomoses. New methods for systemic or targeted collateral blood flow augmentation will also be discussed. Given the importance of these collateral pathways in predicting stroke outcome and response to treatment, our work highlights the potential of collateral therapeutics as an adjuvant or stand-alone therapy for acute ischemic stroke.

Seizures Induce a Severe Ischemic/Hypoxic Episode

G. Campbell Teskey, University of Calgary

Seizures often result in negative neurological outcomes as well as post-seizure sensory, cognitive and behavioural dysfunction similar to ischemic/hypoxic attacks. Given that seizures also disrupt ion homeostasis and neurovascular control of blood flow, we hypothesized that seizures would lead to a subsequent ischemic/hypoxic event culminating in post-seizure behavioural impairment. We discovered a severe hypoxic event ($pO_2 < 10\text{mmHg}$) that begins after the termination of a seizure in both experimental animal models and a person exhibiting spontaneous epileptic seizures. We also observed post-seizure hypoxia accompanied by reduced hippocampal perfusion in awake, freely moving rats. Nifedipine, utilized as a tool that promotes vasodilation and prevents vasoconstriction (ischemia), inhibited the severe ischemia/hypoxia after experimental seizures and prevented ensuing behavioural deficits. This novel finding identifies ischemia/hypoxia as a new mechanism by which seizures may negatively impact brain function and suggests pharmacological treatments that target attenuation of the post-seizure ischemic/hypoxic event could prevent the neurological and behavioural consequences of seizures.

Symposium 2: Genetic and environmental regulation of gene expression and development of vulnerability to psychiatric disorders

Circuit-wide transcriptional profiling in a mouse model of depression

Rosemary Bagot, Icahn School of Medicine at Mt Sinai

Alterations in nucleus accumbens are implicated in the pathophysiology of depression. Chronic social defeat stress (CSDS) produces a depression-like phenotype in mice associated with robust transcriptional alterations in NAC. The NAC receives major excitatory inputs from the medial prefrontal cortex (mPFC), ventral hippocampus (vHIP), and basolateral amygdala (BLA) and alterations in this circuitry regulate depression-like behavior. To examine the mechanistic basis of CSDS effects on this network, we performed next-generation RNA-sequencing on NAC, mPFC, vHIP, and BLA tissue from control animals and mice determined to be either susceptible or resilient to CSDS. We employed both differential expression analyses as well as co-expression network analyses to further characterize circuit-wide transcriptional profiles. Inter-region comparisons of global gene expression patterns across the circuit indicate enhanced synchrony of differential expression between NAC and mPFC in mice resilient to CSDS and increased synchrony between mPFC and vHIP in susceptible mice. We constructed weighted gene co-expression networks in control, susceptible and resilient mice and identified modules of genes exhibiting differential connectivity between conditions. Several of these modules also showed significant enrichment of differentially expressed genes. To identify transcriptional “master regulators” of resilient-specific gene expression profiles, we focused on genes with high intra-module connectivity. Viral-mediated over-expression of one potential master regulator, *sdh1*, in mPFC increased resilience to CSDS while the same manipulation in vHIP increased susceptibility. Our findings suggest that susceptibility and resilience to CSDS associate with distinct circuit-wide transcriptional profiles. Susceptibility may arise from a general dysregulation of transcriptional programs in this circuit.

A novel role for the 6th base: how DNA hydroxymethylation governs adaptive behavior

Timothy Bredy, University of California, Irvine

5-hydroxymethylcytosine (5-hmC) is a novel DNA modification that is highly enriched in the adult brain and dynamically regulated by neural activity. 5-hmC accumulates across the lifespan; however, the functional relevance of this change in 5-hmC and whether it is necessary for

behavioral adaptation have not been fully elucidated. Moreover, although the ten-eleven translocation (Tet) family of enzymes is known to be essential for converting methylated DNA to 5-hmC, the role of individual Tet proteins in the adult cortex remains unclear. Using 5-hmC capture together with high-throughput DNA sequencing on individual mice, we show that fear extinction, an important form of reversal learning, leads to a dramatic genome-wide redistribution of 5-hmC within the infralimbic prefrontal cortex. Moreover, extinction learning-induced Tet3-mediated accumulation of 5-hmC is associated with the establishment of epigenetic states that promote gene expression and rapid behavioral adaptation.

Epigenetics and Early Intervention: a study of DNA methylation in the Nurse Family Partnership

Kieran O'Donnell, Douglas Research Institute

The persisting influence of childhood adversity on vulnerability for mental disorder is well established. Both animal models and clinical studies identify epigenetic regulation of genomic function as a plausible mediating mechanism. It is unknown if early intervention programs, which buffer the effects of early adversity, mediate their treatment effects via epigenetic mechanisms. We have carried out the first epigenetic analysis in the Nurse Family Partnership (NFP), a randomised control trial of a perinatal parenting intervention targeting high-risk women and their infants. We assessed genome-wide DNA methylation (Illumina 450k) in whole blood samples from adult participants (n=69) born to women from treatment and control groups. Methylation data were corrected for cellular heterogeneity and batch effects. Principal component analysis, variance and regression analyses determined associations between DNA methylation, early intervention and current psychiatric symptoms. The NFP program was significantly associated DNA methylation 27 years post-intervention ($F = 5.28$, $p=0.03$). Barlett tests of variance revealed markedly increased variance in methylation profiles in the treatment group. Both early intervention and current symptoms of substance abuse associated a principal component accounting for 8.1% of the variance in DNA methylation. Candidate genes identified from these analyses include PAX8 and CACNA1D implicated in development and risk of psychiatric disorder, respectively. We provide some of the first clinical evidence that early intervention is associated with DNA methylation. These novel

data suggest that epigenetic mechanisms may, at least in part, mediate treatment effects associated with early intervention programmes such as the NFP.

Genomic Embedding of Early Life Experiences

Michael Kobor, University of British Columbia

Symposium 3: Synaptic Adhesion Molecules: From Synapse Development to Complex Behavior

Synaptogenic adhesion complexes for excitatory and inhibitory synapse development

Hideto Takahashi, Institut de recherches cliniques de Montreal

Synapse development requires not only physical contact between axons and target neurons but also chemically matched pre- and post-synaptic assembly. Thus, synaptic organizing complexes, trans-neuronal adhesion complexes with the ability to induce pre- and/or post-synaptic assembly, have been suggested to function as essential molecular signals for synapse development. The neuroligin-neurexin complex has been the most notable synaptic organizing complex and a genetic determinant predisposing to autism. However, synapse diversity suggests many other synaptic organizing complexes for excitatory (glutamatergic) and/or inhibitory (GABAergic) synapses. To identify novel synaptic organizers that induce presynaptic assembly, we performed a functional expression screen based on a neuron-fibroblast coculture assay combined with full-length cDNA library or candidate prediction. Further, to identify their presynaptic receptor, we performed candidate cDNA screen based on a cell-surface binding assay using soluble Fc-fusion extracellular proteins of the synaptic organizers. Using these two screening approaches, we demonstrated that TrkC-PTP σ trans-synaptic complex acts as a bidirectional synaptic organizing complex that selectively regulates excitatory synapse development. We also identified Slitrk3-PTP δ trans-synaptic complex as an inhibitory synapse-specific synaptic organizing complex. Given genetic linkages of our identified molecules with neuropsychiatric disorders, our data suggest that aberrant synaptic organization could be a common pathogenesis of many neuropsychiatric disorders.

Variable effects on brain and behaviour in mouse models featuring loss of function

mutations in Neuroligin3, Neurexin1, and Cntnap2

Jason Lerch, SickKids

Advances in the genetics of neurodevelopmental disorders, in particular autism, have identified a series of causative mutations in synapse adhesion genes. We have, over the last years, set out to phenotype multiple mouse models related to autism spectrum disorders in order to understand commonalities and differences amongst these models. To that end we've been using high-resolution Magnetic Resonance Imaging and assessing subtle alterations in neuroanatomy across the brain. Included in this effort have been several mouse lines with mutations affecting synapse adhesion, including neurexins, neuroligins, and Cntnap2. Intriguingly, the phenotype of each of these mutations is drastically different, with Neurexin1- α showing brain overgrowth, Neuroligin3 reduced brain volume, and Cntnap2 no differences. This talk will explore these differences in phenotype between related synapse adhesion mutation mouse models in greater detail.

Synaptic Adhesion Molecules: From Synapse Development to Complex Behavior

Valerie Mongrain, Université de Montréal

Current hypotheses support that sleep regulation depends on mechanisms controlling synaptic plasticity. Indeed, modifications in neuronal synchrony and firing occurring as a function of vigilance state changes across the 24-h day involve profound alterations in synaptic transmission. Moreover, sleep loss, achieved for instance using sleep deprivation, impairs mechanisms underlying synaptic plasticity in several brain areas. Therefore, we anticipated that synaptic adhesion molecules, which control synaptic strength by regulating both pre- and post-synaptic mechanisms, will be involved in sleep regulation. Results supporting this hypothesis and concerning two different synaptic adhesion systems will be presented. The first findings will focus on the role of Neuroligin 1 in the regulation of wakefulness duration and of sleep intensity. Then, recent data showing an implication of EphA4 in the control of sleep consolidation and of paradoxical sleep duration will be highlighted. Lastly, two different molecular mechanisms targeting transcription that likely contribute to sleep/wake-dependent changes in the expression of these synaptic adhesion elements will be discussed.

Translational control of autism and Fragile-X syndrome

Nahum Sonenberg, McGill University

Symposium 4: Moving toward an understanding of brain functioning using computational approaches***Variability, homeostasis and modulation in neural circuits***

Eve Marder, Brandeis University

Multi-scale in silico modeling of personal ion channel gene mutations as a cause of epilepsy and brain mediated sudden death

Tara Klassen, University of British Columbia

One of the central challenges of personalized medicine is to solve a model where risk is a non-linear summation of contributions from many gene variants. The fourth most prevalent neurological disorder, epilepsy, affects 65 million people worldwide and ~1% of the Canadian population. Epilepsy is a multifactorial spectrum of clinical disorders characterized by unpredictable seizures that vary in type and severity resulting from an imbalance of excitation and inhibition in neural networks. Ion channels regulate and respond to changes in membrane voltage such that mutations in >60 of these genes cause a plethora of neurological and somatic excitability disorders including epilepsy, cardiac arrhythmias and sudden unexpected death in epilepsy (SUDEP). Genetic background has an essential role on variant penetrance and complicates risk prediction because inheritance of identical ion channel mutations can be both dominant and recessive, and causative mutations are found in asymptomatic individuals. Regardless of disease status, genetic variation in a personal channotype is extensive, forming a highly complex pattern of common and rare alleles. To evaluate the contribution of common population polymorphisms to risk in epilepsy and SUDEP we have inverted the classical experimental paradigm employing computational models of proteins, cells and neural networks to evaluate the theoretical (dys)functional contributions from combinations of ion channel mutations. These combinatorial models of voltage and ligand-gated channels have identified compound mutations which result in overt cellular hyperexcitability or quiescence. Cross-comparison with pre-existing channotypes is guiding mutation profile analysis for in vitro experimentation and validation.

Gene networks for understanding brain function and dysfunction

Jesse Gillis, Cold Spring Harbor Lab

A central challenge to understanding neuropsychiatric disorders is determining how candidate variants interact with one another and the environment to produce a disease phenotype. In response to this challenge, gene networks have become a common resource for integrating potentially diffuse functional effects into a single common framework. Ideally, candidate variants not only converge on consistent pathways or interact within a network, but also do so in a way that is perturbed in response to disease or factors relevant to disease. While gene network analyses can be extremely opaque, they are grounded in a few straightforward principles. The central top-down principle in the interpretation of gene networks is 'Guilt by Association' (GBA) and it simply states that genes which share functions are more likely to be associated. Many, perhaps most, analyses of 'novel' sets of candidate disease genes rely on GBA to claim that the genes have some known shared function determinable through their associations. In a recent series of papers, we laid out grounds for treating previous gene network analyses related to function with skepticism. We showed that gene networks (protein interactions, genetic interactions and co-expression) tend to encode very generic information about gene function without learnable specificity, leading to highly multifunctional genes dominating analyses to the point that details of network structure have a surprisingly small impact. We suggest that this property plays a dominant role in most previously reported network analyses. We focus on potential corrections in an analysis of schizophrenia co-expression and disease-associated variant data.

Understanding sensory-to-motor transformations through network models

Gunnar Blohm, Queen's University

Artificial neural network models provide a mechanistic link between behaviour, neuronal recordings and disease that cannot be achieved otherwise. Here, I exploit neural networks to gain insight about the brain's sensory to motor transformation for planning reaching movements. Emergent properties of neural networks learning this complex transformation are in tight accordance with electrophysiological observations. I will show how damage and re-

learning of such networks can reproduce acute and acquired symptoms of posterior parietal cortex lesions due to stroke. We hope that findings from these neural network studies will help understanding stroke symptoms and ameliorate treatment options.

Symposium 5: Large-scale brain dynamics: combining insights from intracranial EEG and fMRI

Thalamic control of cortical dynamics

Yuri Saalman, University of Wisconsin – Madison

Brain networks are commonly defined using correlations between blood oxygen level-dependent (BOLD) signals in different brain areas. Although evidence suggests that gamma-band (30–100 Hz) neural activity contributes to local BOLD signals, the neural basis of inter-areal BOLD correlations is unclear. We first defined a visual network in monkeys based on inter-areal BOLD correlations, and then simultaneously recorded local field potentials (LFPs) from the same four network areas. Low-frequency oscillations (<20 Hz), and not gamma activity, predominantly contributed to inter-areal BOLD correlations. Specifically, the degree of synchrony between low-frequency oscillations in different network areas best predicted BOLD connectivity. The low-frequency oscillations also influenced local processing by modulating gamma activity within individual areas. We suggest that such cross-frequency coupling links local BOLD signals to BOLD correlations across distributed networks. How are these cortical oscillations and the inter-areal synchrony controlled? Based on its anatomical connectivity with the cortex, we hypothesized that the pulvinar, a thalamic nucleus, regulates cortical synchrony. We mapped pulvino-cortical networks within the visual system, using diffusion magnetic resonance imaging, and simultaneously recorded spikes and LFPs from these interconnected network sites in monkeys performing a visuospatial attention task. The pulvinar synchronized activity between interconnected cortical areas according to attentional allocation, suggesting a vital role for the thalamus in regulating information transmission across the cortex according to behavioral demands.

Large-scale patterns of rhythmic suppression in human cerebral cortex

Christopher Honey, University of Toronto

Rhythmic activity in populations of neurons is believed to coordinate neural activity within and across regions of the mammalian brain. However, we lack a framework for understanding which rhythms operate in each region and what their distinct roles are. Here, using intracranial recordings from humans and macaques, we show that, almost all regions of the primate neocortex contain at least one suppressive oscillatory process within the 4-30 Hz range. Population activity is decreased on average when the suppressive rhythm is strong, and this effect is strongest at particular phases of the local oscillation. The frequency of suppressive rhythms varies across regions, and with a similar topography across individuals. Moreover, increases in the activity of the suppressive rhythm occur at the same time as decreases in the BOLD signal, and changes in suppressive rhythms are also coordinated across regions. We consider whether sets of regions with shared suppressive rhythms correspond to the large-scale brain networks that are ubiquitously observed in neuroimaging. Overall, the concept of suppressive rhythms provides a framework for understanding how rhythmic activity modulates function within and across a wide range of mammalian cortical networks.

How single cell activity in prefrontal and anterior cingulate cortex contributes to large-scale network dynamics: State specific burst synchronization at beta and gamma band activity

T. Womelsdorf, York University

Attention is realized in the brain by the rapid formation of a coalition of brain cells into a large-scale attention network. Cells in medial and lateral prefrontal cortex control this formation of attention networks, but how the activity of single cells exerts network control is unknown. This talk will discuss possible mechanisms on how cell activation dynamics link to large scale network dynamics and suggest that burst-synchronization could serve as potent candidate mechanism to achieve network control during attentional states. We find in single cell activity of macaque prefrontal cortex an increase in the firing of brief 200 Hz burst events when subjects shifted attention and engaged in selective sensory processing. In contrast to non-burst spikes, burst spikes synchronized at narrow beta and gamma frequencies. Burst synchronization is anatomically specific, functionally connecting the anterior cingulate cortex with the lateral prefrontal cortex, both key

players of attentional control. These findings identify burst synchronization as a possible mechanism to enable the formation of large-scale attention networks based on 'top-down' information in dorsal anterior cingulate and lateral prefrontal cortex.

The neurophysiological basis of the default-mode network

Karim Jerbi, University of Montreal

Resting-state networks are predominantly investigated by measuring the brain's haemodynamic responses during rest. However, our understanding of its functional role will not be complete without elucidating their electrophysiological underpinnings. We report an extensive intracerebral EEG (iEEG) exploration of the neural correlates of intrinsic correlation patterns. To this end, we acquired data directly from core default-mode network (DMN) areas, including the posterior cingulate cortex (PCC) and the medial prefrontal cortex (MPFC) in stereotactically implanted epilepsy patients. We investigated spontaneous long-range coupling between nodes of the DMN using a combination of time and frequency domain correlation measures. The observed iEEG resting-state correlation dynamics were compared to the functional connectivity patterns obtained in the same subjects with fMRI. Our results reveal a combination of sustained and transient patterns of long-distance neuronal correlations between DMN nodes at various spatial, spectral and temporal scales. In particular, sustained broadband gamma amplitude envelopes in DMN areas reveal slow correlated fluctuations, similar to those observed with fMRI. Our findings help bridge the gap between fMRI and human electrophysiological studies of the brain's large-scale functional architecture at rest.

Symposium 6: Novel cellular and molecular mechanisms in the pathophysiology of parkinsonism

The function of PINK1 and parkin in mitochondrial quality-control

Edward Fon, McGill University

Parkinson's disease (PD) is a common, devastating neurodegenerative disorder. Both genetic and environmental models strongly implicate mitochondrial dysfunction in PD. In particular, PINK1 and Parkin, two recessive PD genes, function in a common pathway regulating mitochondrial quality-control. In healthy

mitochondria, PINK1, a mitochondrial kinase, is rapidly degraded in a process involving both mitochondrial proteases and the cytosolic proteasome. This process is highly dependent upon the membrane potential across the mitochondrial inner membrane ($\Delta\Psi_m$), which drives PINK1 import into mitochondria. Indeed, mitochondrial damage that dissipates $\Delta\Psi_m$ blocks PINK1 import and leads to its accumulation on the surface of mitochondria. PINK1 accumulation triggers the translocation of parkin, an E3 ubiquitin ligase, from the cytosol to mitochondria, where it mediates the elimination of dysfunctional mitochondria by autophagy (mitophagy). From these studies, a concept of PD pathogenesis is emerging whereby defects in PINK1 or parkin function reduce the efficiency with which damaged mitochondria, a major source of toxic reactive oxygen species, are eliminated. We will present recent work, based on the crystal structure of parkin and on genetic screens to identify regulators of parkin function, exploring how parkin is activated upon its recruitment to damaged mitochondria.

Axonal arborization and energetic metabolism of nigral dopamine neurons: a window into selective vulnerability

Louis-Eric Trudeau, Université de Montréal

Parkinson's disease (PD) is associated with severe locomotor deficits accompanied by the selective and progressive loss of a small subset of neuronal populations including dopamine (DA) neurons of the substantia nigra pars compacta (SNc). Why SNc neurons are particularly vulnerable and degenerate in PD is poorly understood. However, mitochondrial dysfunction and oxidative stress have been proposed as key contributors. This presentation will discuss recent data testing the hypothesis that the selective vulnerability of SNc DA neurons, in comparison to other less vulnerable DA neurons including those of the ventral tegmental area (VTA), is due to the fact that these neurons have a higher basal rate of mitochondrial oxidative phosphorylation related to their considerably larger axonal arborization. Work funded by Brain Canada and the Krembil Foundation.

Multiple roles of Lmx1a and Lmx1b in dopaminergic axonal connectivity and maintenance

Martin Levesque, Université Laval

Degeneration of midbrain dopaminergic neurons (mDA) is the principal cause of Parkinson's

disease (PD). Graft of dopaminergic neurons newly generated from stem cells represents a promising therapeutic avenue. However, a major factor limiting success in transplantation studies is the inappropriate re-innervation of the grafted neurons. Thus it is primordial to identify factors regulating axon projection and connectivity of mDA neurons. Here we demonstrate the role of two transcription factors, Lmx1a and Lmx1b, in the regulation of mDA neurons circuit formation. We then reveal Lmx1a/b target genes and identify PlexinC1 as a crucial axon guidance receptor allowing segregation of dopaminergic pathways. Our results show new mechanisms regulating dopamine neurons connectivity and should help in the effort to understand the molecular factors contributing to the efficiency of cell replacement therapies for PD. In addition to their role during development, we demonstrate the requirement of Lmx1a/b in the maintenance of dopaminergic neurons. Inactivation of Lmx1a/b in mDA neurons induces metabolic changes and leads to dopaminergic axon degeneration, alpha-synuclein accumulation, microglia activation and progressive degeneration of mDA neurons. Gene profiling experiments also reveal that Lmx1a/b regulate genes of the mitochondrial respiratory chain. Our results show that the maintenance of dopaminergic systems is underpinned by the continued action of Lmx1a/b beyond the stages of development. These findings also suggest that Lmx1a/b could be promising therapeutic targets to prevent the degeneration of mDA neurons in patients suffering from PD. Finally, mutant mice for Lmx1a/b represent a valuable new model that reproduces cellular characteristics of PD.

Multiple Parkinson's disease-linked proteins regulate synaptic transmission and neurotransmitter receptor trafficking

Austen Milnerwood, University of British Columbia

The etiology of Parkinson's disease (PD) is unknown, symptoms are progressive and patients suffer inexorable decline of cognitive and motor functions then death. Contemporary treatments have limited efficacy, troubling side effects and do not modify progression. Studies of familial parkinsonism have linked pathogenic mutations in several genes, providing the potential for mechanistic insights and novel therapeutic targets. Furthermore, tracking of 'asymptomatic' mutation carriers enables investigation of early disease processes. Mutations in alpha-synuclein (aSyn) and

leucine-rich repeat kinase-2 (LRRK2) are the major genetic risk factors for sporadic and familial PD. Recently, mutations in Vacuolar Protein Sorting 35 (VPS35) and Receptor Mediated Endocytosis 8 (RME8) have also been linked to autosomal dominant, late-onset parkinsonism. As multiple mutations in several proteins produce a similar disease, studying their physiological and pathophysiological activity may uncover a common neuronal dysfunction important to the etiology of many forms of parkinsonism. Longitudinal investigations of transgenic cells and mice, alongside emerging evidence from asymptomatic mutation carriers, are redefining the classical understanding of PD pathogenesis. We demonstrate that LRRK2, VPS35 and RME8 physically interact and, when mutated, alter neuronal connectivity and synaptic function. Furthermore, neurotransmitter receptor function and trafficking are altered by PD mutations in these proteins, and we are beginning to define the links with aSyn function / pathology. We posit that perturbed endosomal and vesicle sorting is a common feature of genetic, and potentially idiopathic, parkinsonism. An improved knowledge of the underlying pathophysiology is vital to define biomarkers and design neuroprotective strategies for PD and related disorders.

Symposium 7: New cuts by calpain to remodel the nervous system

Calpain activity maintains the stability of neurite morphology in Vivo

Tim O'Connor, University of British Columbia

An essential feature of the nervous system is its long-term stability. After development a neuron's capacity for plasticity is restricted. This stability is essential for the maintenance of neuronal identity, morphology, appropriate connectivity, and ultimately network properties. This has engendered a belief that with age, neurons settle into a 'locked-in' default state. However, our recent work leads us to propose a markedly different hypothesis from this long-standing view. Our data uncovers a novel principal for the stable maintenance of neuronal morphology. We have found that maintenance of stable neuronal processes is not a passive default mechanism but instead requires ongoing active repression of sprouting. This stability is established by an antagonistic interaction between the calcium-activated cysteine protease, calpain, and specific proteins including cortactin, that regulate the assembly of the actin cytoskeletal

network. Disruption of calpain activity results in exuberant growth and sprouting along axons in vitro and our most recent data provides evidence that this active stabilization model operates in vivo. Calpain activity is robust in neurons of the developing nerve cord in *Drosophila melanogaster*. After manipulating calpain activity and cortactin expression in specific identified neurons in the developing nerve cord we find that neurons show exuberant growth and branching. We will test whether similar mechanisms regulate neuronal plasticity in the mature nervous system.

Conditional disruption of calpain in the CNS alters dendrite morphology, impairs LTP, and promotes neuronal survival following injury

Mandana Amini, University of Ottawa

Ubiquitous classical (typical) calpains, calpain-1 and -2, are Ca²⁺-dependent cysteine proteases which have been associated with numerous physiological and pathological cellular functions. However, clear understanding of the role of calpains in the CNS has been hampered by the lack of appropriate deletion paradigms in the brain. In this study, we describe a unique model of conditional deletion of both calpain-1 and -2 which more definitively assesses the role of these ubiquitous proteases in brain development/function and pathology. Surprisingly, we show that these calpains are not critical for gross CNS development. However, our study reveals that calpain-1/2 loss leads to reduced dendritic branching complexity and spine density deficits associated with major deterioration in hippocampal long-term potentiation and spatial memory. Moreover, calpain-1/2 deficient neurons were significantly resistant to injury induced by excitotoxic stress or mitochondrial toxicity. Examination of downstream target showed that the conversion of the Cdk5 activator, p35, to pathogenic p25 form, occurred only in the presence of calpain and it played a major role in calpain-mediated neuronal death. These findings unequivocally establish two central roles of calpain-1/2 in CNS function in plasticity and degeneration.

A tail to memorize: cleavage of synaptic GluN2B by calpain to support synaptic plasticity

Paul De Koninck, Université Laval

At excitatory synapses, the mechanisms that support learning may be initiated by long-term alterations in the content of specific dendritic

spines to modify synaptic strength and to prime connections for further activity-dependent modifications. Synaptic potentiation is triggered by NMDA receptor activation and downstream signaling processes that involve Ca^{2+} -responsive enzymes, such as CaMKII and ERK. A particular feature of the NMDA receptor is the long cytoplasmic tails of its GluN2 subunits, which are believed to engage in subunit-specific interactions with signaling enzymes for synaptic potentiation. The interaction between CaMKII and GluN2B is particularly recognized as important for the induction of synaptic potentiation. Meanwhile, calpain is capable of cleaving the cytoplasmic tail of GluN2 subunits, particularly GluN2B, raising the question of whether such cleavage can participate in GluN2B-dependent synaptic signaling. We will discuss the hypothesis that strong synaptic activity can trigger calpain-mediated cleavage of the c-tail of GluN2B, releasing a signaling complex to which CaMKII is associated and which participates in signaling for synaptic potentiation. We will examine this process with the combination of biochemical and molecular methods, advanced optical imaging and electrophysiology in rat hippocampal and cortical cultures and slices. Results from our experiments might reveal a novel view of how NMDA receptors modify synaptic contents for modifying strength in transmission and/or for priming specific synapses for further modifications.

Role of typical and atypical calpains in cleavage of PKCs into PKMs for memory formation

Carole Abi Farah, McGill University

Protein kinase Ms (PKMs) are truncated persistently active forms of protein kinase Cs (PKCs) that play an important role in memory formation both in vertebrates and invertebrates but may be generated differently in these systems. In rodents, the atypical PKM Zeta can be transcribed from an internal promoter in the PKC Zeta gene whereas this transcript is not present outside of chordates. In Aplysia, the atypical PKM Apl III, the orthologue of PKM zeta, is generated by calpain-dependent cleavage of PKC Apl III. To investigate the role of calpains in the formation of PKMs and memory, we have cloned Aplysia calpains and generated constructs and dominant negative forms for the classical, SOL and PalB calpains which we found to be enriched in sensory and motor neurons. To monitor cleavage of the different PKC isoforms and PKM formation in

live cells, we generated PKC FRET (Fluorescence Resonance Energy Transfer) reporters in which Cyan Fluorescent Protein (CFP) was added to the N-terminal and Yellow Fluorescent Protein (YFP) added to the C-terminal. Our results show that different PKC isoforms are cleaved by distinct calpains in diverse cellular plasticity paradigms. The calcium-dependent PKC Apl I is cleaved by classical calpain during associative intermediate-term facilitation (ITF). In contrast, the SOL calpain is important for cleaving PKC Apl III downstream of PKC Apl III activation in a positive feedback mechanism that may be important for persistent formation of PKM Apl III during long-term memory.

Symposium 8: Linking neural circuit dynamics to cognition and behaviour

Role of sleep for motor skill learning

Masami Tatsuno, University of Lethbridge

Increasing evidence suggests that sleep plays an active role in our cognitive and motor functions. One suggestion is that memory is consolidated during sleep or quiet wakefulness, possibly by reactivating neural activity that occurs during waking and driving synaptic changes that strengthen a memory trace. With respect to spatial memory, studies have suggested that reactivation during non-REM sleep and quiet wakefulness is particularly important. With respect to motor skill memory, even though there is substantial behavioural evidence showing the importance of sleep, relatively little is known about the underlying neural activity. We have performed high-density multi-electrode recordings from rats as they are trained with the single-pellet reaching task. Daily recordings (a 3 hr pre-task sleep, a 30 min task, and a 3 hr post-task sleep) were performed 3-4 weeks to cover the entire learning period. By analyzing spike-sorted unit activity, we obtained preliminary evidence that reactivation of the single pellet reaching task occurred during both non-REM and REM sleep. Analysis of multi-unit activity (MUA) during cortical spindles also demonstrated that the modulation of MUA decreases in the post-task sleep and that the decrease of modulation was related to learning. In summary, our experiments have provided the evidence of reactivation for motor skill learning during both non-REM and REM sleep. Additional MUA analysis also demonstrates that the neural dynamics of sleep spindles change with motor skill learning.

Processing objects and space in the hippocampus

Jennie Young, Massachusetts Institute of Technology

The hippocampus plays a key role in the acquisition of new memories for places and events. In rodents, it is believed to provide a spatial framework within which items and events can be integrated to form a coherent representation of the animal's on-going experience. Sensory information arrives in the hippocampus through two parallel processing streams: place-related information from medial entorhinal cortex (MEC) and object-related information from lateral entorhinal cortex (LEC). The unique anatomical arrangement of inputs to CA1 suggests that it may receive both integrated and segregated space/object information - from upstream hippocampal subregions and directly from MEC/LEC, respectively. We carried out large-scale ensemble recordings in area CA1 of mice as they performed novel object-location recognition, a one-trial contextual learning task that occurs in a familiarized environment. We present physiological data that reflects the anatomical segregation of parallel processing streams to the hippocampus, and we find that the formation of new object-place representations requires CA3 input to CA1. Distal CA1 cells, which receive predominantly LEC input, fire selectively at locations relative to objects in both controls and in mice with blocked CA3 transmission. However, only control animals show changes in object-related firing that are specifically associated with object displacement. Our ensemble data support the idea that CA3 inputs provide a more stable representation of the familiarized context to CA1; Meanwhile information on new features in the environment arrives concurrently through the direct entorhinal inputs, where it can be quickly incorporated to reflect the animal's present experience.

Segmentation of spatial experience by theta oscillations

Matthijs van der Meer, University of Waterloo

"Place cells" in the rodent hippocampus are tuned to specific locations within an environment, such that the animal's location can be inferred on a moment-to-moment basis from the ensemble activity of many such cells when recorded simultaneously. This decoding procedure enables access to the content of hippocampal sequential firing patterns that occur

during off-line "replay" and during theta states associated with attentive spatial behavior. Specifically, it becomes possible to determine if the hippocampus represents a trajectory corresponding to one (left) or another (right) spatial choice -- or to an experienced past or imagined future. Historically, hippocampal sequences have been interpreted as a short-term memory buffer that repeats recent experience to facilitate systems consolidation into other brain structures. However, recent results suggest that the content of these hippocampal sequences can dissociate from literal experience. In particular, theta sequences do not represent the environment uniformly, but tend to avoid crossing turns and decision points, resulting in a "chunking" of spatial experience into segments. Awake replay can favor remote, rather than more recent experiences, as well as contain never-experienced trajectories. Thus, the rodent hippocampus appears to broadcast a selective and highly processed record of experience. These observations invite questions of what factors control the content of hippocampal sequences, and how they are interpreted by downstream structures that may be involved in the evaluation of alternatives during decision making and in the formation of semantic-like knowledge structures.

Hippocampal oscillations in monkey and humans during memory-guided visual search

Kari Hoffman, York University

Symposium 9: An unexpected roundtrip journey through the hippocampal trisynaptic excitatory network

Synaptic vesicle dynamics and the timing and efficacy of glutamate release at hippocampal mossy fibre terminals

Katalin Toth, Université Laval

Hippocampal mossy fibres play a key role in translating dense cortical information into sparse hippocampal code. How do mossy fibres shape the fidelity of neurotransmission? How do the unique features of this presynaptic terminal such as the presence of multiple release sites, a very large vesicle pool and a profound short-term facilitation contribute to information processing? Calcium-dependent neurotransmitter release involves a fast synchronous component and a slower asynchronous phase. We investigated how asynchronous release influences postsynaptic action potential coding. Asynchronous release is expected to decrease

temporal fidelity, surprisingly our results show that in the presence of asynchronous release the precision of action potential firing is increased in an activity-dependent manner. Synaptic short-term plasticity is a key regulator of neuronal communication. We found that two distinct presynaptic mechanisms are involved in short-term facilitation, with their relative contribution dependent on extracellular calcium concentration: multivesicular release and the recruitment of additional release sites. Multivesicular release was dominant at lower release probabilities and the recruitment of additional release sites played a key role at higher p. The increase in N could be promoted by calcium microdomains of heterogeneous amplitudes observed in single mossy fiber boutons. Our findings suggest that the combination of multivesicular release and the recruitment of additional release sites act together to increase glutamate release during bursts of activity. This is supported by the compartmentalized spatial profile of calcium elevations in boutons and helps to expand the dynamic range of mossy fibers information transfer.

Astrocytes detect and regulate basal synaptic transmission at single CA1 synapses

Richard Robitaille, Université de Montréal

Basal synaptic transmission is fundamental for information processing in the brain. It occurs at individual synapses and involves the release of neurotransmitters evoked by single action potentials. Over the past two decades, evidence has shown that astrocytes actively regulate synaptic transmission. Classically, it was considered that these glial cells detect and in turn modulate synaptic transmission during intense and sustained neuronal network activity. However, the ability of astrocytes to detect and regulate basal synaptic transmission remained unclear and controversial. We monitored basal, minimal synaptic transmission at CA3-CA1 synapses of rat hippocampus using whole-cell patch-clamp recordings. Simultaneously, we monitored local Ca²⁺ activity in small compartments along astrocytic processes using the line-scan mode of a confocal microscope. We show that astrocytes in CA1 region of hippocampus detect synaptic activity induced by single synaptic stimulation at functional compartments along the astrocytic process. This detection is mediated by metabotropic glutamate receptors subtype 5. Moreover, we uncovered that astrocytes release purines to increase the

efficacy of transmission in CA1 pyramidal cells through activation of presynaptic adenosine A2A receptors. This work provides a new perspective of fundamental brain function since astrocytes are now intimately involved with neurons in the regulation of elementary synaptic communication in the brain. We will discuss how astrocyte can integrate synaptic activity in time and space, providing a unique feedback to the neuronal network.

Developmentally-regulated spatiotemporal features of calcium signaling at CA1 glutamatergic synapses

Jean-Claude Béïque, University of Ottawa

New mechanisms for bidirectional communication in the trisynaptic glutamatergic circuit of the hippocampus

Sylvain Williams, McGill University

The hippocampus plays a central role in memory processing. The seminal work of neuroanatomists Ramon Y Cajal and Lorente de No, and physiologist Per Andersen, proposed that information travels unidirectionally across the tri-synaptic circuit from the CA3, CA1 and subiculum. This canonical unidirectional flow of information through feed-forward glutamatergic synapses across the hippocampus remains central to our understanding of how this structure operates. The objective of this presentation is to revisit this paradigm and determine the direction of information flow in a complete hippocampus in vitro and in freely behaving mice in vivo. The predominant state of the hippocampus is theta rhythm, a 4-8 Hz frequency oscillation found both in the complete hippocampus in vitro as well as during exploration or REM sleep in vivo. By measuring theta rhythm and cell spiking across the CA3, CA1 and subiculum transverse axis in the complete hippocampus, we found that the hippocampus is made-up of two separate networks located in CA3 and subiculum. Surprisingly, we found that theta flows predominantly backward from subiculum to CA3 and that spikes in subiculum most often preceded those in the CA3. Moreover, optogenetic activation of GABAergic interneurons in the subiculum generated CA3 theta activity and modulated the intrinsic CA3 network. Finally, in vivo recordings suggest that this reversed theta influence of subiculum operates in the majority of theta epochs to govern the timing of the CA3. Thus, intra-hippocampal communication between sub-

regions is bidirectional and suggests a new form of communication in hippocampus.

Symposium 10: Novel Pharmacology of Ion-Channels & Transporters

Allosteric potentiation of synaptic inhibition by excitatory neurotransmitters

Yu-Tian Wang, University of British Columbia

Neuronal excitability in the central nervous system (CNS) is fundamental to neuronal function, and is primarily controlled by a fine balance between synaptic excitation and inhibition. In the mammalian central nervous system, synaptic excitation and inhibition are mediated by the excitatory transmitter glutamate, which acts on ionotropic glutamate receptor-gated cationic channels, and the inhibitory transmitter glycine acting on glycine receptor (GlyR)-gated chloride channels in the brainstem and spinal cord and by GABA acting on A type of GABA receptor (GABAAR)-gated chloride channels in the brain. Here we report that glutamate and several of its analog ligands potentiate GlyR- or GABAAR-gated chloride currents in cultured neurons. The potentiation is not dependent on activation of any known ionotropic or metabotropic glutamate receptors, and manifests as an increase in single-channel open probability in single-channel recordings. Moreover, this glutamate potentiation could be demonstrated in HEK293 cells transiently expressing human GlyRs or GABAARs. Using ligand-binding assay and site-directed mutations, we identified the glutamate binding sites at these inhibitory receptors. Our data strongly indicate that glutamate may allosterically potentiate GlyR- or GABAAR-gated chloride channels, thereby blurring the traditional distinction between excitatory and inhibitory transmitters. Such a rapid homeostatic regulatory mechanism may have a significant role in tuning functional balance between synaptic excitation and inhibition in the CNS.

Na⁺/H⁺ exchanger NHE6, X-linked intellectual disability and autism

Anne McKinney, McGill University

Neurodevelopmental disorder encompasses a wide range of diseases, including rare but recognizably distinct syndromes and also much more common disorders such as autism spectrum disorders, idiopathic epilepsy and mental retardation. Despite the high prevalence of such disorders, relatively little is known about

their etiology and thus intervention for affected individuals is lacking. Recently it has become evident that in many forms of severe autism are caused by mutations in the X-linked genes NHE6 and NHE9. NHE6 and NHE9 function to regulate the acidification of endosomes the compartments within the cell that mark a protein for degradation or recycling. In this talk the localization of these NHEs in the brain and mechanisms how these mutations can lead to the neuronal defects will be explored.

Chloride dysregulation; a culprit for several brain diseases

Yves De Koninck, Université Laval

Sodium ion On/Off switch for ionotropic glutamate receptors

Brent Dawe, McGill University

A major challenge for modern medicine is to develop better therapeutic approaches to treat the many disease states of the human brain. Although significant progress has been made in the last decades, a perennial problem has been developing drugs that lack side-effects. This issue has been particularly pertinent to the development of drugs targeted to ionotropic glutamate receptors (iGluRs). Although iGluRs are implicated in many CNS disorders from early childhood development (e.g. autism) to the aging brain (e.g. Alzheimer's disease, Parkinsonism), therapeutic compounds targeted to them have met with limited success. I will present recent data from our lab uncovering the importance of an allosteric cation-binding pocket in the extracellular ligand-binding domain of kainate-type receptors that is absent from all other iGluRs. Ongoing work in the lab is aimed at exploiting the cation binding pocket to develop therapeutically-relevant compounds that will either inhibit or potentiate KAR activity without little or no off-target effects.

Symposium 11: Alzheimer's disease molecular mechanisms and therapeutics

The role of BACE1 in Alzheimer's Disease Pathogenesis

Weihing Song, University of British Columbia

Alzheimer's Disease (AD) is the most common neurodegenerative disorder leading to dementia. Deposition of amyloid β protein ($A\beta$) to form neuritic plaques in the brains is the pathological hallmark of Alzheimer's disease (AD). $A\beta$ is generated from sequential cleavages of the amyloid β precursor protein (APP) by the β - and

γ -secretases. Beta-site APP cleaving enzyme 1 (BACE1) is the β -secretase essential for A β generation. Increased A β levels could facilitate AD pathogenesis and inhibition of A β generation may have therapeutic implications for AD treatment. Our studies showed that regulation of BACE1 expression plays an important role in AD pathogenesis and is a valid target for AD drug development. We found that BACE1 tightly controlled APP processing and A β production in normal condition, and selection of APP β -cleavage site by BACE1 had a dramatic effect on A β production in the pathological condition. Upregulating BACE1 expression by hypoxia facilitated neuritic plaque formation and potentiated behavioral deficits in AD pathogenesis. Alteration of BACE1 trafficking and maturation contributes to increased A β production in Down Syndrome. Furthermore, we found that GSK3 β signaling regulated BACE1 gene expression and AD pathogenesis and that inhibition of GSK3 signaling reduced A β neuropathology and alleviate memory deficits in AD model mice.

Chaperoning neurotoxicity in Alzheimer's disease

Marco Prado, University of Western Ontario

Alzheimer's disease is triggered when the balance between production and elimination of Amyloid-beta peptides increase above a threshold. Cholinergic neurons are particularly vulnerable to Amyloid-beta toxicity, but the long-term consequences of cholinergic dysfunction for brain activity is still not fully understood. We have found that cholinergic dysfunction in the hippocampus changes alternative splicing of pre-mRNAs by regulating the expression of the mRNA chaperone and transcription regulators hnRNP A2/B1 and A1. Mice genetically altered to model cholinergic dysfunction reproduced most of the key alterations in alternative splicing observed in Alzheimer's disease. Moreover, we found that these mice present several cognitive alterations in executive function and hippocampal learning, which resemble those in Alzheimer's disease. In order to understand the basis of neuronal vulnerability in Alzheimer's disease, we investigated mechanisms of amyloid beta toxicity. Recent work has implicated the prion protein (PrPC) in Alzheimer's disease and suggested PrPC as possible receptor for amyloid peptides. Stress Inducible phosphoprotein 1 (STI1) is well-conserved protein thought to regulate cellular function by interacting with Hsp70/Hsp90 or with PrPC. We found that neurons with decreased

levels of STI1 have increased sensitivity to amyloid beta peptide, whereas increased STI1 levels decreased amyloid beta toxicity. Recombinant STI1 prevented amyloid toxicity by inhibiting the binding of Amyloid-beta peptide to PrPC and by activating alpha 7 nicotinic receptors. Our results demonstrate novel mechanisms by which neuronal dysfunction can trigger behavioural alterations in Alzheimer's disease and provide a new pathway to interfere with Amyloid beta-induced neuronal toxicity.

Role of beta oligomers in triggering Alzheimer's like pathology and the role of insulin receptor signalling in neuroprotection

Doug Munoz, Queen's University

Recent evidence shows that the neurotoxins in Alzheimer's disease (AD) comprise soluble A β Os that accumulate in the brain. These A β Os instigate synapse damage, promote neuronal dysfunction, and impair brain insulin signaling. Our hypothesis is that decreased insulin sensitivity triggered by A β Os contributes to neuronal dysfunction and represents a novel risk factor for diseases like AD. Therapeutics designed to boost neuronal insulin signaling could protect neurons against A β Os and open up new avenues for the prevention and treatment of AD. Currently, there is no effective treatment for AD, and the pursuit of new disease-modifying therapeutics is the object of intense investigation. A major impediment lies in the difficulty of translating the mechanisms, behavioural indicators and therapies that work in animals (usually rodents), to the specific human disease condition. To reduce the translational gap, we have developed a non-human primate (NHP) model of AD. Intraventricular (icv) injection of A β Os (100 μ g, 4-6 injections over 2-3 weeks) leads to pathology in the macaque brain that is strikingly similar to that observed in humans with AD. While A β Os may not be the root cause of sporadic AD in humans, they seem to be critical for the disease development. Therefore, reversing the effect of A β O injection into the NHP model may lead to future AD therapies in humans. We have initiated one approach by using the anti-diabetic drug, liraglutide. Pretreatment with liraglutide, starting 1 week before the first A β O injections, produced a reduction in pathology induced by A β O injection, providing one example of how the NHP model can be used.

Multiple roles of cholinergic neurons in the modulation of amyloid production

R. Jane Rylett, University of Western Ontario

Alzheimer disease (AD) is associated with increased amyloidogenic processing of amyloid precursor protein (APP) to β -amyloid peptides ($A\beta$), cholinergic neuron loss with decreased choline acetyltransferase (ChAT) activity, and cognitive dysfunction. Both 69- and 82-kDa ChAT are expressed in cholinergic neurons in human brain and spinal cord with 82-kDa ChAT localized predominantly to neuronal nuclei, suggesting potential alternative functional roles. The presence of 82-kDa ChAT in nuclei of cholinergic neurons decreases with increasing age and is lost in mild cognitive impairment and early AD. By gene microarray analysis, we found that 82-kDa ChAT-expressing neural cells have altered expression of genes involved in diverse cellular functions. Genes for several proteins regulating APP processing are differentially expressed in 82-kDa ChAT-containing cells, and the predicted effect is decreased amyloidogenic APP processing with decreased $A\beta$ production. This was verified as a significant decrease in BACE1 levels and activity and a concomitant reduction in release of endogenous $A\beta$ 1-42 from neurons cultured from brains of AD-model APP/PS1 transgenic mice. Expression of 82-kDa ChAT in neurons increased levels of GGA3, which is involved in trafficking BACE1 to lysosomes for degradation. shRNA-induced decreases in GGA3 protein levels attenuated 82-kDa ChAT-mediated decreases in BACE1 protein and activity and $A\beta$ 1-42 release. 82-kDa ChAT can enhance GGA3 gene expression shown by enhanced GGA3 gene promoter activity in neural cells expressing this ChAT protein. These studies indicate a novel relationship between cholinergic neurons and APP processing, with 82-kDa ChAT acting as a negative regulator of $A\beta$ production. This decreased formation of $A\beta$ could result in protection for cholinergic neurons, as well as protection of other cells in the vicinity that are sensitive to increased levels of $A\beta$. Decreasing levels of 82-kDa ChAT due to increasing age or neurodegeneration could alter the balance towards increasing $A\beta$ production, with this potentiating decline in cholinergic neuron function.

Symposium 12: Comfort Feeding: Functional Interplay Between Feeding Behaviour, Stress and Emotionality

Stress and Obesity: The Ghrelin Connection

Alfonso Abizaid, Carleton University

Obesity is among the most pressing health issues facing Western societies. It is evident that stress is a factor leading to metabolic changes that can lead to obesity. In particular, continuous social stressors can result in increased body weight and abdominal fat deposition, insulin resistance, and cardiovascular disease. These changes, however, do not occur in all individuals, suggesting that there may be traits that make some individuals more likely to develop metabolic alterations when stressed than others. Ghrelin, a peptide hormone produced by the stomach, could be a critical factor mediating the development of obesity following social stressors. In contrast to other peripheral signals regulating food intake, ghrelin stimulates feeding and increases adiposity in laboratory animals and in humans. Interestingly, chronic social defeat in mice results in increased caloric intake and promotes adiposity, and this is mediated by stress induced ghrelin secretion. We have also determined that ghrelin produces these effects acting at central sites. Interestingly, social stressors also generate increases in the secretion of ghrelin in human subjects. In subjects that have high emotional eating scores, ghrelin are lower than those that have low emotional eating scores, but the post-prandial decrease in ghrelin concentrations was absent in high emotional eaters. These results point to ghrelin as a hormone that secreted in the face of social stressors, and one that increases appetite and weight gain. Overtime, increased ghrelin secretion may lead to obesity. In humans, susceptibility to overeat following social stress may be determined by abnormal post-prandial ghrelin responses.

Glucocorticoid hormones recruit endocannabinoid signaling to promote obesity and metabolic syndrome

Matthew Hill, University of Calgary

Obesity, and associated cardiometabolic diseases such as type 2 diabetes, are increasing in modern society and represent a major contributor to morbidity and mortality. Persistent exposure to environmental and psychological stress, and the concomitant increase in circulating glucocorticoids (the primary stress hormones), are believed to contribute to the ever-growing epidemic of obesity and metabolic syndrome. The mechanisms by which glucocorticoids produce these changes in weight regulation and metabolism remains unclear. Multiple studies have shown that glucocorticoids mobilize the endocannabinoid (eCB) system and that this

process is essential for the effects of glucocorticoids to exert feedback regulation, for example. It is also clear that the eCB system has effects on feeding and metabolism that mirror those of glucocorticoids. These observations lead to the hypothesis that stress-induced increases in glucocorticoids result in a hyperactive eCB system, which contributes to metabolic syndrome and obesity. Using a combination of genetic and pharmacological tools to ablate the eCB system, we demonstrate that eCB signalling through the CB1 receptor (CB1R) contributes to the development of obesity and metabolic syndrome in a mouse model of excess glucocorticoid exposure, independent of central feeding effects. These data further substantiate the role of the eCB system in obesity and metabolic syndrome and indicate that the eCB system contributes to hormonally mediated forms of obesity, in addition to diet-induced obesity.

Early life exposure to high fat diet modulates the development and maturation of stress responses

Claire-Dominique Walker, McGill University

Environmental influences during early life are important determinants of adult responsiveness to stress, in particular by altering regulation in the hypothalamic-pituitary-adrenal axis and mesocorticolimbic dopamine (DA) system. During this critical period, maternal dietary changes signal available resources to the offspring and alter the nutritional and hormonal environment of the young, which is important for brain maturation. Maternal high-fat (HF) feeding (30% vs 5%) increases the lipid content of the maternal milk and plasma concentrations of leptin and corticosterone in the offspring. While perinatal HF feeding reduces acute stress responses in neonates through the action of increased leptin, after weaning to a control diet (CD), adolescent HF rats display higher ACTH and corticosterone responses to stress compared to CD controls. As adults, HF offspring display higher accumbens DA responses to an acute stress even though the neuroendocrine response is comparable to that of CD offspring. The effect of the HF perinatal exposure is revealed after repeated exposure to stress where HF adult offspring fail to show the normal neuroendocrine adaptation to a homotypic stressor and exhibit exaggerated responses to a novel stressor. High DA responses in the nucleus accumbens are maintained in HF offspring after repeated stress. These studies demonstrate that early exposure

to HF during the postnatal period only has long lasting consequences on the offspring. Initially, HF exposure might be protective to the developing brain by limiting stress responses, but in adolescence and adulthood, such dietary changes might reduce functional plasticity and adaptation to environmental stressors, thus enhancing vulnerability to disease. Supported by CIHR.

Nutritional, metabolic and neural signals connecting obesity and depression

Stephanie Fulton, Université de Montreal

Obesity and diabetes are significant risks factors for mood disorders. Excessive consumption of high-fat foods not only contribute to the development of obesity but can also promote emotional impairments and heightened responses to stress that can exacerbate intake of palatable, high-calorie foods as a means to offset a negative mood state. Our findings show that long-term consumption of diets enriched with saturated but not unsaturated fat in rodents leads to depressive-like behaviour, decreased behavioural sensitivity to rewards (reward deficiency) and impaired HPA responses. The behavioural and metabolic impairments elicited by saturated fat intake are tied to neuroinflammation and neuroplastic adaptations in the nucleus accumbens, a limbic brain region strongly tied to hedonic and motivational deficits in depression. Our results suggest that dietary derived free fatty acids and the metabolic consequences associated with saturated fat intake impact limbic and midbrain circuitry to affect feeding, emotions and reward function.

POSTER SESSION 1**A – Development****1-A-1 Perinatal environmental enrichment alters the trajectory of mouse brain development****Rylan Allemang-Grand¹, Jan Scholz¹, Ellen Langille², Jason Lerch²**¹Mouse Imaging Centre, ²University of Toronto

In a preliminary study, we found that 3 weeks of housing in an enriched environment lead to volumetric increases in brain areas associated with explorative behaviour and spatial memory. In this study, we wanted to determine how perinatal exposure to an enrichment environment alters brain development and adult neuroanatomy. Pregnant CD-1 dams were housed in enriched environments (multi-level maze and running wheel in a large rat cage) or standard housing (no maze or wheel) conditions. Pups lived in the cage until weaning, at which point they were assigned into the same environment they grew up in. Pups were longitudinally scanned at 2.5, 3.5 and 5 weeks of age with a manganese-enhanced MRI protocol to acquire high-resolution images of the brain. Images acquired at each time point were registered and deformed to generate a consensus average. A mixed effects model was computed at each voxel relating the Jacobian-determinant, a value related to volume, to the fixed effects of Age and Cage. At 2.5 weeks of age, enriched pups had volume increases in regions of the hippocampus and cerebellum as well as volume reductions in the cortex (10% FDR corrected). Interestingly, the trajectory of these differences between enriched and control mice did not change over the time course of the study, suggesting an alteration in early-life brain development. We are currently studying pups (P7) that have not yet opened their eyes or started moving to determine whether the neuroanatomical changes are caused by early life exploration in the enriched cage, or via a maternal epigenetic mechanism.

1-A-2 Paranode Maintenance Requires Netrin-1 Expression by Oligodendrocytes**Jenea Bin¹, Timothy Kennedy¹**¹McGill University

Myelinated axons are divided into several specialized domains: the node of Ranvier, the paranode, the juxtaparanode, and the internode.

The secreted protein netrin-1 is sequestered at paranodes and is required for their maintenance. Both neurons and oligodendrocytes express netrin-1, but it has not been determined whether paranode maintenance requires netrin-1 made by the oligodendrocytes, the axon, or both. To address whether cell-autonomous expression of netrin-1 by oligodendrocytes is required for myelin maintenance, we transplanted oligodendrocyte precursor cells (OPCs) from netrin-1 knockout pups into organotypic cerebellar slices from shiverer mice. In slices transplanted with netrin-1^{-/-} OPCs there was a delay in the sequestering of sodium channels compared to slices transplanted with OPCs from control littermates. In addition, in long term, but not short term, netrin-1^{-/-} OPC transplanted cultures the paranodal caspr immunoreactive domain was extended and the juxtaparanodal kv1.2 channel domains were closer together resulting in a loss of the juxtaparanode-paranode boundary. This leakage of proteins into neighbouring domains could be explained by the loss of contact between the oligodendroglial loops and the axon, as observed by electron microscopy. Together these results indicate a role for netrin-1 expressed by oligodendrocytes in paranode maintenance. Uncovering the fundamental mechanisms that regulate myelin maintenance is an important step in identifying novel therapeutic targets to protect and repair myelin in patients with demyelinating diseases.

1-A-3 Ten-m regulates precise synaptic targeting in Drosophila mechanosensory neurons**Vedrana Cvetkovska¹, Brian Chen¹**¹Research Institute of the McGill University Health Centre

Teneurins are a highly conserved family of cell surface receptors that have important roles during nervous system development for axon guidance, cell adhesion, and synaptic specificity. Drosophila Teneurins have been shown to regulate synaptic partner matching between classes of olfactory neurons and at the larval neuromuscular junction. However, it is not clear how Teneurins regulate axonal targeting decisions at the level of single neurons and its effect on circuit function. To address this issue, we use the Drosophila hard-wired mechanosensory circuit for identification and quantitative analysis of axonal targeting errors, with a functional assessment of synaptic connectivity through behaviour. We found that

RNAi knockdown of Ten-m solely within specific mechanosensory neurons produced stereotyped axonal targeting errors, including mis-routing of axonal branches, inappropriate midline crossing, and premature branch termination or extensions. We assessed mechanosensory circuit function by stimulating mechanosensory bristles to elicit a cleaning reflex in Ten-m RNAi mosaic animals. We found that loss of Ten-m within neurons reduced the ability of the animal to perceive a mechanical stimulus. Our results demonstrate that Ten-m is required for precise synaptic targeting in single sensory neurons and for proper circuit function. In humans, the TEN-M1 gene is located in a region associated with X-linked mental retardation; thus, further examination of the roles of Teneurins in the developing brain may provide insight into the mechanisms of miswiring in disease.

1-A-4 Role of mTOR pathway in GABAergic maturation in the mouse neocortex

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¹CHU Ste-Justine, ²Université de Montréal

mTOR pathway has been implicated in controlling several aspects of neurodevelopment by regulating the rate of protein-synthesis. Mutations in the regulatory components Tsc1 and Tsc2 of mTOR-Complex1 (mTORC1) cause Tuberous Sclerosis (TSC) in humans. The majority of TSC patients develop neurological problems like seizures, mental retardation and autism. The role of mTORC pathway in the development of neocortical GABAergic interneurons and the specific contribution of altered GABAergic cells in disease manifestation remain largely unknown. Here, we investigated whether and how Tsc1 knockdown perturbs GABAergic circuit development, both in vitro and in vivo. In particular, we focused on parvalbumin basket cells (BC), a major GABAergic cell subtype. To investigate the role of mTORC1 activation in BC development, we knocked down Tsc1 expression, by transfecting CRE-GFP driven by a promoter specific for BC in cortical organotypic cultures prepared from Tsc1-lox mice. Tsc1 knockdown in vitro caused a precocious increase in bouton density and terminal branching in BC, which was reversed by Rapamycin treatment. These data suggest that mTOR pathway hyperactivation might affect the timing of basket cell synapse maturation. To investigate the role of mTORC1 in BC in vivo, we bred Tsc1-lox mice with Nkx2.1-CRE mice At

P18, Tsc1^{fl/fl}::Nkx2.1Cre mice showed both mTORC1 hyperactivation in BC and an increased expression of perisomatic VGAT, a presynaptic GABAergic marker. Behavioral studies are currently underway to investigate possible deficits in working memory and social behavior.

1-A-5 Mcl-1 and Bcl-x survival signaling through neurogenesis

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During development of the murine embryonic nervous system cells progress through different stages from neural stem cells to progenitors to neuroblasts and finally differentiated neurons. Cell survival signaling changes during neurogenesis however, are still poorly understood. Here we have examined the role of the anti-apoptotic Bcl-2 proteins Mcl-1 and Bcl-x in promoting survival as cells progress through the stages of neurogenesis. Nestin-mediated conditional deletion of Mcl-1 (Mcl-1 CKO) or Bcl-x (Bcl-x CKO) results in extensive cell death within specific neural populations in the forebrain, hindbrain and spinal cord at different time points during development. In the Mcl-1 CKO, apoptosis begins at embryonic day 10 (E10), the start of neurogenesis and is seen initially within the proliferating cell populations. In the Bcl-x CKO, apoptosis begins in post mitotic cells at E11 in the spinal cord and hindbrain, but not until E17 in the cortex as neurogenesis is concluding. To determine whether changes in the expression of Bcl-2 pro-apoptotic proteins coincide with the stages of neurogenesis, qPCR and in situ hybridization were used to examine the expression profiles of Bcl-2 family members. Differences in cell death in the CKO models suggest Mcl-1 and Bcl-x are critical for cell survival during neurogenesis, however further investigation into the specific cell populations affected will clarify their distinct and overlapping roles. This work was supported by an operating grant from the CIHR, RDC-NL to JV. LF is supported by an NSERC Canada Graduate Scholarship.

1-A-6 Autism-Associated Ankrd11 is a Novel Epigenetic Regulator of Neurogenesis

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Increasing evidence suggests that perturbations in stem cell differentiation during the development of the nervous system may underlie cognitive dysfunction associated with neurodevelopmental disorders. We have identified a novel role for a recently identified, autism-associated gene, ankyrin repeat domain-containing protein 11 (Ankrd11), in the development of the cerebral cortex. Ankrd11 is a nuclear protein capable of regulating transcription through recruitment of histone-modifying enzymes such as histone deacetylases (HDACs), but its physiological role is still unknown. Here, we show that Ankrd11 is expressed in both embryonic cortical precursors and in newly-born cortical neurons. Embryonic cortical precursor proliferation and differentiation were perturbed after acute shRNA-mediated knockdown of Ankrd11 in vivo using in utero electroporation. Furthermore, "Yoda" mice, which are heterozygous for a point mutation in the C-terminal, HDAC-binding domain of Ankrd11 display defects in both embryonic and adult neurogenesis reminiscent of human patients carrying similar mutations. Overexpression of HDAC3 rescues the effects of Ankrd11 knockdown on neural precursor proliferation, suggesting that Ankrd11 mediates its effects in cortical precursors by recruiting and regulating HDACs. To ask whether Ankrd11 is similarly important in human cortical precursors, we developed a method for generating forebrain neural precursors from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). Knockdown of Ankrd11 in human precursors causes the same deficits

1-A-7 TORC1 regulates excitatory synaptic maturation and dendritic development in vivo in the retinotectal system of *Xenopus laevis*

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During early brain development, neurons undergo extensive growth and rearrangement of their connections, contributing to the formation of functional circuits. While TORC1-dependent protein synthesis has been implicated in long-

lasting synaptic plasticity in the mature brain, little is known about its role during development. Using electrophysiological recordings and time-lapse two-photon imaging techniques, we investigated the role of TORC1-dependent translation in synapse stabilization and maturation in vivo in the retinotectal system of the albino *Xenopus laevis* tadpole. We show that TORC1 inhibition, by either raising tadpoles in rapamycin or electroporating tectal neurons with a Raptor Morpholino oligonucleotide, significantly reduced AMPA mEPSC amplitudes and frequencies, as well as AMPA/NMDA ratios, compared to neurons in control tadpoles. On the other hand, neurons that were electroporated to overexpress Rheb, an upstream activator of TORC1, exhibited greatly enhanced AMPA mEPSC amplitudes and frequencies, as well as greater AMPA/NMDA ratios. Interestingly, mIPSCs were not affected by TORC1 activation, resulting in a significant imbalance in the excitatory-inhibitory (E/I) ratio. Moreover, we showed that TORC1 inhibition significantly reduced dendritic arbor size and complexity, whereas TORC1 activation dramatically increased dendritic arbor size and complexity. These experiments therefore suggest that TORC1 activity is critical for regulating the number and maturity of excitatory synapses and may also contribute to setting the E/I balance in the developing brain.

1-A-8 Response of Medullary Catecholaminergic Heme-Oxygenase-2 Coexpressing Neurons to Hypoxia in Chick Embryos

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Monitoring O₂ availability is of critical importance for vertebrate embryos as they prepare for transitioning to air-breathing. A brain area believed to be a primary O₂ sensing site is the volume of tissue which includes the A1/C1 region in the ventrolateral medulla. This region contains A1/C1 catecholaminergic (CA) neurons which are thought to sense oxygen through the action of heme-oxygenase-2 (HMOX2), an enzyme involved also in peripheral oxygen sensing. However, it is possible that non-CA neurons in this region may also be oxygen sensitive, in addition to the CA ones. In order to better study the neuronal populations expressing HMOX2 and their roles in the brain response to hypoxia, we used triple-labelling immunofluorescence for tyrosine hydroxylase (a

marker of CA neurons), HMOX2 and c-fos (indicating neuronal activation) on embryonic day 18 chick medullary tissue sections. Embryos were kept in either normoxic (21% O₂) or hypoxic (10% O₂) conditions for 4 hours before perfusion. A population of A1/C1 CA neurons co-expressed HMOX2, and significantly increased their activity in response to hypoxia. A population of interdigitating HMOX2- expressing non-CA neurons was also present but these did not significantly increase their activity in response to hypoxia. This suggests that in late gestation chick embryos, the A1/C1 CA neurons are the dominant cell population driving brain responses to changes in oxygen availability. Non-CA HMOX2 producing cells are present within the A1/C1 region, but do not appear to participate in O₂ sensing in late gestation chick embryos.

1-A-9 Regulation of Neural Connectivity by DISC1 through a novel Dixdc1-Actin Cytoskeleton Pathway

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Gene mutations associated with schizophrenia(SZ) are shared among neuropsychiatric diseases, such as autism spectrum disorder(ASD). While the pathophysiology of these disorders remains unknown, they are hypothesized to be a neurodevelopment disorder, arising from abnormalities in neural connectivity at different stages of development. To better understand the disruptions in neural connectivity, we are studying the role of a well-established SZ risk gene, disrupted in schizophrenia-1(DISC1). DISC1 plays an important role in the regulation of neural connectivity, however the molecular and cellular mechanisms by which this occurs remain unknown. Using mouse in vitro and in vivo models, we are testing the hypothesis that a DISC1-binding partner, DIX domain containing-1 (Dixdc1), a key regulator of neural connectivity, plays a role in dendrite outgrowth, dendritic spine and synapse formation. Our results show that decreasing expression of DISC1 or Dixdc1 reduces dendritic outgrowth, branching, and spine formation. Interestingly, overexpression of DISC1 or Dixdc1 increases dendritic outgrowth. Finally, we have evidence to suggest that Dixdc1 may mediate its effects through regulation of the actin cytoskeleton. We will determine if the newly discovered genetic variants in Dixdc1, associated with ASD, have an impact on dendritic spine and synapse

function. Together, these experiments suggest the DISC1-Dixdc1 pathway is important for neural connectivity development, revealing a signaling pathway that may underlie the disease pathology of neurodevelopmental and psychiatric disorders.

1-A-10 The distribution of cFos-immunoreactive neurons in the brainstem and hypothalamus of late gestation chicken embryos in response to acute hypoxia

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Research into the effects of prenatal hypoxia has benefited in recent years from the study of avian embryos, as they lack the confounding effects of maternal and placental adaptation during hypoxia exposure. Both bird and mammal embryos display comparable subcortical brain anatomy and similar physiological responses to hypoxia; however it has yet to be determined whether similar CNS structures are involved. Therefore, in concordance with previous studies of late gestation mammalian embryos, this study uses cFos (a measure of neuronal activation) to map the pattern of subcortical activation in late gestation chicken embryos (embryonic age 18 out of 21) after acute exposure to normoxia (21% oxygen), modest hypoxia (15% oxygen), and medium hypoxia (10% oxygen). Results largely confirmed a similar pattern of hypoxia-related cFos activation to that of mammalian embryos: greater cFos labeling in areas of the brainstem (nucleus of the solitary tract, dorsal motor nucleus of vagus, ventral lateral medulla, lateral parabrachial nucleus, and periaqueductal grey) and hypothalamus (arcuate nucleus, ventral zone, and median preoptic nucleus). However, unlike mammalian embryos, reduced cFos activity in hypoxic groups was found in the caudal medullary raphe and its lateral extensions. As this area drives thermogenesis in mammals, the moderate normoxic activation (absent in mammals) may reflect the unique tendency for bird embryos to maintain their body temperature above of incubation temperature; hypoxic deactivation here would act to reduce thermogenesis and therefore oxygen expenditure.

1-A-11 Activity Induced Plasticity in AMPAR composition at the Developing Calyx of Held/MNTB Synapse

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The development of high fidelity synaptic transmission at the calyx of Held synapse requires a postsynaptic shift of slow-gating GluA1 dominant to fast-gating GluA4 dominant AMPARs, but the initiating signal remains unknown. The onset of sound evoked neural responses coincides with the beginning of this gating switch, suggesting activity dependent processes may drive the GluA subunit shift. To test this, pre-hearing synapses were subjected to burst stimulation imitating acoustically evoked activity followed by a >30 min expression phase before membrane rupture to establish whole-cell recording. In these neurons we observed acceleration of the fast decay time constant (τ_f) of evoked EPSCs. Distribution histograms of miniature EPSC (mEPSC) τ_f values for naive and tetanized cells showed two mEPSC populations with τ_f being 0.4 and 0.7ms, respectively, with the relative weight of the fast population increasing in tetanized synapses. Such changes are blocked by NMDAR or mGluR antagonism and inhibitors of CamK or PKC signalling implicated in post-translational AMPAR regulation. These kinetic changes are absent in GluA4^{-/-} synapses, suggesting GluA4 is a key substrate underlying this gating switch. Interference of an interaction between the immediate early gene product, Neuronal Activity Regulated Pentraxin (Narp), and AMPARs, blocks this switch, substantiating a role for Narp in excitatory synapse remodeling. These results show that activity works through NMDAR/mGluR signalling mechanisms to facilitate high fidelity transmission at the calyx of Held synapse in vitro.

1-A-12 Evidence for impaired migration of SVZ-derived neuroblasts in suicide

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Background: Alterations in adult hippocampal neurogenesis have been implicated in depressed mood and antidepressant efficacy. This study investigated neurogenesis in the subventricular zone (SVZ)-olfactory bulb (OB) pathway of medicated and unmedicated suicide completers. Methods: Neurogenic markers in OB and SVZ were assessed by immunoblotting in postmortem tissue samples from subjects having died by suicide (n = 21) and psychiatrically healthy sudden-death controls (n

= 14). Migrating neuroblasts immunostained for DCX in the olfactory tract were quantified and reconstructed in 3 dimensions using the Neurolucida software, and cell densities in the granule cell layer were estimated in Nissl-stained OB sections. Results: Compared to controls, suicides displayed a greater expression of DCX and Sox2 (stem cell marker) in the SVZ, and higher DCX levels in the OB. Antidepressant treatment appeared to normalize SVZ protein expression, but had no effect on OB DCX levels. Although suicides showed no change in OB cell densities, they displayed increased DCX multipolar cells and processes in the olfactory tract, which in turn correlated positively with OB DCX expression. DCX cells varied considerably in size, tended to be aligned rostro-caudally along the tract, and were structurally similar in suicides and controls, with the exception of process volume, which was significantly higher in suicides. Conclusions: These data suggest that the migration and differentiation of SVZ-derived neuroblasts may be impaired in suicides.

1-A-13 Patterned activity instructs axon refinement: a revision of Hebb's rules

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Hebbian plasticity posits that temporal correlation in pre- and postsynaptic cell firing instructs circuit refinement. In *Xenopus* tadpoles, retinal ganglion cell (RGC) axons mainly innervate the contralateral optic tectum, but we found in about 40% of tadpoles that a few axons are misguided to the ipsilateral tectum. We used visual stimuli designed to synchronously or asynchronously activate single ipsilateral RGC axons relative to the contralateral inputs to see how correlated firing instructs circuit formation and plasticity. Using perforated patch recordings of tectal neurons, we found that asynchronous visual stimulation reduced the strength of ipsilateral eye input relative to the contralateral eye. Synchronous stimulation maintained the ipsi-to-contralateral input ratio at baseline levels. Live imaging of ipsilateral RGC axons revealed that asynchronous, but not synchronous stimulation, upregulated branch additions. However, axonal branches formed during synchronous stimulation were more stable. To see how synaptic transmission contributes to axonal

branch formation and stability, we transfected ipsilateral RGCs to express tetanus toxin light chain to prevent synaptic transmission. RGC firing still promotes axonal branching in these cells but, the stabilization of newly formed branches by synchronous stimulation was lost. Blocking NMDARs gave a similar outcome, consistent with their putative role as synaptic correlation detectors. Thus, presynaptic activity promotes axonal branch motility, but correlated firing suppresses both branch addition and stabilization.

1-A-14 Development of melanin-concentrating hormone neurons in the chicken hypothalamus

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Melanin-concentrating hormone (MCH) acts as a neurotransmitter/neuromodulator in the vertebrate brain. MCH neurons are found in the dorsal and lateral hypothalamus in both rodents and birds, and send diffuse projections throughout the brain that regulate a variety of physiological and behavioral functions, including the sleep-waking cycle. The developmental expression of MCH has been studied in rodents, but no previous information is available for birds. In order to better understand the possible role(s) of MCH neurons in the developing avian brain, we studied MCH expression using standard immunohistochemistry on tissue sections obtained from the hypothalamus of chickens at different embryonic (E6-E20) and postnatal (P1 and P21) ages. We occasionally detected labelled neurons lying along the 3rd ventricle at E8. A good number of lightly labeled MCH neurons were seen at E10. The number of labeled neurons and the intensity of staining increased at E12 and older ages. MCH neurons were generally seen in medial locations at anterior levels (retromammillary area, posterior hypothalamic nucleus). More posteriorly, they extended from the hypothalamic periventricular organ laterally into the lateral hypothalamic area. These results suggest that the distribution of MCH neurons in chickens is more complex than originally described. The presence of a substantial number of MCH neurons in the second half of embryonic development suggests that these neurons may play a functional role in developmental processes, and possibly also in regulating brain states in chick embryos.

1-A-15 Maternal care influences susceptibility to anxiety-type behaviours mediated by neurogenesis-dependent mechanisms.

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Variations in maternal care during the first week of life influence the offspring's susceptibility to depression and anxiety in adulthood. We have previously shown that offspring of low licking and grooming (LG) dams display increased depression and anxiety-type behaviours. In an analysis of adult neurogenesis, high LG offspring displayed increased proliferation and survival of adult-born neurons in the dentate gyrus (DG). Given the strong correlation of decreased adult neurogenesis with anxiety, we propose that low maternal care in early life leads to an increase in anxious behaviour in adulthood through a neurogenesis dependent pathway. The anti-depressant fluoxetine hydrochloride has been shown to ameliorate anxiety-type behaviour in correlation with an increase in DG neurogenesis. In order to establish whether this effect is varied in high and low LG offspring, a chronic treatment regiment was undertaken in adult rats. Anxiety and depressive behaviours were subsequently assessed. Fluoxetine-treated low LG offspring displayed significantly decreased levels of anxious behaviour. The decrease in anxiety was correlated with decreased expression ratio of pro/anti-apoptotic factors as well as an increase in neurogenic marker expression in the ventral DG. There is clear evidence that maternal care affects depression and anxiety-type behaviour in adult male rat. We hypothesize that anti-depressant treatment leads to an increase in neurogenesis in the ventral DG, rescuing the effects of low LG on anxiety through an apoptosis-dependent mechanism.

1-A-16 Precipitated cannabinoid withdrawal in the rat brain: Mapping c-fos gene expression in adolescent and adult rats.

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The immediate early gene c-fos is a biological marker of recent cellular activity. Using

immunohistochemistry, we quantified Fos (the protein product of c-fos) in several brain regions of adult and adolescent rats undergoing drug withdrawal precipitated by the selective CB1 receptor antagonist SR141716 (SR). Rats were first chronically treated with Δ^9 -tetrahydrocannabinol (THC, 10 mg/kg, IP, twice daily for 6 days), or its vehicle, and then were injected with SR (3 mg/kg, IP) or its vehicle 2 h after the final THC or vehicle treatment, and 2 h later were perfused transcardially. The anatomical distribution of cannabinoid-responsive brain structures was analyzed by localization of Fos-immunoreactivity (Fos-IR) in response to 1) acute SR, 2) chronic THC, and 3) SR-precipitated withdrawal in rats treated chronically with THC. SR-precipitated withdrawal was associated with increased Fos-IR in reward related and stress responsive brain areas including the nucleus accumbens and the central nucleus of the amygdala. Preliminary analysis suggests that in some brain areas of THC-naïve animals, SR alone was associated with age-dependent c-fos expression. SR-precipitated cannabinoid withdrawal was associated with age mediated differences in Fos-IR in response to the SR challenge in areas including the lateral septum and the ventromedial hypothalamus.

1-A-17 Atypical PKC-CBP Pathway Regulates Murine Adult Neurogenesis

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Our previous study has shown that phosphorylation of serine 436 (S436) in CBP by aPKC is important for CBP to promote embryonic neural precursor differentiation in culture. Recently, intriguing findings that CBP level and/or its activity is also required to regulate adult neurogenesis led us to ask further whether CBP aPKC phosphorylation at S436 is a key modulator of adult neurogenesis. We used two knock-in mouse models to target the aPKC-CBP pathway, CBPS436A and p300G422S, where an aPKC phosphorylation site was modified in CBP and p300 alleles in order to generate phosphorylation-defective (CBPS436A) and phosphorylation-competent (p300G422S) mouse models. By using BrdU in vivo labeling technique, we showed that total number of hippocampal and olfactory bulb newborn neurons (BrdU/NeuN positive) was decreased in CBPS436A mutants, while total

number of hippocampal newborn neurons was increased in p300G422S mutants at the age of 3-month. More interestingly, we found that the percentage of double labeled BrdU/NeuN positive neurons over total BrdU positive cells in the dentate gyrus was reduced in CBPS436A mutants at 6-month, whereas the percentage of double labeled BrdU/Sox2 positive neural stem cells (NSCs) in the same dentate gyrus was surged. However, total number of immature doublecortin positive neurons was not changed. These data suggest that aPKC-mediated CBP phosphorylation is important for normal adult neurogenesis, and it modulates rate of differentiation of NSCs and maturation of newly-born neurons in the hippocampus in an age-dependent fashion.

1-A-18 Functional connectivity changes induced by monocular deprivation during the critical period

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Monocular deprivation is a well-characterized example of how early sensory experience effectively modifies neuronal circuits during development. During the critical period, brief monocular deprivation causes weakening of input from the deprived eye to the contralateral binocular zone. Here we examined functional connectivity changes induced by monocular deprivation across the brain using intrinsic optical signal (IOS) imaging. IOS imaging was performed in C57BL/6 mice anesthetized with isoflurane. We studied functional connectivity by examining spontaneous metabolic activity, using data collected in the absence of stimuli. Studies have indicated that functionally related areas have correlated neural and hemodynamic activity in the resting state. To guide the interpretation of spontaneous data, primary sensory areas were mapped with peripheral stimulation. Centers of maps calculated from sensory-evoked response were assigned as seed pixels in the analysis of spontaneous activity. Seed pixel correlation was performed and correlation coefficients were used as an indicator of strength of functional connectivity. Using this method, we observed robust functional connectivity changes in the mouse brain after monocular deprivation during the critical period. Within the primary visual cortex, the monocular zone contralateral to the deprived eye became less correlated with the rest of the

visual cortex, the binocular zone became more correlated with the ipsilateral visual cortex. Global asymmetry in functional connectivity was also observed in monocularly deprived mice.

B - Neural Excitability, Synapses, and Glia: Cellular Mechanisms

1-B-19 Control of vesicle release by cortical presynaptic NMDA receptors

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Traditionally, postsynaptic NMDARs act as coincidence detectors. Recently, presynaptic NMDARs (preNMDARs) have been found at several synapse types, although their functional role remains debated. Cortical preNMDARs are known to upregulate probability of release (Pr) during high frequency firing, but it is unclear how. Using whole-cell recordings, we compared evoked and spontaneous release onto layer-5 pyramidal cells (PCs) in acute slices of juvenile mouse visual cortex. AP5 blockade of preNMDARs resulted in a characteristic downregulation of evoked Pr, but only at high presynaptic firing rates (>8 Hz). In apparent contradiction, the frequency of spontaneous release (2.4 ± 0.5 Hz) was reduced by AP5. Two-photon imaging indicated that in PC boutons AP5 reduced basal calcium level, which is known to control spontaneous Pr directly. We next asked whether preNMDARs regulate evoked Pr directly or indirectly, e.g. by controlling the readily releasable pool (RRP) size. With Schneggenburger-Neher's approach, we found that preNMDAR blockade decreased both RRP size and replenishment rate. In agreement, fitting a modified Tsodyks-Markram short-term plasticity model to evoked responses before and after AP5 showed that preNMDARs upregulate vesicle replenishment rates and Pr during high but not low frequency firing. To conclude, preNMDARs control evoked Pr indirectly by upregulating RRP replenishment rates in the face of high-frequency firing. However, preNMDARs control spontaneous Pr directly by increasing bouton basal calcium levels, presumably by flickering open at resting potentials.

1-B-20 Targeting the TRPV1 assembly domain attenuates inflammation-induced hypersensitivity

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Transient receptor potential vanilloid 1 (TRPV1) ion channels are major contributors of inflammatory pain. They are composed of four subunits surrounding a central pore. To function as noxious sensors in primary afferent dorsal root ganglion (DRG) neurons, TRPV1 subunits must assemble into tetramers, a process dependent on the C-terminal domain, as well as be exported to the plasma membrane. Using biochemical assay, imaging in live cells as well as electrophysiological recordings of recombinant TRPV1 channel mutants, we identified the TRPV1 assembly domain in the C-terminal region of the channel. We tested whether preventing TRPV1 assembly with a membrane-tethered disrupting peptide mimicking this domain may block channel function and thus inflammatory pain. Results: TRPV1 subunit association was disrupted by truncating the protein shorter than residue G734. Peptides comprised of residues 734-751 and 752-772 both bound the C-terminus of TRPV1 in co-immunoprecipitation and membrane translocation assays. Using mutant TRPV1 channels combined with bioluminescence resonance energy transfer assay (BRET), we found that 734-751 motif governs channel assembly. Finally, we show that interfering with TRPV1 subunit association in vivo attenuated mechanical and thermal hypersensitivity in mice models of inflammatory hyperalgesia. Conclusions: We have identified residues 734-751 of the TRPV1 C-terminus to be critical in TRPV1 subunit assembly. Disrupting TRPV1 channel tetramerization may represent a novel therapeutic way of reducing hypersensitivity in inflammatory pain.

1-B-21 Pharmacological characterization of electrical coupling between identified peptidergic neurons

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Electrically coupled neurons communicate through an assembly of channels called gap junctions, which connect one cell to another and mediate the transfer of metabolites and current.

Electrical coupling is involved in the synchronization of spiking and rapid transmission of action potentials within circuits. In turn, this influences the coordination, bursting pattern, and firing frequency of coupled neurons. The present study concerns an electrically coupled two-neuron system within the CNS of the gastropod mollusc, *Lymnaea stagnalis*. The two neurons, designated Visceral Dorsal 1 (VD1) and Right Parietal Dorsal 2 (RPD2) are readily identifiable, peptidergic, strongly-coupled and, given their role in cardio-respiratory function, crucial for survival. In isolated brain preparations, under dual sharp-electrode current-clamp recording, neurons were exposed to gap junction blockers to disrupt electrical coupling. Hyperpolarizing current was injected into either VD1 or RPD2 to determine the coupling coefficient before and after drug application. Niflumic acid and 5-nitro-2-(3-phenylpropylamino) benzoic acid significantly decreased the coupling coefficient, whereas meclofenamic acid, quinine, and carbenoxolone had no effect. Moreover, uncoupling revealed a distinct firing pattern and frequency in RPD2, suggesting that gap junctions are key to the regulation of synchronous rhythmic control.

1-B-22 The Subfornical Organ: A new target for melanocortins

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Alpha -Melanocyte Stimulating Hormone (α -MSH) is a peptide that has demonstrated involvement in the maintenance of energy homeostasis. Specifically, α -MSH has been shown to have profound inhibitory effects on feeding through actions at the melanocortin 4 receptor (MC4R). Systemic administration of MT-II (an MC4R agonist) has been suggested to similarly inhibit feeding through actions at one of two circumventricular organs the subfornical organ (SFO) or the area postrema (AP) (Trivedi et al 2003), both of which have been shown to express MC4R mRNA (Kishi et al 2003). The current study was therefore undertaken in order to determine the effects of α -MSH on the excitability of SFO neurons. We used the whole-cell patch clamp technique to determine the influence of α -MSH on the membrane potential of dissociated SFO neurons. We found that 50% of neurons responded when treated with α -MSH (250 nM-500 nM). Of the cells that responded, 25% showed a depolarization (mean 33 mV) and 75% a hyperpolarization (mean -11.9 mV). This study suggests the SFO as a potential

central nervous system site at which circulating melanocortins may act to influence energy homeostasis. Supported by the Canadian Institutes for Health Research

1-B-23 Pore properties of heteromeric kainate receptors

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Kainate receptors (KARs) are ionotropic glutamate receptors that modulate synaptic transmission. Intracellular polyamines block KARs in a voltage-dependent manner; this can be abolished by RNA editing of a Q/R site at the apex of the channel pore. Not all KAR subunits are edited, suggesting that many native KARs are formed from unedited subunits. Native KARs are heteromeric; the most widely-expressed are composed of GluK2 and GluK5. The extent to which heteromerization imparts functional changes to the properties of the channel block is currently unknown, largely owing to the challenges of studying heteromers in recombinant systems. We used a single-molecule fluorescent subunit counting approach to determine, for the first time in mammalian cells, that the stoichiometry of GluK2/K5 is fixed at 2:2. This finding enabled the identification of outside-out patches from HEK293T cells containing high levels of GluK2/K5. Electrophysiological analysis of unedited GluK2/K5 receptors revealed that heteromerization reduces channel block by spermine without affecting calcium permeability. This is in contrast to the conventional understanding of polyamine block and provides new evidence that it can be uncoupled from calcium permeability in KARs. Here, we show that a proline located in the pore of GluK5 is responsible for the reduction in polyamine block in GluK2/K5. We propose that the fixed stoichiometry imparted by KAR heteromerization provides neurons with an additional mechanism by which to control the apparent affinity of polyamines, and thus the shape of native KAR responses.

1-B-24 Electrical coupling in Aplysia: identification of innexin genes.

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Electrical synapses are employed throughout the animal kingdom as a means to achieve rapid

and synchronous neuronal communication. In the snail, *Aplysia californica*, a group of electrically-coupled neuroendocrine cells, known as the bag cell neurons, initiate reproductive behaviour via the release of hormone during a lengthy afterdischarge. This burst of action potentials is synchronized across essentially all bag cell neurons due to extensive gap junctional coupling. For invertebrates, gap junctions are mediated by cell-to-cell channels comprised of innexin proteins. We used a bioinformatic approach to search for innexin homologues utilizing both the University of California, Santa Cruz (UCSC) *Aplysia* Genome Browser and the University of Maryland *Aplysia* transcriptome assembly. Following in silico identification of putative genes, standard PCR was used to obtain 18 full-length *Aplysia* innexins. All putative *Aplysia* innexins showed intracellular amino and carboxy termini, four transmembrane domains, and two extracellular loops containing conserved cysteines required for channel-to-channel association between cells. Real time PCR indicated that most of the *Aplysia* innexins were expressed in nervous tissue as well as foot muscle and salivary glands. However, *Aplysia* innexins 7, 8, and 10 were the most abundant in both the bag cell clusters and the abdominal and buccal ganglia. Identification of *Aplysia* innexins will provide the molecular tools needed to study how electrical coupling contributes to the bag cell neuron afterdischarge and the control of reproductive behaviour.

1-B-25 Molecular determinants of recycling of delta-opioid receptor

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Opioids are the most potent analgesics for the treatment of severe pain. The delta opioid receptor (DOR) agonists induce fewer side effects than those of mu receptor, making them a target of interest for chronic pain treatment. However, they induce tolerance to analgesia. Recent hypotheses suggest that drugs tolerance potential is the result of stabilization of different ligand-specific conformations of the receptor, each having different trafficking profiles. We will determine whether DOR ligands display different post-endocytic trafficking (recycling) properties. The experiments were done in cortical neurons transfected with the DOR. Our results indicate that only DPDPE, UFP-512 and TIPP were able to induce DOR recycling (SNC-80 and morphine did not). Recycling of neuronal DOR stimulated by DPDPE was reduced when conducted at

20°C, this temperature is known to block the transport in the late endosome and the receptor is no longer able to go upstream. The receptor co-localizes with the M6PR (marker of trans-Golgi network (TGN) and late endosome (LE)) and depends on Rab9 and TIP47 (two proteins involved in LE to TGN transport) to be recycled to the membrane. This recycling was dependent on the kinase PKD which is involved in the transport of the cargo from the TGN to the membrane. We can conclude that DOR recycle from the LE to the membrane via the TGN. Our outlook is to study recycling mechanisms for all the drugs cited and to correlate these properties with drugs tolerance potential. These results will enable the development of ligands with a longer analgesic activity.

1-B-26 Involvement of spinal nuclear metabotropic glutamate 5 receptors (mGluR5) in persistent pain: anatomical and biochemical evidence

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mGluR5 play a key role in the modulation and plasticity of pain especially in dorsal horn spinal cord. mGluR5's are GPCR's that have been shown to be expressed on cell surface and nuclear membranes of striatal neurons in culture. While it has been shown in neuronal cultures that the activation of plasma membrane versus nuclear membrane mGluR5 stimulates different signalling pathways leading to the activation of different genes, the physiological role of the nuclear-located receptors of the GPCR family remains unknown. Electron microscopy was used to show that mGluR5s are localized on the nuclear membrane of neurons in rat spinal cord dorsal horn. Moreover, we found that mGluR5s on the nuclear membrane are increased in dorsal horn neurons from neuropathic rats, without changes in the plasma membrane compartment. Western blots, demonstrate that mGluR5 is increased in the nuclear fraction from neuropathic rats, while no changes are detected in the plasma membrane fraction. EAAT3 protein level remained unchanged after SNL. Downstream signalling molecules induced by intracellular mGluR5 activation, such as pERK and Arc, showed an increase in the nuclear fraction from neuropathic rats. Intrathecal glutamate-induced activation of spinal dorsal horn mGluR5s led to an increase in Fos immunolabelling which was prevented by

previous injection of an inhibitor of neuronal glutamate transporters. First evidence that nuclear mGluR5s in dorsal horn are functionally active during neuropathic pain. Moreover, this is the first model which shows the physiological function of a nuclear GPCR

1-B-27 Glial cells govern synaptic plasticity of competing nerve terminals at the mammalian neuromuscular junction

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The precise wiring of synaptic connections is shaped by elimination of supernumerary inputs competing for the innervation of the same target cell. At the neuromuscular junction (NMJ), this competition depends on the synaptic efficacy of competing terminals which strengthens one input and weakens the others, leading to their elimination. However, mechanisms responsible for the strengthening and weakening of inputs remain ill defined. Here, we propose that perisynaptic Schwann cells (PSCs), glial cells at NMJs, play a key role in synapse competition since they are known to modulate synaptic efficacy at mature NMJs, decode synaptic strength of competing terminals and eliminate the losing ones. Here, we performed intracellular recordings from dually innervated P7-8 mouse Soleus muscle fibres and monitored PSC activity using confocal Ca²⁺ imaging. In addition, we specifically activated or blocked PSCs by photoactivation of NP-EGTA (caged Ca²⁺ molecule) or Diazo-2 (photoactivable BAPTA) respectively. First, we confirmed that PSCs decode synaptic competition as revealed by tight relationship between the size of Ca²⁺ responses and the synaptic efficacy of competing inputs. Second, at the same NMJ, the strong input showed a long-lasting potentiation of neurotransmission while the weak one displayed only a small transient potentiation. Finally, this plasticity was mimicked by direct induction of PSCs Ca²⁺ responses and prevented by PSCs Ca²⁺ chelation. Altogether, these data indicate that glial cells regulate synaptic efficacy which may influence the outcome of synaptic competition.

1-B-28 Myelin-specific ablation of UNC5B improves motor function in mice

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Distinct axoglial domains - the node, paranode, juxtaparanode and internode - are assembled along myelinated axons to enable saltatory conduction of the action potential. We have previously shown that stability of paranodal junctions requires netrin-1 and its receptor DCC, however it is currently unknown if unc5 netrin receptors also play a role in myelin maintenance. To address this question, we have crossed Olig2-CRE and Unc5b flox mouse lines to ablate Unc5B specifically from oligodendrocytes. Surprisingly, when compared to wild-type littermate controls, Olig2CRE/Unc5Bflox mice exhibit enhanced motor function, including balance, strength and exploratory activity, suggesting that overall motor function is modulated by netrin-1 and its receptors at paranodes. Further characterization of this transgenic line will contribute to elucidating the mechanisms regulating paranode stability and motor function in the adult CNS.

1-B-29 Chondroitin Sulfate Proteoglycan negatively regulate the properties of Adult Spinal Cord Neural Precursor Cells through LAR and PTP σ receptors and activation of the Rho/ROCK pathway

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Multipotent neural stem/progenitor cells (NPCs) reside in the spinal cord and are capable of replacing lost oligodendrocytes following spinal cord injury (SCI). Despite this intrinsic capacity, spinal NPCs mainly differentiate into astrocytes, with only a limited number becoming oligodendrocytes. We recently reported that injury-induced upregulation of chondroitin sulfate proteoglycans (CSPGs) restrict the survival, integration, and oligodendrocytes differentiation of resident and transplanted NPCs in their post-SCI milieu. Given the long-lasting upregulation of CSPGs in NPCs niche after SCI, it is important to unravel the potential mechanisms by which CSPGs influence the properties of NPCs. Using an in vitro model of the extracellular matrix of SCI, we investigated the direct role of CSPGs on NPCs. In primary cultures of adult spinal NPCs, using cell viability, western blotting and immunocytochemistry assays, we show that CSPGs significantly decrease NPC growth and attachment, survival, proliferation and oligodendrocytes differentiation. Genetic down-regulation of CSPG receptors protein tyrosine phosphate receptor sigma (PTP σ) and leukocyte common

antigen-related phosphatase (LAR) in NPCs attenuated the inhibitory effects of CSPGs on NPCs. CSPGs inhibitory effects were mediated through activation of the Rho/ROCK pathway and inhibition of Akt and Erk phosphorylation. Our data suggest the impact of CSPGs and its signaling receptors in governing the response of NPCs in their post-SCI niche, and identify new therapeutic targets for enhancing NPC-based therapies following SCI.

1-B-30 Anatomical characterization of VGLUT3-POSITIVE gabaergic basket cell terminals in the hippocampus

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Glutamate is the most prevalent excitatory neurotransmitter in the central nervous system. It plays an important role in hippocampal functions such as learning and memory. The hippocampus consists mostly of large ensembles of glutamatergic neurons (namely pyramidal and granular cells). A small population of GABAergic interneurons finely tunes the activity of these excitatory neurons. It has been recently shown that a subpopulation of these interneurons (CCK-positive basket cells) expresses the vesicular glutamate transporter 3 (VGLUT3). VGLUTs are considered as anatomical and functional markers of glutamatergic transmission. Therefore, paradoxically, some basket cells in the hippocampus have the ability to co-release GABA and glutamate from the same terminals. In this study we investigated the anatomical characteristics of these basket cell terminals expressing VGLUT3 in the CA1 pyramidal layer of the mouse hippocampus. Using double fluorescent in situ hybridization (FISH), we found that 8.7 ± 0.3 % of all GABAergic neurons express VGLUT3. In the pyramidal layer of CA1, 3D quantification of terminals showed that 98 ± 1 % of VGLUT3-positive terminals were GABAergic but only 13 ± 2 % of GABAergic terminals contained VGLUT3. Electron microscopy revealed that VGLUT3-positive synapses are forming axo-somatic symmetric contacts, supporting their role as inhibitory synapses. Our anatomical data support the hypothesis that basket cells expressing VGLUT3 might co-release GABA and glutamate in the pyramidal layer, a strategic location for regulation of hippocampal functions.

1-B-31 Accessory beta subunits for molluscan Nav1 sodium channels are members of a novel CUB domain containing protein family

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Voltage-gated sodium channels are responsible for the action potentials upstroke and form a complex with accessory beta subunits (Nav β), which in vertebrates are related to the neural cell adhesion molecules (CAMs) with a V-set Ig extracellular loop. We have identified the molluscan equivalent of Nav β subunits, using mass spectrometry analyses of a ~40 kDa protein bound to the Nav channel complex isolated in snail (*Lymnaea stagnalis*) brain homogenate by high affinity, Nav α subunit antibody. LNav β subunits are members of a novel CUB domain containing protein family, which has no structural kinship to the vertebrate CAM-like, Nav β subunits or the insect TipE and Teh Nav β subunits which have a likeness to (Slo/BK) Kv β subunits (eg. KCNMB4), with EGF-like domains. There are four molluscan LNav β candidate genes, three of which contain a string of (>10) repeat sequences in addition to the CUB domain in the extracellular C-terminus and a putative N-terminal transmembrane helix. CUB domains are found in cell adhesion molecules like the neuropilins, and accessory subunits to ligand gated channels such as NETO (kainite receptors) and LEV-10 (acetylcholine receptors). We are presently characterizing the effect of the structurally dissimilar molluscan, insect and mammalian Nav β subunits on expressed snail (LNav1) and mammalian (Nav1.2 and Nav1.4) sodium channels in transfected HEK-293T cells. Our hypothesis is that the Nav β subunits are an example of convergence, which evolved independently in different invertebrates and vertebrate groups to serve similar functions.

1-B-32 Transmembrane Protein Coxsackievirus and Adenovirus Receptor (CAR) Promotes Neurite Outgrowth: Role of Translation Regulation

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Coxsackievirus and Adenovirus Receptor (CAR), a transmembrane receptor for adenovirus and coxsackie B virus, is a cell adhesion molecule that may play a role in brain

development. Although its mechanism of action is unknown, it does promote neurite outgrowth when triggered by ligands. To explore which pathways signal downstream of CAR, we performed pulldown and proteomic analysis with a GST protein fused to CAR's cytoplasmic domain. Surprisingly, we identified many proteins which function in translation, such as ribosomal proteins S6 & L4, translation factors eIF4G1 & eEF1A1, and RNA-binding proteins hnRNP & LRP. Therefore, we hypothesized that CAR may promote neurite outgrowth through regulating translation. Using GST-pulldown, ribosome centrifugation, and confocal microscopy, we showed that CAR interacts with translational proteins. By in vitro and in vivo translation assays, we demonstrated that CAR affects translation level. We then asked whether CAR promotes neurite outgrowth through its regulation of translation. We first verified that CAR's ligands, FN40 (a 40-kDa fragment of fibronectin) and collagen (CL), promote significant neurite outgrowth. We further confirmed that this effect is mediated by CAR as CAR-knockdown reduced FN40- and CL-stimulated neurite outgrowth to control levels. Finally, we showed that FN40- and CL-stimulated neurite outgrowth can be blocked by inhibiting translation using cycloheximide. We are now determining whether CAR directly affects neuronal protein synthesis through transmembrane signaling.

1-B-33 Investigating the role of Staufen 2 and the nonsense-mediated decay factor Upf1 in regulating stalled neuronal polyribosomes.

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In neurons, where there is a pronounced distance between the cell body and sites of protein synthesis, messenger RNA (mRNA) transport and localized translation are important mediators of synaptic plasticity. Some neuronal mRNAs are packaged in translationally-silenced ribonucleoprotein complexes, which we have recently shown to consist of polyribosomes reversibly stalled at the level of translation elongation and/or termination. Stimulation of metabotropic glutamate receptors reactivates translation on these mRNAs, a phenomenon exhibited during mGluR-LTD. This form of plasticity is deregulated in diseases such as Fragile X Syndrome and understanding the mechanism could reveal new approaches to therapies. To this end, we are now exploring the

possible roles of the RNA binding protein Staufen 2 in regulating granules. We found that knockdown of Staufen 2 reduces the number of stalled polyribosomes in distal neurites of primary hippocampal neurons. We also report that the number of dendritic map1b transcripts, a Staufen 2 target mRNA, decreases in neurons expressing Staufen 2 RNAi. These data indicate that Staufen 2 is critical for somato-dendritic transport of stalled polyribosomes. Intriguingly, Staufen 2 was found to interact with Upf1, a protein well-known for its role in nonsense-mediated RNA decay. We found that knockdown of Upf1 in primary hippocampal neurons also leads to a decrease in the number of distal stalled polyribosomes. These data highlight a new role for Staufen 2 and Upf1 in regulating mRNA targets at the level of translation in the neuron.

1-B-34 Cysteine substitutions in the Domain II turret of T-type channels can uncouple ion conductances and alter cation block

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The pore of voltage-gated channels consists of a wide outer vestibule formed by an extracellular turret. Pore helices lead into and out of a narrow selectivity filter and an inner water-filled compartment is formed between the selectivity filter and a bundle crossing of S6 helices that form an inverted teepee and inner channel gate. Our research focus is on understanding how the extracellular turret in the outermost vestibule, contributes to ion selectivity and drug block in T-type channels. We discovered that the extracellular turret in Domain II can be alternatively-spliced in invertebrates to generate highly sodium permeant T-type channels (with exon 12a) or a more calcium selective T-type channel (with exon 12b). A notable feature of the Domain II turret is a conserved pattern of cysteine residues, which consists of a tricysteine and pentacysteine pattern for exon 12a and exon 12b in invertebrates respectively, and a unicysteine framework for vertebrate T-type channels. Creation of unicysteine turrets that resemble the vertebrate condition in the invertebrate LCaV3 channel, caused an: 1) an increase in sodium permeability; 2) an enhanced divalent cation block; and 3) a decoupling of mixed ionic currents, so that the multi-ion pore mostly passes monovalent ions or divalent ions at one time, but not both at once. This research

has given insight to the structural elements in the extracellular turret of Domain II that contribute to ion selectivity and drug block.

1-B-35 Investigating Formation and Substrates of Protein Kinase M During Synaptic Plasticity in Aplysia

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Ongoing activity of Protein Kinase Ms (PKM), truncated, persistently active forms of PKC has been proposed as a mechanism for memory maintenance. Most research has focused on the rat atypical PKM zeta. Using the simple mollusc *Aplysia californica*, in which the PKM form of the zeta homolog, PKC Apl III, is implicated in synaptic plasticity, we sought to 1) confirm endogenous PKM formation during plasticity, 2) identify upstream events required for PKM formation and 3) identify a candidate substrate for memory maintenance. To probe for endogenous PKM formation, ganglia were treated with a protocol that induces long-term synaptic facilitation. Immunoblotting revealed increased PKM in ganglia homogenized 1h after treatment, confirming PKM formation during plasticity. Upstream, we followed up on existing evidence that PKM Apl III formation in *Aplysia* results from calpain cleavage of PKC Apl III that can be monitored by over-expression dependent cleavage. To identify the calpain isoform involved, we overexpressed dominant-negative (DN) forms of specific *Aplysia* calpains and tested for PKM formation. DN Small optic lobes (Sol) calpain blocked cleavage of PKC Apl III, suggesting Sol calpain mediates PKM formation. Downstream, as PKMs are thought to act on glutamate receptor trafficking, we investigated Numb, an endocytic protein regulated by atypical PKC phosphorylation, as a PKM Apl III substrate. PKM Apl III phosphorylated Numb in vitro and in neurons, making it a candidate substrate. These results suggest new elements for a model of events surrounding PKMs in plasticity.

1-B-36 Sequence variability leads to distinct roles for M4 as an allosteric regulator of pentameric ligand-gated ion channels

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Pentameric ligand-gated ion channels (pLGICs) play a central role in rapid communication between neurons in the central and peripheral nervous systems. pLGICs are also the sites of action of numerous pharmaceuticals, which alter channel gating to influence synaptic communication. The prototypic pLGIC, the nicotinic acetylcholine receptor (nAChR), is influenced by endogenous and exogenous compounds that interact with the transmembrane domain. M4, the most lipid-exposed of the transmembrane α -helices, likely plays a key role in lipid sensing, protein folding, and the action of many transmembrane domain allosteric modulators. Surprisingly, M4 exhibits a high degree of variability from one nAChR subunit to another. Here, we use two prokaryotic homologs of the nAChR, GLIC and ELIC, to explore the functional consequences of M4 sequence variability. We show that mutations in GLIC M4 invariably lead to a reduction in channel gating, suggesting that interactions with the adjacent transmembrane α -helices, M1 & M3, are optimized to promote channel gating. In contrast, ELIC retains function even in the absence of M4, while mutations in ELIC M4 typically result in a gain of function, suggesting that interactions are poorly optimized, with intrinsically weak coupling of agonist binding to gating. Our data suggest that the importance of M4 as an allosteric regulatory element may vary from one nAChR subunit type to another. This variability may influence the susceptibility of different nAChRs to allosteric modulation by compounds that interact with the transmembrane domain.

1-B-37 Electrophysiological properties of rat subfornical organ neurons expressing calbindin d28K

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The subfornical organ (SFO) is a sensory circumventricular organ, an area of the central nervous system characterized by lack of a blood-brain-barrier. The SFO is well-known to play key roles in regulation of thirst, water balance, cardiac output and energy balance. Several subtypes of neurons underlie the various roles of SFO: most neurons of SFO are either calbindin-positive (CalB) or calretinin-positive (CalR). CalB neurons project axons to the magnocellular neurons of the paraventricular nucleus (PVN), stimulating vasopressin and oxytocin release, while CalR neurons project

axons to the parvocellular PVN and regulate pre-autonomic output. In this study, we used a CalB mRNA-specific fluorescent probe (SmartflareTM) to enable identification of CalB neurons. Patch clamp recording was carried out and intrinsic electrophysiological properties were compared between CalB and non-CalB neurons. The CalB SFO neurons demonstrated significantly lower spontaneous action potential frequency and higher coefficient variation compared to non-CalB SFO neurons. While CalB neurons exhibited lower Na current density, there was no difference in activation or inactivation of Na currents. Consistent with a previous study demonstrating that SFO neurons projecting to the PVN of the hypothalamus have unique electrical properties, this study indicated that SFO neurons exhibit unique electrophysiological fingerprints. Our results demonstrate the validity of the Smartflare reagent to identify biochemically defined subpopulations of neurons in culture for use in patch clamp studies.

1-B-38 The role of CaMKII beta in regulating structural plasticity of dendritic spines

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Structural plasticity involves activity-dependent modification of synaptic structure and is essential for reorganization of synaptic function and structure. Here, we study how CaMKII β (calcium/calmodulin-dependent protein kinase type II β), an abundant F-actin binding Ser/Thr kinase, regulates structural plasticity of dendritic spines. Actin is the major cytoskeletal protein in dendritic spines and the mechanism for its regulation is a key locus in spine structural plasticity. Previously, we found that CaMKII β bundled F-actin and stabilized actin dynamics to maintain dendritic spine structure. Moreover, when CaMKII β was activated, it unbundled actin filaments in vitro. These results suggest that CaMKII β kinase activation status may regulate activity-dependent reorganization of the actin cytoskeleton in dendritic spines during structural plasticity. In this study, we found that novel autophosphorylation sites in the F-actin binding domain of CaMKII β are crucial for unbundling F-actin. Expressing a CaMKII β phosphoblock-mutant resulted in the blocking of structural plasticity. Whereas, mimicking the dissociation of CaMKII β from the actin cytoskeleton by

chromophore-assisted light inactivation (CALI) rescued the block of structural plasticity. Furthermore, the dissociation of CaMKII β from the actin cytoskeleton was followed by an influx into the spine of cofilin1, a potent actin binding and regulating protein. These results suggest that CaMKII β plays an important role in regulating the activity-dependent structural modification of synaptic structure.

1-B-39 Altered neuron-glia interactions at the neuromuscular synapse during normal aging

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Whether it is for memory loss, difficulties in voluntary movement executions and reduced muscle strength, age-induced alterations could be related to an altered synaptic transmission. A growing body of evidence revealed the role of glial cells as a modulator of synaptic transmission at adult synapses, however little is known about their contribution to synaptic activity during normal aging. In this study, we characterized glial cell activation at the old neuromuscular junction (NMJ). We recorded synaptic transmission of old NMJ (620 days) following high frequency electrical (HFS) stimulation of the levator auris longus nerve since it has been demonstrated that perisynaptic Schwann cells (PSC), glial cells at the NMJ, regulate synaptic plasticity at adult NMJ. As a sign of weakness, old NMJs were less likely to potentiate compared to adult NMJ during both short and long-term plasticity. We then measured PSC activity during HFS by calcium imaging at the old NMJ since PSCs ability to detect and modulate synaptic transmission is based on their calcium activity. We observed that PSCs detect synaptic activity but with a reduced contribution of muscarinic receptors. Since these are very important for synaptic maintenance, we studied the morphological integrity of the three synaptic elements of the old NMJ by immunohistochemistry. Regardless of the changes at the presynaptic and the postsynaptic element, PSCs were abnormally quiescent. Together these results suggest that PSCs interactions with pre and postsynaptic elements at the synapse is inadequate at NMJ from old animals.

1-B-40 Synaptic inputs to somatostatin interneurons display transcription- and

translation-dependent long-term synaptic plasticity**Alexandre La Fontaine¹, Isabel Laplante¹, Jean-Claude Lacaille¹**¹Université de Montréal

The hippocampus contains a heterogeneous population of inhibitory interneurons (INs). The role that subtypes of INs play in hippocampal learning and memory remains largely unknown but likely relies on cell type-specific plasticity mechanisms at IN synapses. Previously, we uncovered a persistent long-term potentiation at excitatory synapses onto CA1 oriens/alveus interneurons induced by chemical activation of mGluR1 [mGluR1-mediated chemical late LTP (cL-LTPmGluR1)]. Oriens/ alveus neurons showing cL-LTPmGluR1 are potentially somatostatin-positive interneurons (SOM-INs). Our hypothesis is that cL-LTPmGluR1 is specific to SOM-INs and defines cell type-specific roles in network plasticity underlying learning and memory. First we showed that repetitive mGluR1 stimulation activates, in an ERK dependent fashion, the CREB transcriptional and mTOR translational pathways required for persistent plasticity. Next we determined if cL-LTPmGluR1 is present at excitatory synapses onto SOM-INs. EPSCs recorded in SOM-INs were potentiated after mGluR1 activation, compared to sham-treated slices. Application of U0126 (MEK inhibitor) or PP242 (mTOR inhibitor) during induction blocked this cL-LTPmGluR1, consistent with a requirement for ERK and mTOR activation. cL-LTPmGluR1 was prevented by the mGluR1 antagonist LY367385. These data indicate that transcription- and translation-dependent plasticity (cL-LTPmGluR1) is present at excitatory synapses onto CA1 SOM-INs and requires mGluR1-dependent activation of the mTOR and ERK pathways. Funded by CIHR, CRC and FRQS.

1-B-41 Synapse-specific plasticity in the neocortical layer 5 microcircuit**Txomin Lalanne¹, Julia Oyrer², Rui Costa³, Andrew Chung¹, You Chien Chou¹, Mark Farrant², Jesper Sjöström¹**¹McGill University, ²University College London,³University of Edinburgh

Long-term plasticity is cell-type specific and depends on differences in synaptic molecular machinery. Little is known about neocortical inhibitory neuron (IN) plasticity, in part because INs are difficult to classify. We examined

plasticity at pyramidal cell (PC) synapses onto basket (BCs) and Martinotti cells (MCs) in layer 5 of acute mouse visual cortex slices. With 50-Hz induction, which potentiates PC-PC synapses, we observed non-Hebbian depression of both PC-BC and PC-MC connections. In hippocampus, calcium-permeable (cp) AMPARs underlie non-Hebbian plasticity, so we looked for cpAMPARs in PCs, MCs, and BCs. With internal spermine, both spontaneous and evoked currents onto BCs rectified, suggesting the presence of cpAMPARs. Controls showed that rectification required internal spermine. In agreement, the cp-AMPA blocker Naspm reduced both spontaneous and evoked release in BCs. In contrast, PC-MC connections did not rectify with internal spermine, suggesting excitatory inputs onto MCs may not have cp-AMPA. To ensure Naspm acts post-synaptically, we uncaged NPEC-AMPA with a 405-nm laser while washing-in the drug, which still reduced responses in BCs but not MCs or PCs. We tuned a network model to our data, which showed that cp-AMPA differentially impact fast BC but not slow MC feedback inhibition onto PCs. To conclude, both PC-MC and PC-BC synapses show non-Hebbian long-term depression, yet cp-AMPA are specifically expressed at PC-BC but not PC-MC connections. Such synapse-specific plasticity may critically influence information processing in cortical networks.

1-B-42 The role of PAK signaling in synaptic transmission and plasticity using a tetracycline inducible system in mice**Celeste Leung¹, Shouping Zhang², Zhengping Jia²**¹University of Toronto, ²Hospital for Sick Children

Neurodevelopmental disorders including autism, Alzheimer's disease and intellectual disability are among the most devastating deficits of mental and neurological diseases. These brain diseases are associated with a diversity of potential causes, including single gene mutations. PAKs (p21-activated kinases) 1-3 are a family of serine/ threonine protein kinases that are target enzymes of Rho small family GTPases and central regulators of actin cytoskeleton and neuronal morphology. In vivo studies reveal that PAKs are involved in synaptic and behavioural plasticity. Mutations in the PAK gene are implicated in various brain diseases however we do not understand how

these mutations cause synaptic and behavioural deficit. We employ a tetracycline inducible system where the dominant negative PAK3 mutation can be spatiotemporally modulated. We found that mutant PAK3 mice had profound impairments in spatial and associative memory. Furthermore, the learning deficit in the mutant mice can be rescued with a tetracycline analog that blocks the expression of the mutant PAK3 transgene, which suggests that the memory impairments are not perturbed at development and are caused by deficits in mature synapses. We showed that mutant mice had reduced basal synaptic strength and plasticity that were not due to alterations in presynaptic function. Our data indicate that the molecular pathways through which PAK3 may mediate the Rho signalling process through cofilin dependent actin regulation in the cortex and hippocampus has a central role in the regulation of cognitive and synaptic function.

1-B-43 The Kinesin Khc-73 regulates BMP signaling and Synaptic Homeostasis at the Neuromuscular Junction

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At *Drosophila* larval neuromuscular junctions, reduction in postsynaptic receptor activity triggers a compensatory increase in presynaptic neurotransmitter release. Loss of function in Khc-73, a kinesin motor protein suppresses this effect, indicating a role for Khc-73 in the synaptic homeostasis pathway. The Bone Morphogenetic Protein (BMP) pathway is required in synaptic homeostasis and relies on endocytic pathways to shuttle activated receptors to the cell body in a retrograde direction. Activated BMP signaling increases synaptic transmission and nuclear accumulation of phosphorylated Mad (Mothers against decapentaplegic). Loss of Khc-73 suppresses both these effects of enhanced BMP signalling. Accordingly, overexpression of Khc-73 enhances synaptic transmission. Electrophysiological recordings ruled out Khc-73 effects on synaptic vesicle turnover, thus pointing to receptor containing endosomes. Consistent with a role in endosome sorting, Khc-73 synaptic termini exhibit large vesicles and multivesicular bodies. Khc-73 likely functions during late endosomal stages since Khc-73 mutants lack the synaptic overgrowth phenotype found in mutants of early endosome formation.

Furthermore, western blot analysis of Khc-73 mutants show an increase in BMP receptor protein levels. We propose Khc-73 is required for retrograde routing of receptor containing endocytic vesicles during the expression of synaptic homeostasis.

1-B-44 Two-photon optogenetic control of cAMP dynamics in dendritic spines for studying synaptic plasticity

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Understanding how synaptic function is modulated by neural activity is essential for elucidating the mechanisms of learning and memory. Here we study the role of cyclic AMP (cAMP) in the structural plasticity of dendritic spines by developing two-photon optogenetics and live imaging techniques. cAMP is a ubiquitous second messenger involved in a variety of cellular events, particularly synaptic plasticity such as the late-phase of long-term potentiation (L-LTP). However, the precise dynamics and role of cAMP in dendritic spines remains elusive. We have therefore prepared a genetically-encoded cAMP sensor utilizing Förster resonance energy transfer (FRET), and visualized the spatiotemporal dynamics of cAMP in dendritic spines during synaptic plasticity by two-photon FRET microscopy. In addition, we have developed a two-photon optogenetic approach to non-invasively manipulate local cAMP levels with light by a combination of two-photon microscopy and photoactivatable adenylyl cyclase (PAC). Using this two-photon optogenetic tool, we found that light-dependent postsynaptic cAMP production enhanced dendritic spine enlargement after LTP induction by caged-glutamate uncaging. This suggests a role for postsynaptic cAMP in the maintenance of reorganized dendritic spine structure during structural plasticity in dendritic spines. Thus, this two-photon optogenetic approach provides a powerful tool to elucidate intracellular signaling mechanisms, such as synaptic plasticity in living neurons.

1-B-45 eEF2 acts as a biochemical sensor during synaptic plasticity, coupling diverse activity patterns to translational control

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Activity-dependent regulation of protein synthesis is important for enduring changes in synaptic function. Distinct activity patterns can be transduced by the neuronal translation machinery into different outputs. At the sensory-motor neuron synapse of *Aplysia* both spaced or continuous (massed) applications of serotonin (5-HT) induce increases in synaptic strength that requires new protein synthesis but not gene transcription, a form of intermediate-term facilitation (ITF). However, these two forms of ITF (spaced vs massed) are expressed by different pathways. Here, we demonstrate that eukaryotic elongation factor 2 (eEF2), a GTP-dependent translocase which catalyzes nascent peptide translocation through the ribosome, acts as a biochemical sensor that is specifically tuned to the pattern of neuronal stimulation. Phosphorylation of eEF2, a modification known to inhibit translational elongation, is increased by massed training but decreased by spaced training. Consistent with eEF2 as a downstream effector for differentiating patterns of stimulation, the increase in eEF2 phosphorylation observed after massed 5-HT was blocked with inhibitors of PKC and the decrease in eEF2 phosphorylation by spaced 5-HT was blocked by inhibitors of PKA. An inhibitor of eEF2 Kinase, or expressing a form of eEF2 kinase that could not be activated by 5-HT, blocked ITF after massed 5HT. In contrast, the inhibitor of eEF2K did not block ITF induced by spaced training and indeed it was sufficient to induce ITF. These results suggest that eEF2 is a sensor integrating neuronal activity patterns.

1-B-46 Extracellular glucose and lactate rapidly increase in the motor cortex after initiation of movement

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We measured extracellular concentrations of glucose and lactate using fixed-potential amperometry in freely moving CD-1 mice. Mice were implanted with bilateral guide cannulas in the limb area of the primary motor cortex. A lactate electrode was inserted on one side and a glucose electrode on the other side. Simultaneous measures were obtained during a period of several hours. Locomotion were associated with a 50% simultaneous increase in glucose and lactate followed by a progressive decline once movement stopped. Glucose and lactate increases occurred within 1 second of

movement initiation. The amplitude and frequency of extracellular lactate and glucose changes associated with movement were dramatically increased in fasted animals and after receiving a 0.4IU/kg insulin ip injection. However insulin led to only a small decrease in extracellular glucose while there was a modest rise in extracellular lactate. When the animals received a 2g/kg glucose injection, the extracellular glucose levels rose and the amplitude of movement-elicited lactate increases were blunted. Running on a running wheel was associated with a steady and sustained increase in lactate and glucose levels. Usually, the animals stopped running after the extracellular glucose and lactate reached a plateau for a few seconds. These results support the idea that lactate and glucose levels follow closely neuronal activity in the motor cortex and that systemic glucose levels modulate both glucose and lactate levels.

1-B-47 Unlearning: NMDA receptor mediated metaplasticity in the anterior piriform cortex following early odor preference training in rats

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The NMDAR undergoes activity-dependent plasticity, which likely influences the threshold of, and capacity for, future plasticity - a phenomenon termed metaplasticity. The functional significance of NMDAR-mediated metaplasticity for learning is not understood. Here we investigated NMDAR plasticity and metaplasticity in early odor preference learning in the anterior piriform cortex (aPC) in rat pups. Western blots of synaptoneurosomes showed that the NMDA NR1 subunit was down-regulated 3 h following odor training and up-regulated at 24 h. Ex vivo fEPSP recordings revealed the same patterns of NMDAR plasticity at the afferent synapse in the aPC. Concomitantly, 3 h following training, AMPAR LTP was excluded while LTD became inducible. Behaviorally, one trial, 10 min peppermint-odor stroking conditioning produces a peppermint preference memory lasting 24 h. Pups lost the normally acquired peppermint preference 24 h later if they were re-exposed to peppermint stroking 3 h following initial peppermint stroking training. This unlearning is pathway-specific since pre-training with another odor did not

affect the later peppermint preference memory. Down-regulating NR1 with SiRNA prevented odor preference memory. Finally, blocking NMDAR with MK-801 abolished LTD induction ex vivo 3 h following odor learning, and eliminated the loss of peppermint odor preference when MK-801 was injected before the 2nd peppermint stroking training at 3 h. These results demonstrate an NMDAR-mediated metaplastic effect in a cortical structure that has broad implications for learning optimization.

1-B-48 Variations in Early Life Maternal Care Program Molecular Determinants of Hippocampal Plasticity and Intrinsic Excitability

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Naturally occurring variations in early life maternal care modulate hippocampal development and function to program distinct cognitive phenotypes which persist into adulthood. Adult rat offspring which received low maternal licking and grooming (low LG offspring) show reduced long term potentiation (LTP), and impairment in hippocampal-dependent spatial memory tasks. However, previous studies focused uniquely on the dorsal hippocampus (DH). Emerging evidence suggests a distinct role of the ventral hippocampus (VH) in mediating fearfulness and fear memory formation, behaviours in which low LG offspring are increased. We have presently found that the early life environment differentially programs hippocampal function along the dorsal-ventral axis. Low LG offspring were suppressed in DH-LTP, but enhanced in VH-LTP. CA1 pyramidal neurons in the VH of low LG offspring fired action potentials at lower threshold voltages, which were larger in amplitude and faster in rise rate than those recorded in high LG offspring. Furthermore, we found a specific enhancement of synaptic potential-to-spike coupling (E-S coupling) in VH of low LG offspring. Expression of the voltage-gated sodium channel NaV1.2-coding mRNA SCN2A is specifically enhanced in the VH of low LG offspring, providing a candidate molecular mechanism. Taken together, these findings suggest that low maternal care in early life programs intrinsic excitability specifically in VH, which in turn associates with enhanced synaptic plasticity and the capacity for fear-associated hippocampal cognition in these offspring.

1-B-49 Investigating the Role of the mRNA Binding Protein FXR1P in Brain Plasticity

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Fragile X Related Protein 1 (FXR1P) is one of two autosomal homologues of the Fragile X Mental Retardation Protein (FMRP), a protein whose expression is significantly reduced in Fragile-X Syndrome. Like FMRP, FXR1P is an mRNA binding protein that is implicated in regulating the synthesis of specific target proteins. However, in comparison to FMRP, little is known about the function of FXR1P in brain function. Our lab has recently discovered that FXR1P co-localizes with translational machinery near synapses suggesting that it could play a role in locally controlling the levels of proteins involved in synaptic plasticity and learning and memory. In order to test this, we have generated an FXR1 conditional knockout mouse model where the FXR1 gene is conditionally ablated from neurons in the forebrain, including the hippocampus (a brain region important for learning and memory). Interestingly, after screening for potential synaptic proteins whose expression may be altered in the knockout mice, we found that FXR1P is critical for regulating the expression of the AMPA-type glutamate receptor subunit GluA2; a protein known to have a profound role in synaptic plasticity, learning and memory, and autism spectrum disorders. Moreover, we have also found that FXR1P conditional knock-out mice have significant changes in synaptic plasticity, synaptic morphology, and cognitive function. We are currently investigating the mechanism through which FXR1P regulates GluA2 expression, and in turn understand the implications of this mechanism in learning and memory.

1-B-50 Immature phenotype in Perisynaptic Schwann cells after denervation and during reinnervation

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Synaptic contacts in the neuromuscular junctions (NMJs) can be re-established following nerve injury. Some events during synapse reformation, like synaptic competition prior to the establishment of the NMJ, recapitulate events taking place during development. We hypothesize that PSCs should change following denervation reverting to a more immature state, compatible with synapse plasticity during synaptogenesis. To evaluate this hypothesis we compared the excitability of PSCs at NMJs from chronically denervated mouse soleus muscles to PSCs excitability at different development postnatal ages. PSCs excitability is based on the variations in intracellular Ca²⁺ visualized using a calcium indicator and elicited either by endogenous transmitter release induced by motor nerve stimulation or by local application of agonists (muscarine or ATP). Muscarine application revealed that muscarinic receptors were functional during development but their contribution to synaptic-induced activity in PSCs was marginal as shown by minimal effects of muscarinic receptor antagonist. Synaptic-induced responses in PSCs were driven by purinergic receptors, confirmed by their blockade with RB2, a P2 receptor antagonist. In denervated NMJs, PSCs excitability to muscarine application was significantly smaller in comparison to innervated NMJs. This decrease in PSC sensibility to muscarine was maintained during reinnervation. These results indicate that PSCs at denervated mature synapses revert their phenotype to an immature one providing the required plasticity to complete the reformation of the NMJ.

1-B-51 The role of KCC2 interacting partners in maintaining stability and function at the membrane

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GABAergic transmission is critically dependent on neuronal Cl⁻ regulation. The neuronal Cl⁻ gradient is largely established by the neuron-specific K⁺-Cl⁻ cotransporter KCC2, which maintains a low intracellular concentration of neuronal Cl⁻ ([Cl⁻]_i). The molecular regulation of KCC2 trafficking and stability at the membrane is of utmost importance for understanding neurological disorders such as neuropathic pain and for developing therapeutic treatments for epilepsy. Recently, our lab discovered KCC2

interacts with the kainate receptor subunit GluK2 and can positively regulate KCC2 function at the membrane. Here we demonstrate the role of GluK2 in regulating normal KCC2 trafficking and stability at the neuronal membrane surface using live cell imaging, immunohistochemistry, electrophysiology, and surface biotinylation assays. Using these approaches we demonstrate the dynamic regulation of KCC2 stability by GluK2 to maintain normal Cl⁻ regulation in hippocampal neurons.

1-B-52 Age-dependant plasticity of cortical GABAergic innervation lessens seizure severity in Cacna1a mutants.

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Background. CACNA1A deletions result in ataxia and epilepsy in humans. We showed that a prenatal Cacna1a deletion in forebrain GABAergic interneurons (IN) results in synaptic impairment of parvalbumin (PV) fast-spiking basket cells and induces epilepsy in mice. As CACNA1A-associated epilepsy improves with age, we propose that specific compensatory mechanisms occur in the face of cortical disinhibition to re-establish inhibition/excitation balance with time. Methods. We generated conditional mutant mice carrying a post-natal deletion of Cacna1a in telencephalic PV neurons (PVCre;Cacna1ac/c). Results. PVCre;Cacna1ac/c mutants develop ataxia and a mild epileptic phenotype with spike-wave seizures and behavioral arrests after P45. Surprisingly, these mutants display a two-fold increase in the frequency of miniature inhibitory synaptic currents (mIPSC) in cortical pyramidal cells (PC) at P60, whereas Nkx2.1Cre;Cacna1ac/c mutants display a significant decrease of mIPSC frequency in cortical PC at P20 suggesting a compensatory age-dependent plasticity of GABAergic circuits. We show a comparable reduction of GABAergic perisomatic boutons on cortical PC and a similar impairment of synaptic release from PV INs in both pre-natal (Nkx2.1Cre) and post-natal (PVCre) mutants. However, we demonstrate a significant increase of functional dendrite-targeting GABAergic projections from somatostatin (SOM) INs in post-natal mutants. Interpretation. Reorganization of dendritic inhibition by SOM INs restricts cortical excitability and lessens seizure severity in PVCre;Cacna1ac/c mutants.

1-B-53 The role of Disrupted-in-Schizophrenia 1 (DISC1) in synaptic plasticity

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Schizophrenia is a severe psychological disorder that affects about 1% of the population. One of the key characteristics of this disorder is a deficit in cognitive function such as learning and memory. Synaptic plasticity is a feature of the brain that is believed to be the basis for learning and memory and is represented by changes in synaptic strengths between presynaptic and postsynaptic neurons. Increases in postsynaptic response, termed 'potentiation', or decreases in this response, termed 'depression', underlie the mechanisms of synaptic plasticity and are correlated with dendritic spine regulation. Disrupted-in-Schizophrenia 1 (DISC1) has emerged as a strong genetic risk factor for psychological disorders such as schizophrenia, bipolar disorder, and major depression. DISC1 has been shown to be important in neurodevelopment through its involvement in neuronal proliferation and migration. In this study we find that this protein may also play a role in the regulation of synaptic plasticity. Using DISC1 mutant mice L100P and Q31L we find that hippocampal long-term depression and potentiation are altered in these mutant mice. We also found changes in the Rac1 signalling pathway, a well-established signaling pathway involved in the regulation of actin cytoskeleton and dendritic spines, which may mediate the observed changes in synaptic plasticity. We hypothesize that DISC1 play a role in hippocampal synaptic plasticity by regulating the Rac1 signaling pathway. To test this hypothesis we will use a combination of electrophysiological, imaging and biochemical techniques.

1-B-54 The T-type calcium channel from basal eumetazoan Trichoplax adhaerens highlights the evolutionary history and fundamental features of the Cav3 channel family

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T-type channels (or Cav3 channels) are ubiquitous in animals, where they help regulate membrane excitability by amplifying sub-threshold depolarization. Key to this are their relatively fast kinetics and low voltages of activation, which functionally distinguishes them from high voltage-activated calcium channels classically associated with excitation-contraction coupling in myocytes (i.e. L-type channels), and excitation-secretion coupling at the synapse (N- and P/Q-type channels). Molecular cloning and expression of a T-type calcium channel homologue from eumetazoan *Trichoplax adhaerens*, a basal animal that lacks neurons and muscle, suggests that the key distinguishing features of T-type channels existed before the nervous system, and that in turn that these features were exploited by the evolving nervous system to regulate cellular excitability.

1-B-55 Population bursts in large excitatory network models of hippocampus with biological constraints

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Previous modeling and theoretical studies have clearly shown that it is possible to obtain robust network bursting in excitatory networks in which the individual cells exhibit spike frequency adaptation. The mechanism involves a balance between the amount of adaptation and the excitatory synaptic strengths and drive. What is less clear is whether such a mechanism contributes to the generation of population bursts in biological networks. To examine this, we have used: (i) excitatory cell models that are based on pyramidal, excitatory cells in hippocampus, (ii) theoretical insights from mean field theory to determine whether and in what parameter regimes population bursts could occur, and (iii) high performance computing. Our simulation results show that networks with tens of thousands of cells can robustly produce population bursts when connection strengths are within physiological ranges, and this could occur even for sparse connectivity as is present in hippocampus CA1. We find that burst frequency increases with increasing excitatory drive mainly due to a decreasing inter-burst interval, whereas the burst frequency decreases with increasing

excitatory synaptic strength, and the number of excitatory cells involved in population bursts can vary considerably. We conclude that cellular adaptation mechanisms are in play in hippocampal network bursts and that our models can provide insight into the conditions underlying their generation.

1-B-56 Cholinergic effects on temporal summation of theta and gamma-frequency synaptic inputs in the parasubiculo-entorhinal pathway

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Neurons in the superficial layers of the entorhinal cortex provide the hippocampus with the majority of its cortical sensory input, and also receive the single major output projection from the parasubiculum. This puts the parasubiculum in a position to modulate the activity of entorhinal cortex neurons that project to the hippocampus. These brain areas receive cholinergic projections that are active during periods of theta and gamma-frequency EEG activities which are expressed when an animal navigates its environment. The purpose of this study was to investigate how cholinergic activity affects the strength of repetitive synaptic responses at these frequencies in the rat parasubiculo-entorhinal pathway and to assess the cellular mechanisms involved. Whole-cell patch clamp recordings of layer II medial entorhinal neurons were conducted in acute slices, and responses to 5-pulse trains of stimulation delivered to the parasubiculum were recorded. The cholinergic agonist carbachol suppressed the amplitude of single synaptic responses, but also enhanced temporal summation of responses recorded during theta and gamma-frequency stimulation. The Ih channel blocker ZD7288 mimicked the enhanced temporal summation of synaptic responses induced by carbachol, indicating that carbachol may produce these effects by inhibiting Ih. Cholinergic input to the entorhinal cortex may enhance synaptic responses during theta and gamma frequency EEG activities due to enhanced summation of EPSPs induced by an increase in input resistance associated with closure of Ih channels.

1-B-57 Fine-tuning of cation channel function by TRP channel modulators in Aplysia bag cell neurons

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Reproduction in *Aplysia californica* is initiated by a prolonged period of enhanced excitability, known as the afterdischarge, in the bag cell neurons. During this period, several 2nd messengers control a calcium-dependent, voltage-gated, non-selective cation channel to provide depolarizing drive required for maintaining the afterdischarge. Using single-channel recording in excised, inside-out patches, the calcium-activation of the cation channel and its response to 2nd messengers was investigated. In addition to confirming that the channel is calcium-dependent, we now find that it also desensitizes in the presence of high calcium. We investigated if diacylglycerol (DAG), a lipid produced during the afterdischarge, modulates the cation channel, similarly to transient receptor potential (TRP) channels. Like TRPC6, DAG transiently increased the activity of the channel followed by a decrease below the initial level. The *Aplysia* channel exhibits other TRP channel properties, and is blocked by the general TRP channel blockers flufenamic acid and SKF-96365, as well as the TRPM4/5-specific inhibitor, 9-phenanthrol. In an effort to functionally compare the *Aplysia* cation channel to TRP channels, we have cloned and are expressing *Aplysia* TRP-M, -C and -V homologues in HEK293 cells. Characterizing the native *Aplysia* cation channel, and comparing its properties to the *Aplysia* TRP channels, will provide a molecular foothold towards determining the specific role for TRP channels in regulating long term changes in excitability like the afterdischarge.

1-B-58 Hippocampal development and neural excitation modulate H2A histone variant epigenetic responses

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The profound morphological and functional changes that mark early brain development are rooted in dynamic gene activation. It is imperative that variations in gene expression are precisely coordinated temporally and spatially. Otherwise, deleterious neuropathologies can ensue. DNA encodes genetic information and is packaged with histone proteins to form chromatin. Chromatin controls genomic information through the complex interplay

between the DNA, histone proteins and other chromatin proteins. Chromatin structural variability mediates changes to gene accessibility in many ways. Some involve the inclusion of specialized histone variants within chromatin or histone post-translational modification (PTM). This work explores the dynamic regulation of histone variants and their PTMs during rat hippocampal development and neural activation. The protein and mRNA of H2A variants, H2A.Z and H2AX, and their PTMs decrease significantly as perinatal development progresses. The reduced levels are sustained into adulthood. To assess a relation between these development-dependent changes and neuron activation, primary hippocampal cultures were treated with KCl. H2A.Z and H2AX displayed variable responses to excitation, which may reflect their unique roles regulating chromatin metabolism. Glutamate treatment recapitulated these results. Genomic regions developmentally controlled by H2A variants are being defined by chromatin-immunoprecipitation sequencing. These novel findings suggest that H2A variants modulate genomic responses during early postnatal development and neural activity.

1-B-59 Astrocyte Ca²⁺ transients respond to changes in blood flow: Revealed by two-photon imaging in awake mice

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Neurovascular coupling is an essential process ensuring blood flow is matched to the metabolic needs of the brain. The current model stipulates that abrupt increases in neuronal activity lead to rapid elevation in astrocyte and endfoot free Ca²⁺, which triggers the release of diffusible messengers that initiate either vasodilation or vasoconstriction to change blood flow. However, recent in vivo studies call this model into question. Our objective was to uncover the spatiotemporal basis of the communication between astrocytes and the vasculature using two-photon imaging in awake, behaving mice. The vasculature was filled with FITC-dextran and astrocyte activity was captured using Rhod-2/AM Ca²⁺ indicator or transgenic mice in which only astrocytes express the genetically encoded Ca²⁺ indicator GCaMP3. We found that endfoot Ca²⁺ transients and cell-wide Ca²⁺ transients followed, rather than preceded, sensory evoked vasodilation of arterioles as well as sensory evoked increases in red blood cell flux in capillaries. Furthermore, similar astrocyte Ca²⁺

fluctuations could be evoked by intraluminal acetylcholine delivered IV, suggesting arteriole diameter/blood flow changes per se can drive astrocyte Ca²⁺ transients. Our data redefine the potential role of astrocytes as responders to blood flow, rather than rapid initiators of flow control.

1-B-60 Fructose feeding increases catecholamine release from rat adrenal chromaffin cells

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An increased consumption of fructose has been linked to the development of metabolic syndrome. Male Sprague Dawley rats fed with 10% fructose in their drinking water for 6 weeks developed hypertension. Since an augmentation of the sympatho-adrenal outflow has been linked to both the genetic and dietary components of essential hypertension, we examined whether fructose feeding affects the secretion of catecholamine (CA) from chromaffin cells of the adrenal medulla. Using carbon fiber amperometry, we found that the amount of cholinergic agonist-evoked CA release from individual chromaffin cells, which were acutely isolated from the hypertensive fructose-fed rats, was larger than that released from the normotensive age-matched controls. This augmentation of CA release involves an increase in the number of release events during the "sustained phase" of secretion. In addition, fructose feeding increased the amount of CA released per granule (quantal size) and altered the release kinetics of individual amperometric signals. However, fructose feeding did not change the amplitude of the cholinergic agonist-evoked calcium signal or the voltage-gated calcium current. These data suggest that the development of hypertension with high fructose consumption involves an adaptation of the stimulus-secretion coupling in chromaffin cells to metabolic stress which in turn leads to an augmentation of CA release.

1-B-61 δ GABAA receptors facilitate long-term potentiation in the CA3 subfield of the hippocampus

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Introduction: γ -Aminobutyric acid type A receptors (GABAARs) are novel targets for memory-enhancing drugs. Recently, we showed that a drug selective for δ subunit-containing GABAARs (δ GABAARs) receptors enhanced hippocampus-dependent memory and neurogenesis. Long-term potentiation (LTP) of synaptic transmission, a cellular correlate of memory, is modulated by GABAARs. In the hippocampus, LTP at Mossy fiber-CA3 pyramidal cell synapses (MF-CA3 LTP) is facilitated by presynaptic GABAARs. δ GABAARs may be expressed at presynaptic MF terminals and could have an excitatory action that facilitates MF-CA3 LTP. Here, we tested this hypothesis. Method: Extracellular field postsynaptic potentials (fPSPs) were recorded from the CA3 subfield in hippocampal slices from wild-type (WT) and δ subunit null (Gabrd^{-/-}) mice. Basal neurotransmission and presynaptic plasticity were measured using input-output plots and paired pulse responses, respectively. MF-CA3 LTP was induced using a tetanic stimulation. fPSPs recorded in the first minute after stimulation were used to measure post-tetanic potentiation (PTP) while fPSPs recorded from 55-60 minutes after stimulation were used to measure LTP. Results: A pronounced PTP and LTP was observed in both genotypes. However, PTP and LTP were reduced in Gabrd^{-/-} slices. The input-output plots and paired pulse ratios were similar between genotypes. Conclusions: δ GABAARs facilitate MF-CA3 LTP. It is possible that pharmacologically enhancing δ GABAAR activity could improve memory functions regulated by the CA3 subfield, including working memory.

1-B-62 General anesthetics cause memory deficits after drug elimination by increasing a tonic inhibitory current in the hippocampus

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General anesthetics cause memory deficits that persist after the anesthetic has been eliminated. We previously showed that pharmacologically inhibiting γ -aminobutyric acid type A receptors that contain the $\alpha 5$ subunit ($\alpha 5$ GABAAR) reversed memory deficits after anesthesia (Anes Analg 2012: 114 (4): 843-55). Here, we tested the hypothesis that $\alpha 5$ GABAARs are necessary for the postanesthetic memory deficits and that the activity of these receptors is enhanced after

anesthesia. Wild-type (WT) and $\alpha 5$ GABAAR null-mutant (Gabra5^{-/-}) mice were treated with the anesthetic etomidate (8 mg/kg i.p.) and memory was assessed 24 h, 72 h and 1 week later with the object recognition task. The activity of GABAARs was studied 24 h - 2 weeks after anesthesia by measuring miniature inhibitory postsynaptic currents (mIPSCs) and the tonic inhibitory current in CA1 pyramidal neurons. Additionally, surface biotinylation and Western blot were used to detect changes in surface expression of GABAAR subunits. WT but not Gabra5^{-/-} mice exhibit impaired memory for 72 h. mIPSCs are not affected by etomidate treatment. However, the amplitude of the tonic current is increased 24 h ($P < 0.001$) to 1 week after anesthesia ($P < 0.01$). Lastly, the surface expression of $\alpha 5$ GABAARs is increased 24 h after etomidate treatment. These results show that post-anesthetic memory deficits may be caused by a long-term increase in the expression and function of $\alpha 5$ GABAARs. These results provide the first evidence that a general anesthetic causes an increase in the expression of its target receptors after it has been eliminated.

C - Disorders of the Nervous System

1-C-63 Involvement of autophagy in the neuroprotective properties of Oleuropein against 6-hydroxydopamine-induced neuronal death

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Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with the destruction of dopaminergic neurons in the substantia nigra. The goal of this study was to investigate whether Oleuropein (Ole), a phenolic compound found in olive leaf, may prevent neuronal cell death observed in dopaminergic neurons. For this purpose, we used a cellular model of PD, neuronal PC12 cells differentiated in dopaminergic neurons by nerve growth factor (NGF), and exposed them to the potent neurotoxin 6-hydroxydopamine (6-OHDA). Cytotoxicity as well as measures of specific apoptotic DNA fragmentation demonstrate that Ole significantly decreases 6-OHDA-induced neuronal death and apoptosis. Besides, the antioxidant effect of Ole against 6-OHDA-induced production of radical oxygen species

(ROS) was evaluated by MitoSOX red and dihydrorhodamine. Currently, a growing body of evidence shows that autophagy plays a key role in the pathogenesis of PD. Indeed, we also observed autophagic vacuoles in the cytoplasm of neuronal cells treated with Ole and we identified them by labeling with Cyto-ID and by measuring reliable markers of autophagy-related processes such as LC3, HDAC6, LAMP2, mtHSP70, and P62 expression by immunofluorescence and Western blots. These preliminary results suggest that Ole may act on the delicate balance between pro-survival and anti-survival parameters to regulate neuroprotection against 6-OHDA-induced neurotoxicity.

1-C-64 How fast cocaine is delivered to the brain is decisive in determining the motivation to take the drug

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The faster drugs reach the brain, the more addictive they are. Indeed, drug users who favour rapid routes of consumption (e.g., smoking or i.v. injection versus intranasal use) show a greater likelihood and severity of addiction. Those who smoke or inject drugs also consume more. As such, 'how fast' is confounded with 'how much'. The question addressed here is, is a more abrupt rise in brain drug levels sufficient to promote excessive motivation for drug? Rats self-administered rapidly rising (delivered i.v. over 5 s) or more sustained (90 s) cocaine infusions during daily 6-h sessions. We then measured the motivation for cocaine using a progressive ratio schedule of reinforcement (PR). Experienced human cocaine users achieve spiking rather than continuously high brain levels of drug during a binge. To mimic this during the 6-h sessions, cocaine was available intermittently (12 x 6-min cocaine bins, separated by 26-min drug-free bins; Zimmer et al., 2012). To ensure that the 5- and 90-s groups would take the same amount of cocaine, infusions were limited to 2 per 6-min bin. Cocaine intake did not differ between the groups. In spite of this, the 5-s rats were more motivated to take cocaine under PR than the 90-s rats. Thus, even when cocaine intake is held constant, increasing the rate of rise of brain cocaine levels promotes increased motivation for the drug. Thus, drugs, formulations, and routes of administration that result in rapid drug uptake might be more addictive because rapidly

spiking brain levels of drug promote compulsive drug use.

1-C-65 Cucurbitacin E is a novel neuroprotective phytosterol with autophagy-modulating activities on dopaminergic neurons

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Neuroprotective therapies may have the potential to reduce the incidence of neurodegenerative disorders such as Parkinson's disease (PD). Natural neuroprotective molecules are currently under study to protect cells from a variety of toxic effects often linked to neuronal death, oxidative stress, organelle dysfunction and protein aggregation. In this study, we analyzed the neuroprotective potential of Cucurbitacin E (CuE), a triterpene phytosterol extracted from the Cucurbitaceae *Ecballium elaterium*, on an in vitro model of PD consisting of PC12 cells differentiated in dopaminergic neurons using nerve growth factor (NGF). We exposed these neurons to 1-methyl-4-phenylpyridinium (MPP⁺), a well known neurotoxin provoking a PD-like syndrome. Even if cytotoxicity and apoptosis assays have proven the neuroprotective effects of CuE, its mechanism of action remains unknown and does not seem to involve antioxidative properties, as demonstrated by oxidative stress measurements. Cellular macroautophagy, a complex degradation and recycling pathway for old and damaged cell components, has been studied as a potential target of CuE. Epifluorescent autophagic flux assays have shown enhanced autophagy following CuE treatment. Further studies have illustrated that CuE modulates the expression of HDAC6, a key regulator of the degradation process, as well as its colocalization with LC3, a common marker of autophagy. These interesting properties of CuE point to an autophagy-related mechanism of neuroprotection in parkinsonian dopaminergic neurons and open the way to neuroprotective strategies.

1-C-66 Neurons Regulate the Cell Surface Proteome of Central Nervous System Glia

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The objective of this study is to investigate how the signals from soluble factors of neurons are transduced into glial specific functional responses. To identify molecules on glial cells modulated by neuronal signals, a proteome screen was performed on cell surface enriched proteins of glial cells following exposure to neuronal conditioned media (NCM). While protein levels in total cell lysates remained the same, NCM caused reciprocal regulation of adhesion molecules (N-cadherin and β -catenin) along with neurotrophin receptors on the surface of astrocytes compared to oligodendrocytes (OLs). Secreted neurotrophins (BDNF) and the shed ecto domain of N-cadherin (NCED) were found to be present in NCM. Using function blocking antibodies and recombinant proteins, we show that the astrocytic surface expression of Trk-B and N-cadherin are modulated by secreted BDNF and NCED respectively. Furthermore, neuronal factors also modulated the cell-substrate adhesion of astrocytes and OLs in a reciprocal manner. While there was no effect of neuronal factors on migration of OLs, NCM exposure led to increased migration and polarisation of astrocytes. Our data show that short exposure to neuronal factors modulate cell surface proteins and function of glial cells, a system that may be useful for examining neuro-glial cross-talk. Our ongoing studies focus on elucidating how inflammatory mediators present in the inflamed MS CNS may impact such cross-talk and how therapies known to access the CNS (eg FTY720; BAF) that signal through neurons and glial cells may impact the repair process.

1-C-67 Deletion of the IL-1 α gene upregulates expression of the survival factor TOX3 in neurons and oligodendrocytes and protects these cells from death after spinal cord injury

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Spinal cord injury (SCI) causes the release of danger signals by stressed and dying cells, a process that leads to neuroinflammation. Evidence suggests that inflammation plays a role in both damage and repair of injured neural tissue. Here, we have analyzed the synthesis, release mechanisms and function of IL-1 α and IL-1 β in neuroinflammation, tissue damage and recovery after SCI. We found that microglia at

sites of injury rapidly express IL-1 α , and that infiltrating Ly6G⁺/4 neutrophils and Ly6C⁺ high 7/4 monocyte-derived M1 macrophages subsequently produce IL-1 β . While infiltration of these two cell types was equally reduced in IL-1 α ^{-/-} and IL-1 β ^{-/-} mice compared to wild-type (WT), IL-1 α ^{-/-} mice exhibited better locomotor recovery as early as day 1 post-SCI. This early recovery seen in IL-1 α ^{-/-} mice persisted over time and correlated with reduced lesion volume. Transcriptome analysis of SCI tissue at day 1 identified 18 transcripts differentially regulated in IL-1 α ^{-/-} mice compared with IL-1 β ^{-/-} and WT. Notably, the list includes the survival factor TOX3. IL-1 α ^{-/-} mice have markedly increased levels of TOX3 in their neurons and oligodendrocytes, beginning early in development and persisting through adulthood. Accordingly, neuronal and oligodendrocyte survival was significantly increased in IL-1 α ^{-/-} mice compared to IL-1 β ^{-/-} and WT at 1 day post-SCI. Thus, we show for the first time that IL-1 α represses expression of survival factors such as TOX3 in neurons and oligodendrocytes, suggesting that inhibiting IL-1 α may help reduce neurodegeneration during CNS insult.

1-C-68 The phosphodiesterase inhibitor Ibudilast attenuates glial cell reactivity, production of proinflammatory cytokines and neuronal loss in experimental glaucoma

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Ibudilast, a phosphodiesterase inhibitor with glial cell modulation, anti-inflammatory and vasodilator properties, has been used for the treatment of asthma and stroke. Here, we characterized the role of Ibudilast on the response of glia and retinal ganglion cells (RGCs) in experimental glaucoma. Ocular hypertension (OHT) was induced by injection of hypertonic saline solution into an episcleral vein in rats. Ibudilast or vehicle were administered by intravitreal injection. Animals were euthanized at 3 weeks after OHT induction. Tumor necrosis factor α (TNF α), interleukin 1 (IL-1 β), interleukin 6 (IL-6), macrophage migration inhibitory factor (MIF), glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1 (Iba1) expression were evaluated by immunohistochemistry and western blots. RGC soma and axon densities were quantified on retinal whole mounts or optic nerve cross sections, respectively. Our data demonstrate a

decrease in the number of GFAP-positive astrocytes and Iba1-labeled microglia in Ibudilast-treated glaucomatous retinas and optic nerves compared to vehicle-treated controls. Ibudilast treatment also led to marked reduction of pro-inflammatory cytokines TNF α , IL-1 β , IL-6, and MIF in ocular hypertensive eyes. Ibudilast promoted robust RGC soma and axonal protection, while not affecting intraocular pressure. Our data demonstrate that Ibudilast attenuates glial cell reactivity, reduces production of pro-inflammatory cytokines, and promotes RGC soma and axon protection in experimental glaucoma.

1-C-69 Evaluation of wild partridgeberry polyphenols as potential natural health product to reduce the risk of Alzheimer's disease

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Alzheimer's disease (AD) is characterized by the excessive production of amyloid- β 1-42 (A β 1-42) that promotes oxidative stress and neuronal cell death. Diets abundant in polyphenols are known to decrease A β 1-42-induced neurotoxicity, reduces the risk of developing AD. Using rat primary cortical and hippocampal neurons, we have examined the inhibitory effects of four polyphenol preparations of wild partridgeberry (crude extract, anthocyanin-, flavan-3-ol- and flavonol-rich fractions) on A β 1-42-induced oxidative stress and cell death. The flavan-3-ol-rich fraction exhibited the highest antioxidant activity ($p < 0.05$) as indicated by multiple antioxidant assays. The flavonol- and flavan-3-ol-rich fractions (EC₅₀ < 9 μ g/mL) protected rat primary cortical neurons and rat primary hippocampal neurons from A β 1-42-induced cell death as measured by MTS cell viability assay. The flavan-3-ol-rich fraction profoundly attenuated neural membrane damage produced by A β 1-42 in primary cortical and hippocampal neurons with EC₅₀ values of 0.03 and 0.01 μ g/mL, respectively. All four polyphenol preparations also inhibited the production of reactive oxygen species (ROS) by A β 1-42. The flavan-3-ol-rich fraction exhibited the greater ability to maintain neural redox balance that was associated with elevated cellular concentrations of superoxide dismutase and catalase relative to the control ($p < 0.05$). These findings indicate that at brain concentrations readily achieved by dietary supplementation (50-100 nM), wild partridgeberry polyphenols reduce A β 1-42-

induced oxidative stress and neuronal cell death.

1-C-70 Excitatory and inhibitory contributions to seizure like events in in vitro human neocortical tissue

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Intractable epilepsy remains a burden on a great number of individuals supporting the necessity for continued research initiatives into its potential underlying cause and novel treatment plans. The present study exploits the unique opportunity to record from human neocortex and to assess the contributions of GABAergic and glutamatergic synaptic activity to seizure activity in the human brain. Human cortical slices were isolated from patients with medically refractory Medial Temporal Lobe Epilepsy, from which recordings of spontaneous seizure like events were made in vitro after drug treatment with kainate, then carbachol (Florez et al., 2013). Local field recordings were used to isolate these spontaneous events, while whole cell activities were recorded using patch clamp techniques. Excitatory and inhibitory synaptic currents were then observed in voltage clamp, holding cells at the reversal potentials for either GABA or glutamate. At least two types of seizure like events were obtained and could be categorized based on whether or not they have long preictal oscillations. The ratios of excitatory and inhibitory synaptic conductances were analyzed at different points during seizure initiation, as well as spike field coherence done to reveal possible mechanisms for the underlying dynamics of the transition to seizure in human cortex. CM Florez, RJ McGinn, V Lukankin, I Marwa, S Sugumar, J Dian, L-N Hazrati, PL Carlen, L Zhang, and TA Valiante. In Vitro Recordings of Human Neocortical Oscillations. 2013. Cerebral Cortex

1-C-71 Intracerebral thrombin infusion causes acute neuronal atrophy and cell death, but does not lead to chronic degeneration: implications for hemorrhagic stroke

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Intracerebral hemorrhage (ICH) is a devastating type of stroke, causing significant mortality rate

and long-term disability. Brain injury after ICH arises from blood vessel rupture leading to primary (mechanical) and secondary (delayed) damage. Notably, thrombin production, needed to stop bleeding, also causes early cell death and edema. In some rodent models of ICH, peri-hematoma neurons die over weeks. Hence we evaluated whether thrombin could be responsible for this chronic degeneration as well as sub-lethal changes known to contribute to functional impairment. Adult rats had an intra-striatal infusion of thrombin (1 U) or saline followed by behavioral assessment using the corner turn test 7 days later. After this they were euthanized and tissue stained with Golgi-Cox to assess dendritic morphology. In a second experiment, rats survived 7 or 60 days after thrombin infusion in order to histologically determine lesion volume. Thrombin caused early cell death and considerable atrophy in surviving peri-lesion neurons. However, total tissue loss was comparable at 7 and 60 days. Acute thrombin infusion, a reductionist model of ICH, causes early cell death and neuronal atrophy in nearby surviving striatal neurons, but not on-going tissue loss. Thus, the chronic degeneration found after ICH in rats is not simply and solely due to acute thrombin production. Nonetheless, thrombin likely contributes to behavioral dysfunction owing to causing cell death and marked dendritic injury.

1-C-72 A novel optogenetic kindling model of epilepsy

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Epilepsy is a devastating neurological disease, yet the microcircuit changes that underlie it remain unclear. Many epilepsy models rely on chemical or electrical induction in rodents. In these models, however, brain injury and nonspecific targeting of cell subtypes make it difficult to study the microcircuit changes that accompany epileptogenesis. To overcome these limitations, we developed a novel optogenetic kindling model of epilepsy. To target glutamatergic neurons, we injected AAV-CaMKIIa-hChR2 (E123T/T159C)-p2A-EYFP into primary motor cortex (M1) of male C57BL/6J mice. After 21 days, we confirmed expression of ChR2 in L2/3 and L5 of M1 by acute slice electrophysiology and 2-photon microscopy. We then stimulated M1 of awake behaving animals

using repeated trains of 445-nm laser pulses. We collected EEG data several minutes before, during, and after each stimulation sessions and scored behaviour using modified Racine criteria. Sessions were repeated at least 20 times. Repeated stimulation progressively led to seizures in 6 out of 6 animals. Epileptogenesis correlated with significant EEG changes, such as altered dynamics of evoked responses, and increased delta band power. Next, we will investigate in acute slices the local circuit changes that are associated with disease progression in epileptic animals. With our novel model, it may be possible to identify candidate circuit changes that underlie epileptogenesis in otherwise healthy animals.

1-C-73 HPRT Deficiency in Human Neural Progenitor Cells

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Lesch-Nyhan disease (LND) is a rare genetic disorder caused by the disruption of the gene HPRT1, which plays a key role in purine metabolism. LND causes a variety of metabolic and neurological symptoms including crystals in the urine, gout, dystonia, intellectual disability, and chronic self-injury. While the metabolic symptoms are easily treated with allopurinol this has no effect on the neurological symptoms, the causes of which remain unknown. We have created a novel model of LND by knocking down HPRT1 expression in human ventral midbrain derived neural precursor cells. Using transcriptome sequencing and western blots we demonstrated substantial alterations in the expression of genes related to protein translation and metabolism.

1-C-74 The role of leptin in the augmentation of heroin seeking induced by chronic food restriction.

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Dietary manipulations during drug abstinence can increase the risk of relapse. We have demonstrated that chronic food restriction augments heroin seeking following a period of abstinence. The precise mechanisms mediating this effect remain unknown. Leptin, a hormone

involved in the regulation of energy balance and food intake, attenuates acute food-deprivation induced reinstatement of heroin seeking. However, the metabolic consequences of acute food deprivation and chronic food restriction are distinct. Therefore, we examined the effect of exogenous leptin on heroin seeking in abstinent, chronically food restricted rats. Rats self-administered heroin for 10 days. Next, rats were moved to the animal colony for 14 days, and given either free access to food or underwent chronic food restriction to maintain them at 90% of their original body weight. In experiment 1, rats were injected with leptin (0.0 or 2.0 µg; i.c.v.) immediately prior to a heroin seeking test under extinction conditions. In Experiment 2, rats were administered two injections of leptin (2.0 µg; i.c.v.) or vehicle, 24 hours before and immediately prior to the heroin seeking test. Chronic food restriction augmented heroin seeking during abstinence. A single leptin injection did not affect the augmentation of heroin seeking induced by chronic food restriction; however, two injections may attenuate heroin seeking. Leptin might modulate the augmentation of heroin seeking induced by chronic food restriction.

1-C-75 S-adenosylmethionine reduces amyloid pathology severity in the McGill-Thy1-APP mouse model of Alzheimers disease

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Late onset Alzheimer's disease (AD) is a complex multi-faceted disorder likely brought about by the interaction of genetic, social and environmental factors. Increasing evidence suggests epigenetic involvement, resulting in the deregulation of genes that participate in AD development and progression. As such, it is now well known that AD is accompanied by DNA hypomethylation and decreased S-adenosylmethionine (SAM) levels. Data from our lab demonstrates that strong DNA hypomethylation is present in our McGill-Thy1-APP mouse model of AD-like amyloid pathology at 5 months of age, one month after the accumulation of the first amyloid plaques. In this study, we examined if the administration of SAM could revert the general DNA hypomethylation and ameliorate or delay the amyloid pathology and related deficits in the McGill mouse model. SAM administration started before plaques

could be detected in the brain and was maintained for 3.5 months. Animals were then tested on behavioral tasks before the brains were harvested for molecular and biochemical analyses. Here we demonstrate that SAM administration prevented the behavior deficits in the novel object recognition and Morris water maze tasks. It also led to the modulation of proteins involved in the amyloidogenic cascade and its resulting products such as Amyloid-beta42 and BACE levels and activity. Effects on DNA methylation were also examined. Our results indicate that SAM supplementation can impact and delay the progression of AD neuropathology.

1-C-76 Chronic haloperidol enhances amphetamine-induced conditioned reward: altered reward circuits?

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Drug addiction is common in schizophrenia. Chronic antipsychotic treatment might contribute to this co-morbidity by altering the reward system. We have shown that rats withdrawn from continuous haloperidol (HAL) treatment (via minipump) pursue reward cues more vigorously than HAL-naïve rats following systemic amphetamine (AMPH). AMPH-induced potentiation of conditioned reward is mediated by the nucleus accumbens (NAc). Here we evaluate the hypothesis that the NAc is both necessary and sufficient for enhancement of AMPH-evoked potentiation of conditioned reward in HAL-treated rats. Rats were trained to associate a light-tone cue with water, and then given continuous HAL via minipump. Following treatment cessation, we assessed lever pressing for the light-tone cue (now a conditioned reward) after intra-NAc AMPH injections or inhibition of the NAc with muscimol/baclofen. Surprisingly, intra-NAc AMPH enhanced operant responding for the conditioned reward only in control rats. Furthermore, in HAL-treated rats, inhibiting the NAc did not alter potentiation of conditioned reward by systemic AMPH. The NAc is a brain region that normally subserves AMPH-induced potentiation of conditioned reward. However, continuous HAL treatment modifies reward circuitry such that the NAc becomes neither necessary nor sufficient to evoke this response. The challenge now is to identify the neural circuits that underlie the ability of HAL to augment the pursuit of reward cues. This could shed new light on the neurobiological substrates

by which antipsychotic treatment alters reward function.

1-C-77 Granulocyte macrophage-colony stimulating factor and erythropoietin influence striatal regeneration in a prodromal model of Parkinson's disease.

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Parkinson's disease (PD) is believed to have a protracted prodromal state, wherein the degeneration of midbrain dopamine neurons is ongoing and by the time the cardinal motor signs of the disease manifest, upwards of 50-80% of these neurons have been lost. Hence, it is critical to find methods of interfering with the progression of this prodromal state or inducing compensatory recovery processes. To this end, we presently utilized a 6-OHDA partial lesion model to model the PD prodromal state, in which a modest but significant loss of striatal dopamine terminals was observed (~10%). Importantly, intervention with two cytokines that possess trophic properties, namely erythropoietin (EPO) and granulocyte macrophage-colony stimulating factor (GM-CSF), administered systemically following the 6-OHDA lesion provoked a time-dependent re-innervation of the striatum. Moreover, enhanced GAP43 and Tau-1 staining in the striatum indicated that local GABA neurons might have also responded to the cytokine administration. Curiously, however, subventricular zone and hippocampal neurogenesis was not increased but actually decreased by the EPO and GM-CSF treatments. Hence, these neurogenic niches were likely not directly linked to the re-innervation of the striatum. Taken together, these data hint at the possibility of using certain immune modulator molecules that have trophic properties to "rescue" nigrostriatal pathology during the early stages of PD.

1-C-78 Axonal arborization and mitochondrial metabolism as key contributors to the selective vulnerability of substantia nigra dopamine neurons.

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Parkinson's disease (PD) is the second most common neurodegenerative disorder. The disease is associated with severe locomotor

deficits accompanied by the selective and progressive loss of dopamine (DA) neurons of the substantia nigra pars compacta (SNc). The exact mechanism by which SNc neurons degenerate in PD is poorly understood. However, mitochondrial dysfunction has been proposed as a key contributor. We tested the hypothesis that the selective vulnerability of SNc DA neurons, in comparison to those from the closely located VTA, is due to higher mitochondrial metabolism related to their larger axonal arborization. In the present study we quantified basal and activity-dependent mitochondrial energy metabolism in primary cultures of postnatal mesencephalic mouse DA neurons, grown on an astrocyte monolayer, using a Seahorse XF24 analyzer. Using confocal imaging, we also quantified the extend of the neurons' axonal arborization and the density of mitochondria after lentivirus-mediated overexpression of Mito-DsRed. Our data show that the more vulnerable SNc DA neurons differ from VTA DA neurons in that they show higher basal mitochondrial respiration, higher axonal mitochondrial density, a larger axonal arborization and an increased susceptibility to the neurotoxin MPP+. Our data are compatible with the hypothesis that SNc DA neurons are more vulnerable because their elevated mitochondrial function leads to more extensive oxidative stress.

1-C-79 Activation of caspase-6 and cleavage of STK3 in an early event in an in vitro model of stroke.

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In Canada, the US and Europe, stroke is the leading cause of long term adult disability and the 3rd leading cause of death. Activated caspase-6 (casp6) has been observed in post-mortem tissue from brains of patients who died following stroke and casp6^{-/-} mice demonstrate reduced stroke injury and improved sensorimotor abilities. These data provide important validation for the activation of casp6 as key step in the pathogenesis of stroke. There is strong evidence to support a critical role for NMDAR-mediated excitotoxicity as a mechanism underlying stroke injury. Inappropriate caspase activation in stroke could result from increases in calcium levels due to excitotoxic stress and lead to generation of proteolytic fragments as a result of caspase

substrate proteolysis. Using rat primary cortical neurons \pm NMDA we demonstrate that at early time points post NMDA no increase in cell death is observed. However at 24h a significant increase in cell death is detected (ANOVA $p < 0.0001$). Assessment of caspase mRNA levels demonstrates that increased casp6 mRNA is observed at 1, 4 and 24hrs post NMDA (ANOVA $p = 0.016$). No differences in casp8 or casp9 levels were observed. Increased casp6 activity post NMDA was also detected ($p < 0.05$). Preliminary data also shows that a significant decrease in full-length levels (ANOVA $p = 0.03$) of the novel casp6 substrate STK3, and increase in STK3 fragments (ANOVA $p = 0.009$) occurs prior to cell death. These data provide a clear link between excitotoxicity and proteolysis and suggest that activation of casp6 is an early event in the pathogenesis of stroke.

1-C-80 Differences in the phagocytic response of microglia and peripheral macrophages after spinal cord injury and its effects on cell death

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Macrophages in the injured spinal cord arise from resident microglia and infiltrating, peripherally derived monocytes. The aims of this study were to investigate the phagocytic response and clearance of damaged axons and tissue debris by these distinct subsets of macrophages and assess their viability after spinal cord injury (SCI). The lysozyme M EGFP-knock in (lys-EGFP-ki) mouse tags hematogenous macrophages, but not microglia. Using neuronal tracing techniques we show that microglia contact damaged axons early (24 h) after SCI and are the main type of macrophage to contain phagocytic material at 3 days. Thereafter, infiltrating macrophages become the predominant cell in contact with degenerating axons and contain more phagocytic material, which in contrast to microglia, persists for up to 42 days. The proliferation marker Ki67 was found predominantly to the nuclei of resident microglia, suggesting a mechanism by which microglial division contributes to phagocytic load management. Furthermore, after phagocytosis of myelin in vitro, macrophages are much more susceptible to apoptotic and necrotic cell death, which is mirrored in vivo with apoptotic TUNEL-positive cells of infiltrating macrophage origin. This work suggests that microglia play a major role in the early response to SCI, by phagocytosing injured tissue efficiently and

remaining viable. Later, macrophages of peripheral origin contribute predominantly to phagocytosis but are less efficient at processing CNS debris, and their death, in situ, may contribute to the secondary damage after CNS injury.

1-C-81 Recombinant IL-4 injection into the brain alters the inflammatory response and grey matter injury in a rat model of ischemic stroke

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Following a stroke, there is a CNS inflammatory response involving activation of resident microglia, and infiltration of peripheral innate immune cells (mainly macrophages and neutrophils). This response can last for hours to days, making it more amenable to treatment than acute neurotoxicity. Microglia can exist in several activation states, and in vitro, their alternative activation is readily evoked by the cytokine, IL-4. Here, we modeled transient focal ischemia by injecting the vasoconstrictor peptide, endothelin-1 (ET-1), into the rat striatum, either alone (untreated) or with 500 ng of rat recombinant IL-4 (treated) to enhance alternative activation. Gene expression analysis revealed an increase in the alternative activation markers, CD163 and CCL22 at 24 hr after ischemia onset, ARG1 at 3 days, and molecules related to IL-4 signaling (IL-4 α , STAT6) at 7 days. Immunohistochemistry was used to quantify inflammation, neuronal, and glial responses in the ischemic core. Surprisingly, early IL-4 treatment increased the number of degenerating neurons (FluoroJade B labeled), and apparently phagocytic neutrophils (labeled with anti-PMN to identify neutrophils, and anti-ED1 for activated lysosomes) at 24 hr. Moreover, early IL-4 treatment did not alter the number of activated microglia/macrophages or the extent of white matter injury at 1, 3 or 7 days post-ischemia. Thus, IL-4 treatment delivered at ischemia onset might be harmful. Future studies are needed to evaluate whether IL-4 administration at later times is beneficial.

1-C-82 Dissociating dopamine functions with new models of dopamine depletion specific to the mesocorticolimbic or nigrostriatal pathways

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The dopamine (DA) system has been involved in a large number of neuropsychiatric disorders. However, the exact implication of DA involved in the two different pathways remains difficult to explore with the current animal models. The goal of this proposal is to create new models of dopamine depletion. We generated a conditional VMAT2 KO (vesicular monoamine transporter, knockout) using the Cre recombinase to splice out the VMAT2 floxed gene specifically in DAT-positive neurons. This model was validated by an absence of VMAT2 mRNA in the ventral tegmental area (VTA) and substantia nigra (SN) and a complete DA depletion in the whole brain. However, the VMAT2DATcre KO mice die at postnatal week 3. Therefore, we generated a VMAT2 KO model which enabled us to dissociate the mesocorticolimbic and nigrostriatal pathways. An adeno-associated virus encoding the Cre recombinase (AAV2-Cre) was injected either in the VTA or in the SN of VMAT2lox homozygous adult mice. The VMAT2AAVcre KO mice were validated by a lack of VMAT2 mRNA expression in the site of injection, and by specific DA depletion in the nucleus accumbens and prefrontal cortex (VTA projections) or in the dorsal striatum (SN projections). Despite conservation of DA neurons, all VMAT2AAVcre KO mice died after 16 weeks post-injection due to a drastic weight loss. In parallel, mice injected in the SN demonstrated motor alterations appearing from postnatal week 8 onwards. In conclusion, the VMAT2AAVcre KO models of dopamine depletion will allow to unravel DA functions in the etiology of psychiatric or neurological pathologies.

1-C-83 GDNF Mimetics for Neuroprotection in the Retina

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Glial cell-line derived neurotrophic factor (GDNF) and its receptors (GDNF-R) are expressed in the developing and the adult nervous system. GDNF yields pro-survival signals by activating AKT and related pathways in cells expressing GDNF-R. GDNF signalling has shown promise in degenerative diseases including Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis and

Retinitis Pigmentosa (RP). The GDNF/GDNF-R axis is a validated target for the treatment of many conditions, and it has been applied successfully in multiple animal models. Moreover, GDNF has been used in human clinical trials. Nonetheless, its clinical development faces major obstacles inherent to proteins, such as high cost, poor delivering to the target tissue, poor stability, and poor selectivity. Our laboratory has promoted a conventional pharmacological approach by designing drug-like small molecule GDNF mimetics with agonistic activity, and high receptor selectivity. By using small, non-peptide molecules with improved stability and relatively easy delivery, it may be possible to sustain neuronal function beyond previous achievements. We will present data from in vitro, ex vivo and in vivo studies on the development, the selectivity, the efficacy, the potency, and the mechanism of action of our novel drug-like GDNF mimetics. Our ongoing trials with these mimetics have yielded evidence of therapeutic merit. Further development or optimization of the lead compounds is likely to yield clinical candidates for treatment of progressive neurodegenerative disorders.

1-C-84 Identifying microRNA regulators of neuronal viability and repair in multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune disease characterized by the infiltration of peripherally activated immune cells into the central nervous system. Current MS therapies are immunomodulatory rather than neuroregenerative, making neural repair and viability an ideal direction for future work. Previously we have identified neurite outgrowth inhibition by immune cells and their conditioned media (CM). Our lab is interested in how immune cells and their products affect neuronal viability and repair. MiRNA are short RNA sequences, a single miRNA is able to target several different mRNA. Altered miRNA expression has been identified in blood cells of MS patients, as well as active and inactive MS lesions. An investigation of neuronal miRNA expression can provide further insight into immune-neural interactions in MS. We developed a list of candidate miRNAs to investigate during neurite outgrowth inhibition in mouse cortical neurons following treatment with

peripheral blood mononuclear cell CM (PBMC-CM). Specifically, levels of miR-27a-3p increase in response to stimulation with PBMC-CM raising the possibility that it may be involved in neurite outgrowth inhibition. Preliminary data however shows that treatment of neurons with miR-27a mimic during control conditions promotes outgrowth and process formation, suggesting that the up-regulation of miR-27a during PBMC-CM treatment functions as a neuroprotective response. Probing for different mRNA targets of miR-27a can unravel its function in neuronal viability and repair.

1-C-85 The Frequency of Coagulopathy And Its Significance In An Emergency Neurotrauma Facility.

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Background: Most data on coagulopathy are from the West. We sought to determine frequency of coagulopathy in our population and determine the relationship of coagulation parameters and other clinical variables with unfavorable outcomes of patients with TBI. **Methods:** This was an observational cohort study conducted in a tertiary care facility from 1st January 2010 to 31st December 2012. All patients with isolated traumatic brain injury presenting within 24 hours of injury were included in the study. Coagulation parameters at presentation were recorded and Glasgow Outcome Scale calculated on the last follow up. Outcomes were dichotomized into favorable and unfavorable outcomes. Relationship of coagulopathy with GCS, GOS, RTS and unfavorable outcomes was calculated using Pearson's correlation and Receiver Operator Curve analysis. **Results:** 121 patients were included in the study. Mean age was 38.86 years (± 16.71). Overall frequency of coagulopathy was found to be 6 %. In severe head injury group it was 14%. Area under curve (AUC) for aPTT was found to be 0.702 (95%CI =0.602-0.802, $p < 0.001$) indicating its strong predictive value. Predictive value of platelets and INR was not found to be significant. APTT, INR, RCTS, Age, GCS and RTS were found to be significantly correlated with GOS. **Conclusion:** Overall incidence of coagulopathy in our population was 6 %. APTT, INR, RCTS, Age, GCS and RTS in emergency are correlated with outcomes of patients with TBI. **Keywords:** aPTT, Coagulopathy, Unfavorable outcomes,

Traumatic Brain Injury. Abbreviations: TBI; Traumatic Brain

1-C-86 Pathological sharp waves and hyperexcitability in a mouse model of fetal alcohol spectrum disorder (FASD)

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Fetal Alcohol Spectrum Disorder (FASD) refers to the wide variety of physiological and behavioral postnatal pathologies induced by in utero exposure to alcohol, which includes an increased prevalence of seizures. However, to date, there exists limited information regarding the underlying pathophysiological mechanisms. The hippocampus is strongly implicated in FASD, and is a source of seizure activity in various epileptic conditions. The hippocampal CA3 region generates population activities called sharp waves (SPWs), and emerging evidence suggests that dysregulation of SPWs induces epileptiform activity. We therefore hypothesize that mice prenatally exposed to alcohol will have increased hyperexcitability in the CA3 hippocampal region and dysregulated SPWs. Pregnant mice received 10% v/v ethanol for the first trimester-equivalent of gestation by voluntary consumption. Spontaneous in vitro SPW were recorded from the CA3 hippocampal region using acute brain slices prepared from ethanol-exposed pups and controls of ages PD 15-21. Prenatal ethanol exposure significantly altered in vitro SPW waveforms, with a 50% increased duration and amplitude, when compared to controls. Application of the GABA-A receptor antagonist bicuculline (BMI) led to the transition of SPWs into a longer duration and amplitude in both ethanol-exposed mice and controls. Interestingly, the percent change induced by BMI was significantly lower in ethanol-exposed mice, indicative of a loss of inhibition. These results may account for the increased seizure susceptibility observed in individuals with FASD.

1-C-87 The genetic landscape of infantile spasms

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Background. Infantile spasms (IS) is an early-onset epileptic encephalopathy of unknown etiology in 40% of cases. We hypothesised that unexplained IS cases represent a large collection of rare single-gene disorders. **Methods.** We investigated 44 children with unexplained IS using comparative genomic hybridisation arrays (aCGH) (n=44) followed by targeted sequencing of 48 epilepsy genes (n=8) or whole-exome sequencing (WES) of familial trios (n=18) to search for rare inherited or de novo mutations. **Results.** aCGH revealed de novo variants in 7% of patients (n=3/44), including a 16p11.2 duplication, a 15q11.1q13.1 tetrasomy and a 2q21.3-q22.2 deletion. Furthermore, it identified a pathogenic maternally inherited DupXp11.2. Targeted sequencing was informative for ARX (n=1/14) and STXBP1 (n=1/8). By contrast, sequencing of a panel of 48 known epilepsy genes (n=8) did not identify further mutations. Finally, WES (n=18) was very informative, with an excess of de novo mutations identified in genes involved in neurodevelopmental processes and intolerant to functional variations. Several pathogenic mutations were identified, including de novo mutations in STXBP1, CASK, and ALG13, and recessive mutations in PNPO and ADSL, together explaining 28% of cases (5/18). Furthermore, WES identified 1-3 de novo variants in 64% of remaining probands, revealing several interesting candidate genes. Our results indicate that IS are genetically heterogeneous with a major contribution of de novo mutations and that aCGH with WES is highly effective in achieving a molecular diagnosis in IS.

1-C-88 Haloperidol-induced dopamine D3 receptor upregulation in the direct pathway correlates with tardive dyskinesia in non-human primates

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Tardive dyskinesia (TD) is a delayed and potentially irreversible motor complication arising in patients chronically exposed to dopamine receptor antagonists. Typical antipsychotic drugs, metoclopramide and several modern (so-called atypical) antipsychotic drugs are associated with generation of TD. But, the pathophysiology of TD remains elusive and difficult to treat. To

investigate the neurochemical basis of TD, we exposed adult capuchin (*Cebus apella*) monkeys to prolonged treatments with haloperidol (N=11) or clozapine (N=6). Six untreated animals were used as controls. Five haloperidol-treated animals developed mild TD movements similar to those found in humans. No TD was observed in the clozapine group. Using receptor autoradiography, we measured dopamine D1, D2 and D3 receptors, as well as glutamate mGluR5, adenosine A2A and serotonin 5-HT2A receptor levels. Haloperidol, but not clozapine, strongly induced D3 receptors in monkey putamen, but spared D2 receptor binding sites. Interestingly, dopamine D3 receptor mRNA levels were upregulated in substance P positive striatal cells. In addition, dopamine D3, but not D2, receptor levels correlated with TD intensity. These results indicate that D3 receptor upregulation in the direct pathway is associated with TD in this non-human primate model, as opposed to rodent data, which have rather suggested D2 receptor involvement. Thus, the D3 receptor could provide a novel target for drug intervention in human TD. Supported by CIHR (MOP-300152). SM and OP hold fellowships from FRQS and GRUM/Hydro-Québec, respectively.

1-C-89 The hemisphere-specific effect of chronic stress on the dendritic morphology of rat prefrontal cortical callosal neurons

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Exposure to chronic stress induces dendritic atrophy of pyramidal neurons in the rat medial prefrontal cortex (PFC). Importantly, these morphological alterations appear to be lateralized in nature. The PFC regulation of stress responses is also lateralized, suggesting that in order to mount an appropriate stress response, the hemispheres must somehow communicate. PFC callosal neurons project to the contralateral hemisphere and are thought to be involved in the interhemispheric communication of stress-relevant information. We tested the hypothesis that callosal neurons would have a lateralized pattern of remodelling in response to chronic stress. A retrograde tracer was injected into rats' PFC prior to 21 days of restraint stress. After completion of the stress regimen, labelled callosal neurons in the PFC were loaded with Lucifer Yellow dye to visualize their dendrites. We observed a reduction in the total dendritic length and

branching of callosal neurons in both the right and the left hemispheres. However, the distribution of these stress-induced alterations in dendritic morphology differed between hemispheres: both proximal and distal dendrites underwent stress-induced changes in dendritic morphology in the right hemisphere, while in the left hemisphere, only distal dendrites were affected. Such hemisphere-specific alterations in morphology of callosal neurons could affect the inter-hemispheric exchange of stress-relevant information, and ultimately contribute to the prefrontal cognitive deficits associated with chronic stress. Funded by the CIHR.

1-C-90 Characterizing upregulated miRNAs during preclinical prion disease: possible roles in neuroprotection

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Prion diseases are caused by the conversion of normal prion protein (PrP^C) into the infectious form (PrP^{Sc}) leading to neuronal dysfunction, degeneration and death. However, how this conversion affects neuronal mechanisms which leads to neuronal dysfunction remains unknown. To address this gap in knowledge we performed global high-throughput transcriptomic and miRNomic temporal screens on a neuronal-rich region (CA1 hippocampus) to obtain profiles of transcripts and their regulatory small RNAs, respectively. We identified the presence of a neuroprotective response that occurs in these neurons only during preclinical disease. We validated 7 miRNAs to be upregulated during early disease of which 3 had known neuroprotective function. Using prediction programs we confirmed that the remaining 4 miRNAs were also potential regulators of numerous neuronal-specific genes, many of which affect neuronal morphology. Post-mitotic mouse hippocampal primary neuronal cultures were employed to further study possible effects of these 4 miRNAs on neuronal morphology; a characteristic that highlights relative fitness of neurons and was previously shown to be affected by neuroprotective miRNAs. Over-expressing of miR-26a-5p showed increased arborization and spine density in hippocampal neurons suggesting a possible neuroprotective role. We are currently characterizing the molecular pathway regulated by miR-26a-5p. Since miRNAs are global gene regulators, better understanding of their contribution to neuroprotective pathways may reveal possible avenues for therapy development.

1-C-91 Overlap in transcriptional and epigenetic signatures between sleep deprivation and epilepsy

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Relationships between sleep and epilepsy have been described since antiquity. Notably, epileptic seizures interfere with normal sleep patterns whereas specific sleep-wake stages have been associated with the occurrence of epileptic seizures (ES) and of interictal epileptiform discharges (IEDs). Moreover, sleep deprivation (SD) facilitates the onset of ES. However, the molecular basis of the development and predisposition for seizures caused by sleep loss are poorly understood. Therefore, we compared mRNA expression profiles obtained by RNASeq of mouse cortex after an acute SD and of mouse hippocampal slices exposed to kainate, a model of epileptogenesis. We observed a significant overlap ($p=3.9E-265$, hypergeometric test) of 1702 genes that are enriched in biological pathways such as cell death, neurotransmission or mRNA processing. Moreover, the comparison of the genome-wide epigenetic alterations associated with SD with the ones induced by the kainate treatment, reveal 1115 and 41 common genes differentially hydroxymethylated ($p=3.6E-65$) and methylated ($p=0.04$), respectively. The epigenetic affected genes scarcely overlap with the genes differentially expressed and are related to the morphology of the nervous system, metabolism and apoptosis. These common transcriptional and epigenetic signatures may provide insight into the immediate and long-lasting adaptive or maladaptive processes involved in the relationship between SD and epilepsy.

1-C-92 Atrophy and cortical demyelination predict the severity of depressive symptoms among people with relapsing-remitting multiple sclerosis

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Background: Although people with relapsing-remitting multiple sclerosis (RRMS) can live for years without motor or sensory symptom exacerbations, the prevalence of depression among people with this disease is very high.

Past research has identified a link between brain volume and depression experienced by such patients. However, these studies did not control for some important factors (e.g. motor disability), nor has magnetization transfer (MT) imaging, a method sensitive to demyelination within normal appearing brain tissue, been used. Objective: Among RRMS patients in clinical remission, we sought to estimate the extent to which global white and grey matter damage predicts depressive symptoms. Methods: Sixteen people with RRMS participated. Neuroimaging data (conventional; MT) and clinical data (Hospital Anxiety and Depression Scale; Self-reported Fatigue Scale; Multiple Sclerosis Functional Composite (MSFC)) were collected. Brain volumes (normalized to skull size) were measured using SienaX. Mean regional MT ratios (MTR) were computed. Results: When adjusting for fatigue and MSFC score, more severe depressive symptoms were predicted by lower normal appearing white matter volume ($\beta = -0.87$, $p < 0.01$), normal appearing grey matter (cortical deep) volume ($\beta = -0.75$, $p < 0.001$), and cortical grey matter MTR ($\beta = -0.47$, $p = 0.05$). MTR within deep grey matter and white matter did not predict depressive symptoms ($p > 0.05$). Conclusion: Atrophy and cortical demyelination may contribute to depressive symptoms of RRMS patients, irrespective of fatigue and motor/cognitive disability.

1-C-93 Involvement of ERK1/2 in Tau phosphorylation

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Alzheimer disease is characterized by the deposition of intracellular aggregates of hyperphosphorylated Tau protein. This phenomenon has been attributed in part to the increase of kinases and/or the decrease of phosphatases activities. ERK1/2 are activated in the first stages of AD and they are proposed as therapeutic target for the treatment of this pathology. However, although the phosphorylation of Tau by ERK1/2 has been demonstrated for a long time in cell-free system, this capacity remains controversial in vivo. Here, we showed that pharmacological ERK1/2 inhibition in mice and in SH-SY5Y cells did not reduce basal levels of phospho-Tau or hypothermia-induced Tau hyperphosphorylation. We also found that treating cells with the specific MEK1/2 inhibitor PD0325901 prevented

the activation of ERK1/2 by hyperthermia but not hyperthermia-induced Tau dephosphorylation. Finally, immunoblots indicated that Tau phosphorylation is not altered in Mek1^{-/-} mice compared to wild-type mice. In conclusion, these results do not support the notion that ERK1/2 are involved in Tau phosphorylation in intact cells.

1-C-94 Unraveling the function of norepinephrine in anxiety and depression with a new NE depletion model: the conditional VMAT2 knockout mouse

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The noradrenergic (NE) neurons, mainly located in the locus coeruleus (LC), have been linked to psychiatric disorders involving anxiety and depression. Previous studies have shown that changes in NE transmission from the LC influence anxi/depressive-related behaviors. The purpose of this study was to develop a viable model of NE depletion in the central nervous system without affecting the periphery to investigate NE functions. The NE depletion model was developed using the cre recombinase expressed by the dopamine β -hydroxylase (DBH) genes, which splices out the floxed VMAT2 gene (Vesicular Monoaminergic Transporter). The VMAT2DBHcre KO mice were validated by a lack of VMAT2 mRNA expression specifically in the LC, and NE depletion in the entire brain. The assessment of development and motor abilities confirmed VMAT2DBHcre KO as viable subjects for further testing. VMAT2DBHcre KO mice demonstrated less anxiety- and depression-related behaviors. Moreover, tissue level of DA and 5HT were altered in different brain structures associated with mood and motivational behavior. While basal corticosterone levels were not affected in the VMAT2DBHcre KO mice, acute stress through physical restraint revealed a faster return to basal corticosterone levels in VMAT2DBHcre KO mice. In addition, prior to the restraint, dexamethasone injections yielded further differences between wildtype and KO mice. All together, these observations showed a predisposition for VMAT2DBHcre KO mice to exhibit an altered susceptibility to developing anxiety and depression related psychiatric disorders.

1-C-95 Calretinin striatal interneurons: characterization and distribution in a murine model of Parkinson's disease

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Despite their small number, interneurons play a crucial role in the organization of the striatum, a major basal ganglia integrator that is severely affected in Parkinson's disease (PD). Here we compare the state of striatal interneurons expressing the calcium binding protein calretinin (CR) in both normal and PD (6-OHDA) conditions in mice. Immunostaining for CR revealed the presence of two morphological distinct types of CR striatal interneurons (CR1 and CR2), whose distribution and density were determined stereologically in normal, sham and 6-OHDA-injected mice. The CR1 cells were small (9-12 μ m), round and intensely fluorescent, they had beaded processes and abounded in the rostral striatum, whereas CR2 neurons were larger (15-20 μ m), less intensely fluorescent, had poorly branched dendrites and were more uniformly distributed than CR1 cells. Immunostaining for CR in BAC double transgenic mice expressing tdTomato protein under the control of dopamine D1 receptor promoter and GFP under the control of D2 promoter showed that CR1 and CR2 cells are devoid of D1 or D2 receptors. In PD mice, no major difference in the density of CR1 cells was detected between intact and 6-OHDA-lesioned sides, or between sham and lesioned animals. In contrast, the CR2 cell density was decreased in both intact and lesioned sides in 6-OHDA-injected mice, as compared to shams. The present study has provided the first detailed description in mouse of a unique population of striatal interneurons that might play a crucial role in the functional organization of the basal ganglia.

1-C-96 Impact of molecular motor disruption on mitochondrial dynamics and function

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Due to the unique architecture of neurons, trafficking of mitochondria throughout processes to regions of high energetic demand is critical to

sustain neuronal health. Disrupted mitochondrial trafficking may play a role in neurodegenerative diseases. In our study we investigated the impact of changing mitochondrial motility on mitochondrial form and function using disruptors of kinesin and dynein mediated transport in cultured neurons. The elongated morphology of a mitochondrion is maintained by a balance between fission and fusion. Following disruption of molecular motors, mitochondrial motility, morphology, ROS and ATP production were examined by fluorescence microscopy. In addition to the expected disruption of transport, we found that mitochondrial morphology was profoundly affected, displaying a punctate form. We also found that punctate mitochondria produced differing levels of ROS and ATP. Interestingly, neurons with disrupted kinesin transport exhibited greater survival than controls after excitotoxic treatment. Our results suggest a novel role for molecular motors. In addition to mediating mitochondrial transport, motors may play a role in the mechanism of regulating mitochondrial morphology. Furthermore our results suggest that molecular motor mediated mitochondrial dynamics may play an important role in regulating various mitochondrial functions and in turn cellular health.

1-C-97 Synaptic Transmission is Depressed in Sympathetic but not Parasympathetic Ganglia of Diabetic Mice due to Different Susceptibility to Oxidative Stress

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Excitatory cholinergic-nicotinic synapses in autonomic ganglia form a vital link between the integrative activities of the CNS and peripheral autonomic effector mechanisms. Normally, these synapses produce large suprathreshold EPSPs; however, recently, we reported that in diabetic mice, synaptic transmission in sympathetic ganglia is depressed. We showed that hyperglycemia elevates reactive oxygen species (ROS) in sympathetic neurons, causing the postsynaptic $\alpha 3$ -containing nicotinic receptors to inactivate. The question is: how widespread is this mechanism in the autonomic nervous system? To address these issues we have studied synaptic transmission in three branches of the autonomic nervous system: a prevertebral sympathetic ganglion, the superior mesenteric; a parasympathetic ganglion, the submandibular; and the adrenal medulla. In all three, we find that the nerve-evoked EPSPs are

mediated by $\alpha 3$ -containing nAChRs. Synaptic transmission is markedly depressed in the superior mesenteric ganglia and in the adrenal medulla within 1 week of diabetes. Unexpectedly, however, synaptic transmission in the parasympathetic submandibular ganglion is only marginally affected by diabetes, even after 4 months. Using combined ROS imaging and electrophysiology, we show that this differential effect on synaptic transmission occurs because sympathetic neurons are more vulnerable to hyperglycemia-induced oxidative stress than parasympathetic neurons. Supported by JDRF, CIHR, NSERC.

1-C-98 Dynamics of interictal spikes and high-frequency oscillations during epileptogenesis in temporal lobe epilepsy

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Mesial temporal lobe epilepsy (MTLE) is characterized in humans and in animal models by a seizure-free latent phase that follows an initial insult and is associated to plastic changes in temporal lobe excitability. Here, Sprague-Dawley rats were implanted with electrodes in the hippocampus CA3 region and entorhinal cortex (EC), after a pilocarpine-induced status epilepticus. EEG recordings and video-monitoring were then performed to study the occurrence of interictal spikes and high frequency oscillations (HFOs; ripples: 80-200 Hz, fast ripples: 250-500 Hz) from 48 h before to 96 h after the first seizure. Interictal spikes were classified as type 1 (characterized by a spike followed by a wave) or type 2 (characterized by a spike with no wave). We found a switch in the distribution of both types of interictal spikes before and after the first seizure: during the latent phase both types of interictal spikes predominated in EC whereas during the chronic phase they predominated in CA3. In addition, type 2 spike duration decreased in both areas from latent to chronic phase. HFO analysis showed that type 2 spikes associated to fast ripples occurred during the latent phase at higher rates in EC than in CA3 but at similar rates in both areas in the chronic phase. Finally, rates of fast ripples outside of spikes were higher in EC than in CA3 during the latent phase. Our findings demonstrate dynamic changes in interictal spike and HFO expression in EC and CA3 during the transition from latent

to chronic phase. These changes may represent biomarkers of epileptogenicity in MTLE.

1-C-99 The Parkinson disease gene LRRK2 works in concert with clathrin-light chains to limit activation of Rac1

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Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common cause of dominant-inherited and sporadic Parkinson disease yet our understanding of the physiological function(s) of LRRK2 remains incomplete. LRRK2 is a large molecular weight protein composed of multiple modules including a Ras of complex proteins (ROC) domain, a functional GTPase. Using affinity-selection assays with the isolated ROC domain we identified clathrin-light chains (CLCs) as novel LRRK2-binding partners and mapped LRRK2 binding to a 10-residue stretch in the C-terminal region of CLCs. We previously demonstrated that CLCs, through an unknown mechanism function as inhibitors of actin polymerization. We now demonstrate that knock down of CLCs and/or LRRK2 enhance activation of Rac1, but not Cdc42 and lead to alterations in cell morphology. LRRK2 expression rescues Rac1 activation and cell morphology changes resulting from CLC knock down, placing LRRK2 downstream of CLCs. Our data reveal a novel pathway in which CLCs function upstream of LRRK2 to control Rac1 activation.

1-C-100 Characterization of cognitive function in the 3xTg-AD mouse model of Alzheimer's disease at 6 months of age

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The 3xTg-AD strain is a commonly used mouse model of Alzheimer's disease (AD). These mice have three transgenes, two associated with familial AD, APP^{swe} and PS1^{M1461}, and one associated with tau pathology, Tau^{301L}. Currently, there are conflicting reports about when this strain develops cognitive deficits, and the nature of these deficits. Therefore, we tested male and female 3xTg-AD mice and their wildtype (WT) controls (B6129S/F2) at six months of age on a battery of tests to determine which cognitive deficits are present in these mice at this age and to determine which

behavioural tests are the most sensitive for detecting these deficits. Our preliminary results indicate that there were no differences between the 3xTg-AD and WT control mice in spontaneous alternation behaviour in the Y-maze, a test of spatial memory, or in a novel object recognition task with a 15-minute delay, which is a task that measures short-term memory. Interestingly, the 3xTg-AD mice spent more time freezing than WT control mice in cued fear conditioning, which may indicate they have enhanced fear learning. However this could also be a result of a higher level of anxiety in 3xTg-AD mice. In the Barnes maze, 3xTg-AD made more errors than the control mice at the end of the acquisition phase, which indicates that they have a deficit in spatial learning. Overall our preliminary findings indicate that there are modest deficits in spatial learning in the 3xTg-AD at six months of age, and that the Barnes maze is most sensitive task for measuring this deficit.

1-C-101 Deciphering the mechanisms of action of parkin during mitophagy using a structure-based FRET-reporter system

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To understand the structural basis of parkin's function and to gain insight its mechanism of activation, Gehring and coworkers have determined the structure of parkin using X-ray crystallography in collaboration with our group. The structure reveals that parkin exists in an auto-inhibited state and its activation must be associated with conformational changes at the C-terminal domain. We investigated the structural changes of parkin during the activation of parkin using fluorescent probes, and set out to characterize the activity of parkin in mitochondrial autophagy (mitophagy) based on insights obtained from the structure of parkin. Time-lapse fluorescent microscopy was used to examine the kinetics of wild-type and mutant parkin recruitment to the mitochondria after their depolarization with carbonyl cyanide m-chlorophenyl hydrazone (CCCP). We use a Förster Resonance Energy Transfer (FRET) - reporter to monitor conformational changes in parkin during the course of mitophagy. Preliminary experiments with the FRET reporter constructs show that labelling at specific positions does not interfere with parkin's function, and that a FRET signal is detected.

Thus it is possible to use FRET to monitor conformational changes in parkin.

1-C-102 Longitudinal imaging of thalamocortical projections after stroke

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The large majority of stroke survivors must cope with chronic disability, often affecting the upper limbs. Improved use of the stroke-affected limb is accompanied by neuroplasticity in peri-infarct areas. Modulating this plasticity should promote further gains in recovery. One largely unknown issue in stroke research involves the role of the thalamus, the brain's relay center for sensory information en route to the cortex, in recovery of function and cortical remapping after stroke in the forelimb area of the somatosensory cortex (FLS1). Thus, the aim of the current study was to elucidate the role of thalamocortical projections in recovery of function. We hypothesized that rewiring of thalamocortical projections to peri-infarct cortex occurs after FLS1 stroke and may be crucial for stroke recovery. Adult C57BL/6 mice underwent a surgical procedure to inject a green fluorescent protein tagged adeno-associated virus (AAV-GFP) into the ventroposteriolateral nucleus of the thalamus, which sends projections to FLS1. Immediately following virus injection, an imaging window was implanted over forelimb and hindlimb S1. Axon terminals and cerebral vasculature were imaged in vivo using two-photon microscopy before and at various times after stroke to assess acute and long-term changes in vascular and neuronal structure and function. Preliminary data indicate that stroke causes an acute loss of axonal density followed by increases in axon branching, axon length, and turnover of varicosities early after the stroke followed by later stabilization as recovery progresses.

1-C-103 Increased microglial priming and perivascular macrophage density in the dorsal anterior cingulate white matter of depressed suicides

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Despite increasing evidence supporting the neuroinflammatory theory of depression, little is known about the distribution of microglia in

individuals suffering from major depression. In this study, we investigated the morphology and distribution of microglia and perivascular macrophages in postmortem samples of dorsal anterior cingulate cortex (dACC) white matter. Fixed dACC samples from depressed suicides (n=14) and matched sudden-death controls (n=8) were obtained from the Douglas-Bell Canada Brain Bank. Tissue sections were immunostained for the macrophage-specific marker IBA1, and immunoreactive (-IR) microglial phenotypes were assessed using a combination of stereology and cell morphometry, and blood vessels were characterized as being associated with either a high or a low density of IBA1-IR perivascular macrophages. The ratio of primed over ramified ("resting") microglia was significantly increased in depressed suicides. The ratios of reactive and amoeboid microglia over ramified microglia, however, remained statistically similar between groups. Strikingly, the proportion blood vessels that were surrounded by a high density of perivascular macrophages was more than twice as high in depressed suicides than in controls (87% vs 42%, respectively), and this difference was strongly significant. These results suggest a higher incidence of microglial priming and increased densities of perivascular macrophages around blood-vessels in dACC white matter of depressed suicides, and support the notion of central neuroinflammatory processes in depression and suicide.

1-C-104 DNA Methylation in the Striatum of Dependent Cocaine Abusers

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Background: Cocaine dependency, like many psychiatric conditions, is characterized by phenotypic heterogeneity and variable response to treatment. While genome-wide association studies have identified common variants associated with addiction, they fail to explain all the biological variation associated with these disorders. Epigenetics, chemical modifications to the structure of DNA without changing the genetic sequence, may explain some of the variability. Animal studies describe numerous epigenetic alterations in response to chronic cocaine administration. Importantly, these modifications relate to behavioral changes

associated with compulsive drug seeking. Despite these findings, little is known about the epigenetic factors associated with cocaine dependency in humans. Of particular interest is DNA methylation as it represents a mitotically stable epigenetic mark. Methods: We used Reduced Representation Bisulfite Sequencing (RRBS), on nucleus accumbens and caudate tissue from dependent cocaine users and controls, to detect differences in DNA methylation. RRBS is a high throughput sequencing technique that enriches DNA fragments for CpG dinucleotides, capturing promoter regions at relatively high coverage. Results: Our preliminary findings suggest that DNA methylation in the striatum is lower, in some genetic regions, in cocaine dependent abusers than in controls. Further investigations will determine the cell-type specific pattern of these changes.

1-C-105 Betacellulin regulates the formation of myelin incisures and conduction of nerve impulses in the regenerating peripheral nerve

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When a nerve fiber is cut or crushed, the axon segment that is separated from the soma degenerates distal from the injury in a process termed Wallerian degeneration (WD). *Ola/WldS* mutant mice exhibit delays in WD, resulting in considerable lags in clearance of inhibitors of axonal regeneration that are associated with myelin debris, thereby delaying nerve regeneration. In our previous work, thousands of genes were screened by DNA microarrays and >500 transcripts were found differentially expressed in *Ola/WldS* compared with wild-type mice (WT). One of these genes, betacellulin (Btc), was selected as it was yet uncharacterized in the nervous system, despite being known as a ligand of the ErbB receptor family, which play a key role in remyelination. The goal of our study was therefore to investigate the role of Btc in WD, nerve regeneration and remyelination. First, we compared recovery of locomotor function in mice deficient in Btc, transgenic mice overexpressing Btc (Tg-Btc) and their respective WT littermates after a sciatic nerve crush injury. We found that Btc overexpression improved

recovery 7, 14 and 21 days post-injury. Using Coherent anti-Stokes Raman spectroscopy to visualize Schmidt-Lanterman clefts and nodes of Ranvier, we showed that Tg-Btc have increased numbers of myelin incisures compared to WT. Ex vivo electrophysiology analysis further revealed significant differences in nerve conduction velocity and excitability threshold values between Tg-Btc and WT mice. Taken together, these results suggest a novel regulatory role of Btc in myelin formation and repair.

1-C-106 The quest for early reversible changes in Alzheimer's disease: mass spectrometric imaging of gangliosides in a novel transgenic rat model of prodromal AD

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Identifying the earliest cellular changes that lead to Alzheimer's disease (AD) is an important step towards preventing the disease. In search of such changes, we investigate membrane lipids called gangliosides that are essential for cell signaling and survival. The expression pattern of ganglioside species can change in response to stress, and in turn, altered membrane lipid composition can increase a cell's vulnerability. We hypothesize that changes in membrane lipid composition, including gangliosides, may be evident in prodromal AD. To model the prodromal stage of AD in the elderly brain, we use a novel transgenic rat strain. APP21 transgenic (tg) rats express human APPSwe/Ind in high quantities but do not develop histological hallmarks of AD spontaneously as they age. Yet, these animals are susceptible to developing pathological hallmarks of AD when challenged, for example with AD brain extracts. In rat brain sections, we can image and profile gangliosides based on their chemical structure using matrix-associated-laser-desorption/ionization imaging mass spectrometry (MALDI-IMS). This innovative technology allows us to visualize changes in ganglioside expression profiles within a neuroanatomical context, and to relate these profiles to neuropathological events. A comparison of brain ganglioside expression between APP21 tg rats and wildtype rats will be presented. If our hypothesis is confirmed, an intervention aimed at stabilizing membrane lipid composition in individuals at risk of developing AD may make the aging brain more resistant to neurodegenerative challenges.

1-C-107 Impact of Bronchopulmonary Dysplasia on Brain Development

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Background: Many premature newborns develop bronchopulmonary dysplasia (BPD), a chronic lung disease resulting from prolonged mechanical ventilation and oxygen exposure. BPD survivors typically suffer long-term injury to the lungs, but also to the brain. However, it is currently not clear if the brain injury in these newborns is related only to their prematurity, or also to BPD. Objective: We investigated whether BPD has an effect on brain injury. Methods: A rat model of BPD was used to examine the direct effect of hyperoxia on the developing brain. Rat pups were exposed to hyperoxia (95% O₂) from postnatal day 4-14 (P4-14); at P14, rats were then housed in room air until two months of age. Controls were only exposed to room air. At two months of age, rats were sacrificed and their brains extracted. Hematoxylin & Eosin staining was performed on brain sections. Areas of different brain structures were measured and compared between the two groups. Results: Hyperoxia exposure resulted in a significantly smaller corpus callosum, anterior commissure, and optic tract, compared to room air controls. The optic chiasma also tended to be smaller in the hyperoxia group. Discussion: Hyperoxia exposure seems to have a direct impact on brain development. The rat model of BPD may be used to further study brain injuries related to hyperoxia.

1-C-108 The effects of intra-hippocampal histamine on dorsal and ventral hippocampal theta rhythm

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Recently it has been suggested that the suppression of evoked hippocampal theta frequency is a reliable neurophysiological signature of anxiolytic drug action. This is based on the observation that all clinically proven anxiolytic drugs tested so far (benzodiazepines, 5-HT_{1A} agonists, and SSRIs) reduce the frequency of reticularly-elicited dorsal hippocampal theta brain rhythm, while drugs

that do not selectively affect anxiety (e.g. antipsychotics and procognitive drugs) do not modulate theta frequency in this way. While considerable pharmacological evidence supports this model, there is emerging evidence that drugs belonging to different therapeutic classes (e.g. the anticonvulsant drug phenytoin, see Yeung et al., 2012a; the bradycardic agent ZD7288, see Yeung et al., 2012b), can also suppress hippocampal theta and produce robust anxiolytic effects in behavioral models of anxiety (e.g., the plus-maze; Yeung et al., 2012a). Here we investigate the effects of intra-hippocampal histamine on both dorsal and ventral elicited theta rhythm. Dorsal hippocampal microinfusions of histamine had no significant effect on evoked hippocampal theta frequency, but did produce behavioral anxiolysis in the plus-maze. In contrast, ventral hippocampal infusions strongly increased the frequency of evoked hippocampal theta at both the dorsal and ventral level, but did not produce behavioral anxiolysis. Taken together with previous results (Chee et al., 2014), our results directly challenge the theta suppression model of anxiolytic drug action.

D - Sensory and Motor Systems

1-D-109 Contribution of TRPC3 to calcium homeostasis and inflammatory nociceptive pathways in DRG sensory neurons

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Pathologically abnormal calcium levels in DRG neurons are linked to sensory hyperexcitability and chronic pain. Tight regulation of extracellular calcium entry is key to calcium homeostasis and STIM1-dependent SOCE (store-operated calcium entry) has been implicated as a major mechanism for regulating intracellular calcium levels. The TRPC (transient receptor potential, canonical) channel family has a documented role in both store- and receptor-operated responses linked to phospholipase C, a key pathway in inflammatory sensitization of DRG neurons. Our in situ hybridization data on the distribution of the entire TRPC family showed that TRPC3 is strongly expressed in adult rat DRG sensory neurons, particularly in small and medium diameter nociceptors. Using ratiometric calcium imaging, pharmacology, gene knockdown and overexpression, we gathered evidence for a significant involvement

of TRPC3 in both store- and GqPCR-operated channels in recombinant preparations as well as in rat DRG neurons. Overexpression of TRPC3 in rat DRG neurons resulted in a strong increase of calcium influx upon endoplasmic reticulum (ER) store depletion, implicating the channel's role in SOCE. This was complemented with shRNA-mediated knockdown, where we measured a significant decrease in calcium entry following ER store depletion. Furthermore, we observed a functional link between TRPC3 and the inflammatory metabotropic receptors P2Y2 and PAR2. Our results suggest that TRPC3 contributes to inflammatory sensitization through both store- as well as receptor-operated mechanisms.

1-D-110 Cortical mechanisms for transaccadic feature integration in spatiotopic vs. Retinotopic coordinates: an fMRI study.

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The neural substrates underlying visual processing of object features across eye movements remain unclear. In 2013, Dunkley & Crawford (SfN Abst. 2013) found that the bilateral supramarginal gyrus (SMG) is involved in orientation discrimination of 2D images across eye movements. Here, we aimed to further explore the frames of reference of orientation processing. In particular, we tested whether the adaptation effect to orientation in SMG is maintained also across different spatial locations. We used an fMRI adaptation paradigm in which participants performed an orientation-discrimination task while fixating on one of two possible fixation crosses. An obliquely oriented stimulus was presented in either the same or different orientation in two successive events of a trial. Therefore, we manipulated the orientation of the stimulus in 3 conditions: 1) same retinotopic, different spatiotopic position; 2) same spatiotopic, different retinotopic position; or 3) different retinotopic and spatiotopic position. Results (n=7) show adaptation to stimulus orientation in bilateral pre-supplementary eye fields (SEF), right SMG and left intraparietal sulcus for condition 1. Adaptation in condition 2 is observed in left SEF, hand motor area (HMA), superior parietal lobe (SPL) and SMG. For condition 3, adaptation is seen in right pre-SEF, left frontal eye fields, HMA, precentral gyrus, superior frontal gyrus, SMG, SPL and inferior

parietal lobe. These results suggest a critical role of the SMG in orientation discrimination in different frames of reference.

1-D-111 A simplified up-down (SUDO) method for measuring mechanical nociception in rodents using von Frey filaments

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The measurement of mechanosensitivity is a key tool for the study of pain in animal models. This is often accomplished with the use of von Frey filaments in an up-down testing paradigm. The up-down testing method described by Dixon (1965) and adapted by Chaplan et al. (1994) for mechanosensitivity testing in rodents remains one of the most widely used methods for measuring pain in animals. However, this method results in animals receiving a varying number of stimulations, which may lead to animals in different groups receiving different testing experiences and influence their later responses. To standardize this approach we propose a simplified up-down (SUDO) method for determining PWT with von Frey filaments that uses a constant number of five stimuli per test. We further refined the PWT calculation to allow estimation of PWT directly from behavioural response to the fifth stimulus, omitting the need for look-up tables. The PWT estimates derived using SUDO strongly correlate ($r > 0.96$) with the PWT estimates determined with the conventional up-down method of Chaplan et al., and this correlation remained very strong across different levels of tester experience, different experimental conditions, and in tests from both mice and rats. Finally, the use of either testing methods produced similar PWT estimates in behavioural tests with mice. SUDO thus offers an accurate, fast and user-friendly replacement for the widely used up-down method of Chaplan et al.

1-D-112 Cortical mechanisms for reaching to a remembered visual location during target memory and motor planning periods: an fMRI study

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Although the human brain areas involved in reach planning have been extensively studied,

the cortical mechanisms underlying the representation of target location and movement planning have not been disentangled yet. Here we investigated the neural circuits involved in these two mechanisms by separating the representation of target location from reach planning in a pro-/anti-reaching event-related fMRI design. Subjects are shown a target location, then after a delay (target representation period) are instructed with an auditory cue to perform a pro- or anti-reach. Following a 2nd delay (movement planning period), subjects perform the instructed movement by reaching-to-touch a touchscreen with their right hand. In a control condition, subjects indicate the colour of the initial target. Preliminary analysis ($n=3$) indicates that bilateral dorsal premotor (PMd) and extrastriate cortex as well as the superior parieto-occipital cortex (SPOC) and midposterior intraparietal sulcus in the left hemisphere show higher activation during the target representation period than the colour report condition. During the movement planning period, bilateral PMd and superior parietal cortex, as well as SPOC and lateral occipital cortex (LO) in the left hemisphere, show higher activity for the pro-reach condition when compared to the colour report condition. This suggests that although target memory and motor planning recruit similar parieto-frontal networks, parietal and occipito-temporal regions are selectively involved in one of the two mechanisms.

1-D-113 Expression of endocannabinoid enzymes diacylglycerol lipase alpha (DAGL α) and monoacylglycerol lipase (MGL) during postnatal retinal development

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In the last decades, there has been an increased interest in the physiological roles of the endocannabinoid (eCB) system and its receptors, cannabinoid receptor types 1 (CB1R) and 2 (CB2R). Some constituents of the eCB system were found in the retina of several species. To our best knowledge, no studies were conducted on the developmental expression of the synthesizing and catabolic enzymes of the cannabinoid endogenous ligand 2-arachidonoylglycerol (2-AG). Due to their lipophilic nature, eCBs are synthesized on demand and are not stored in vesicles. Consequently, the enzymes responsible for their

synthesis and degradation are key regulators of the physiological actions of the eCBs. Therefore, knowing their expression pattern during development is crucial for a better understanding of the role played by eCBs during the formation of the retina. We investigated the expression pattern of the enzymes responsible for the synthesis (DAGL α) and the degradation (MGL) of the principal eCB of the retina, 2-AG, in young and adult rats. The specific aim of this study was to determine the profile of DAGL α and MGL expression for each retinal cell types, from birth to adulthood. Our results indicate that DAGL α is expressed early in postnatal development. It is also largely expressed in the retina, including in the photoreceptors, horizontal, amacrine, and ganglion cells. MGL appears later during retinal development and is expressed in limited number of retinal cells. Overall, these results suggest that the eCB system could play a key role in the development and function of the retina.

1-D-114 Proprioceptive Precision is Impaired in Ehlers-Danlos Syndrome

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Ehlers-Danlos Syndrome (EDS) is a group of genetic connective tissue disorders associated with collagen malformation, which is proposed to include Hypermobility Syndrome (HMS) and Benign Joint Hypermobility Syndrome (BJHS). One of the main clinical features present in EDS is generalized joint hypermobility and it has been suggested by Rombaut (2010) that EDS patients may have proprioceptive impairments; perhaps because there is mutated collagen in proprioceptors which may be providing suboptimal signals. Recently Clayton et al. (2013) found that EDS patients were less precise in estimating their felt hand position in the peripheral workspace compared to healthy controls, and that patients who were the most hypermobile were also the least precise. Here we investigated this further by testing patients on a greater number of locations across a larger workspace. While EDS participants were just as accurate as healthy controls (as shown by similar absolute errors), they were not as precise as healthy controls. Precision of these reaches, measured by computing the areas and axes of the elliptic fits, were all significantly larger for EDS than healthy controls. This suggests a possible proprioceptive impairment in EDS.

1-D-115 Optogenetic modulation of GABAergic activity in mouse primary visual cortex affects contrast adaptation

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Prolonged viewing of high contrast gratings alters perceived stimulus contrast, and produces characteristic changes in the contrast response functions of neurons in the primary visual cortex (V1). This phenomenon is referred to as contrast adaptation. Contrast adaptation has been associated with membrane hyperpolarization, although the underlying cellular and network mechanisms mediating this change are unknown. Having previously established broad similarities in V1 contrast adaptation between mice and higher mammals, we sought to use a mouse model of optogenetic perturbation to study a possible role for GABAergic interneurons in this adaptation associated hyperpolarization. We performed extracellular recordings from V1 neurons in transgenic mice that express channelrhodopsin-2 (ChR2) in GABAergic neurons, and coupled contrast adaptation stimulus protocols with V1 photostimulation. We found that optogenetic activation of GABAergic neurons when the adapting grating was present caused a decline in activity during this period that altered the amount of adaptation observed to the test stimulus. These preliminary results indicate that the hyperpolarization produced by GABAergic neurons may play a role in controlling the magnitude of contrast adaptation in V1 neurons.

1-D-116 Loss of alpha-9 nAChR in the efferent vestibular pathway affects vestibulo-ocular reflex responses to horizontal rotation in mice

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Little is understood about the exact mechanism and function of the efferent vestibular system but many parallels have been drawn from the efferent auditory system given their anatomical similarities. In particular, its role in modifying the development of the vestibular hair cells has been proposed through cholinergic efferent innervations, much like the alpha-9 nicotinic acetylcholine receptor (nAChR) has been implicated to do so for the inner hair cells via the efferent auditory pathway. In order to determine

that the alpha-9 subunit of the nAChR is indeed functionally relevant to proper vestibular function, as it has been shown for auditory function, the vestibular-ocular reflex (VOR) in mutant knock-out strains of mice was characterized. Analysis of VOR gains in response to horizontal sinusoidal rotation demonstrated a significant decrease of approximately 40% across all frequencies greater than 0.2Hz within alpha-9 knock-out mice with no consequent effects on the phase of the response. Main sequence analysis of fast-phase eye movements for these mice also showed no significant difference from wild type, thus excluding the possibility of defective eye muscle development affecting the VOR in alpha-9 mutants. Given these results, it is evident that the alpha-9 subunit - found in the hair cells of the vestibular end organ as targets for vestibular efferent signaling - does not play a crucial role in proper VOR signaling and poses a likely target for investigating the developmental role of the efferent vestibular pathway.

1-D-117 Snout mechanosensory influence on arm extensor response in newborn opossums, *Monodelphis domestica*

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The opossum is born very immature but crawls, unaided, with its forelimbs from the mother's birth canal to a nipple where it attaches to pursue its development. Sensory clues are needed to guide it and trigger its attachment to the nipple. We have shown that stimulations of the trigeminal ganglion induce forelimb movement, trigeminal ganglion fibers distribute to the facial skin and slowly adapting mechanosensory receptors Merkel cells (AM1-43 positive) are present in the face epidermis. To determine the involvement of Merkel cells in locomotion of newborn opossums, we have applied calibrated forces to the snout and recorded the bursts of the triceps brachii as an indicator of forelimb responses in *in vitro* preparations. Pressure applied to the face induced bilateral triceps responses in proportion to stimulation intensity. During consecutive every 1-2 min. stimulations for 1h, the responses showed a trend toward decrease. Removing the facial skin nearly abolished the responses, indicating that the previous effects were specific to skin stimulation. Bath applications of the glutamate metabotropic receptor antagonist YM298198, which strongly

affects the firing of SAI fibers, decreased by half the triceps responses. AM1-43 entering cells via purinergic-2X receptors, we tested the effect of bath applications of the antagonist PPADS and saw a decreased response to 24% of the control. These results support that touch sensitivity of the snout is functional in newborn opossums and may influence forelimb locomotion, possibly contributing to guiding the animal to the nipple.

1-D-118 SK channels convert burst NMDAR-dependent LTD to LTP in communication sensory neurons

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Feedback and descending projections from higher brain centers play a prominent role in all vertebrate sensory systems. Feedback might be optimized for the specific sensory processing tasks in their target brain centers, but it has been difficult to connect the properties of feedback synapses to sensory tasks. We used the electrosensory system of a gymnotiform fish to address this problem. Cerebellar feedback to pyramidal cells in the first central electrosensory processing region, the electrosensory lateral line lobe (ELL), is critical for canceling spatially and temporally redundant electrosensory input. The ELL contains four electrosensory maps and we have previously analyzed the synaptic and network bases of the redundancy reduction mechanism in a map (CLS) involved in electrolocation behavior. In the CLS only long-term depression was induced by pairing feedback presynaptic and pyramidal cell post-synaptic bursts. Here we turn to an ELL map (LS) known to encode electrocommunication signals. We find remarkable differences in synaptic plasticity of the morphologically identical cerebellar feedback input to the LS. Pyramidal cell SK channels permit LTP of feedback synapses when pre- and post-synaptic bursts occur at the same time. We hypothesize that LTP in this map is required for enhancing the encoding of weak communication signals. We conclude that feedback inputs that appear morphologically identical in sensory maps dedicated to different tasks, nevertheless display different synaptic plasticity rules contributing to differential sensory processing in these maps.

1-D-119 Primary motor cortical neurons reflect torque-related activity from ipsilateral limb

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It is commonly accepted that the primary motor cortex (M1) is involved in controlling contralateral limbs. However recent work has shown that many M1 cells respond during ipsilateral as well as contralateral movement. As feedback plays an important role in voluntary motor actions, we were interested in exploring whether neural activity was also related to mechanical perturbations applied to the ipsilateral and contralateral arm. For our tasks, we recorded the activity of M1 cells from a micro-electrode array in a non-human primate (NHP). During the task, the NHP stabilized a cursor representing its right hand at a target. After 1000ms, flexor or extensor step torques were applied to the shoulder, the elbow, or both (9 load conditions). The NHP had 1000ms to return and stabilize at the target for another 1500ms hold period. This paradigm was repeated for the left arm, and then for both limbs simultaneously. We found that a large proportion of M1 cells produce perturbation responses and modulate their activity to static torque for either limb. Cells often displayed preferred torque directions (PTD) that were different for each arm, such as increasing firing for shoulder flexion in the left arm, but increasing firing for elbow flexion in the right arm. When torques were applied to both arms, cell PTDs tended to reflect an average of the PTDs for each arm alone. Our results indicate that M1 represents perturbation and torque-related activity from both limbs.

1-D-120 Differential neural responses to naturally occurring envelopes in the electrosensory system**Chengjie Huang¹, Maurice Chacron¹**¹McGill University

Natural sensory stimuli frequently consist of a fine structure whose amplitude (i.e. envelope) varies more slowly. Previous studies have demonstrated that envelope signals are involved in several sensory systems and are necessary for perception. However, the neural mechanisms underlying the encoding of envelope signals are poorly understood in general. Gymnotiform wave-type weakly electric fish constitute an attractive model system for studying how envelopes are processed in the brain because of well-characterized anatomy and physiology. In our study, we recorded extracellularly in vivo

from the pyramidal cells (PC) of three Electrosensory lateral line lobe (ELL) segments of the weakly-electric fish using well-established techniques. Simultaneously, we presented mimics of the envelope stimuli that occur naturally ranging from 0.05Hz to 10Hz. We hypothesized that pyramidal neurons in different ELL segments will be tuned differently for lower envelope frequencies (<1Hz) corresponding to movement and for higher envelope frequencies (>1Hz) corresponding instead to social interaction. We used quantitative data analysis techniques such as information theory in order to determine how much information about envelope stimuli is transmitted by individual and populations of pyramidal neurons. Our results therefore provide new results elucidating how envelopes are processed in the brain.

1-D-121 MEG gamma oscillations in primary visual cortex are correlated with resting GABA-A receptor density**Jan Kujala¹, Julien Jung², Sandrine Bouvard³, Carolina Ciumas², Françoise Lecaigard², Romain Bouet², Philippe Ryvlin², Jerbi Karim⁴**¹O.V. Lounasmaa Lab, Aalto University, ²Lyon Neuroscience Research Center, INSERM-CNRS-University of Lyon I, ³CERMEP imaging center Lyon, ⁴Université de Montréal

The mechanisms that underly cortical gamma-band oscillations remain poorly understood. Previous reports point to a key role of GABA-mediated inhibition (Buzsaki & Wang, 2012). Recent studies combining magnetoencephalography (MEG) with magnetic resonance spectroscopy (MRS) GABA-concentration measures suggest that the dominant frequency of cortical gamma-oscillations is correlated with the concentration of GABA at rest (Muthukumaraswamy et al., 2009; Gaetz et al., 2011). To investigate the relationship between gamma oscillations and GABA-A receptor density we recorded MEG and positron emission tomography ([¹¹C] Flumazenil-PET) data from a population of 10 healthy participants. The flumazenil uptake recordings were conducted during rest, and the MEG data were collected during an N-back visual working memory task. We investigated task-related modulations of gamma power and performed correlation analyses between GABA-A receptor density and both the peak frequency and amplitude of gamma power in V1. We detected memory-load dependent task-induced increases in gamma-band (60-90 Hz) power

across a widely distributed network and a notable increase in V1 gamma power. Across subjects, measures of GABA-A at rest correlated positively with the peak gamma frequency and negatively with gamma amplitude in V1. These results extend previous MEG-MRS findings by providing the the first direct link between GABA-A receptor density and task-related gamma oscillations and allow us to fine-tune our understanding of the link between GABAergic inhibition and gamma-band oscillations.

1-D-122 Effects of transcranial direct current stimulation, tDCS, of primary somatosensory cortex, S1, on tactile detection.

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tDCS is a non-invasive technique whereby weak, direct current stimulation is applied to cortex. This is reported to enhance, anodal (a), or decrease, cathodal(c), cortical excitability. Few studies have tested the effects of S1 tDCS on tactile perception, and the results to date are mixed. We tested the effects of tDCS (a-, c- and sham) applied to the right S1 hand representation, 2 cm posterior to C4, on tactile detection of vibration (0.5 s duration, 20Hz, amplitudes of 2, 6 and 10µm) applied to the distal pad of the left middle finger. Tactile detection was measured before, during and after 20 min of tDCS (1mA). A bias-free signal detection theory approach was used: half of the trials contained a stimulus; 50% had no stimulus. Subjects indicated whether a stimulus was present or not and rated their degree of confidence in this using a 5-point scale. These data were used to generate ROC (receiver operating characteristic) curves. The area under the ROC curves was calculated for each vibrotactile intensity. From this we interpolated detection threshold (0.75). Mean baseline detection threshold was 4.2 µm (n=13). To date, the results are variable. With a-tDCS, 4 subjects showed the predicted decrease in threshold (to 61% of control), but 4 others showed an increase (144% of control). With c-tDCS, the results were general negative (no change, 4/6). It is expected that the results will lead to a better understanding of the neuronal mechanisms underlying the effects of tDCS on tactile perception. Supported by CIHR, GRSNC.

1-D-123 TRPV1 sensitization is essential for the development of chronic, but not acute pain following colonic inflammation

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Inflammatory bowel disease (IBD) is associated with debilitating abdominal pain, which can persist throughout quiescent phases of the disease. While a growing body of evidence suggests a role for the transient receptor potential vanilloid 1 (TRPV1) in neurogenic inflammation and visceral hypersensitivity, functional studies have yet to assess its function in post-inflammatory pain. This study aimed at establishing the role of TRPV1 in peripheral sensitization and pain during the acute and remission phases of colitis. Methods: Male C57BL/6 mice were separated into controls, acute DSS (2.5% DSS, 7 days), and recovery (acute DSS, followed by a five-week recovery period) groups. Results: Mice treated with DSS showed signs of visceral pain five weeks post-DSS discontinuation, at which point inflammation had resolved. This effect appears to be TRPV1-dependent, as deletion of the channel prevented the development of visceral pain in the remission phase of the disease. Importantly, the deletion of TRPV1 failed to alleviate both inflammation and pain in the acute phase of colitis. In vitro experiments revealed that prolonged exposure to substance P potentiates capsaicin-evoked responses by mediating TRPV1 channel accumulation at the plasma membrane. Conclusion: Our results demonstrate that although TRPV1 is not required for acute inflammation and visceral hypersensitivity, it plays a pivotal role in the development of persistent visceral pain during the resolution phase of colitis, and could therefore represent an attractive therapeutic target in the management of IBD-related pain.

1-D-124 Role of residual dorsolateral pathways in locomotor recovery after spinal hemisection in cats

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After an incomplete spinal cord injury (iSCI), locomotor recovery is achieved through changes at both supraspinal and spinal levels. However, the relative importance of these mechanisms on

locomotor recovery has never been elucidated. In this study, we evaluated the role of intact pathways coursing in the dorsolateral funiculus (DLF) of the spinal cord which mainly contains the cross components of the cortico- and rubrospinal tracts. Our choice was based on the fact that, in intact conditions, DLF pathways are not crucial for treadmill locomotion but are known to modulate the basic locomotor rhythm suggesting that they could play a compensatory role after iSCI through its interactions with the spinal circuitry. Four adult cats were submitted to a dual spinal lesion protocol consisting in a left hemisection at T10 level followed, 3 weeks later, by a lesion of the right DLF at the same spinal level. Locomotion was evaluated on a treadmill and overground in the intact state and then 3 weeks after each spinal lesion. We showed that the DLF lesion abolished for at least 3 weeks (time of experiment) bilateral hindlimb locomotion that had previously recovered from the hemisection. Such a disruption of bilateral hindlimb locomotion suggests that, after hemisection, DLF pathways had gain the capacity to activate the muscles of both hindlimbs, probably through their interactions with the spinal circuitry.

1-D-125 Serotonin modulates electrosensory processing and behavior via 5-HT2 receptors

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Efficient sensory processing of the environment is a critical function for any organism to survive and is accomplished by having neurons adapt their responses to stimuli based on behavioral context in part through neuromodulators such as serotonin (5-HT). We have recently shown that one critical function of the serotonergic system in weakly electric fish is to enhance sensory pyramidal neuron responses within the electrosensory lateral line lobe (ELL) to a specific stimulus class: those caused by same sex conspecifics. This enhancement is accomplished by making pyramidal neurons more excitable through downregulation of potassium channels. However, the nature of the 5-HT receptors that mediate this effect is not known. Here we show that the 5-HT2 receptor antagonist ketanserin can effectively block the effects of 5-HT on pyramidal neuron excitability in vitro. Indeed, 5-HT application subsequent to ketanserin application did not cause any significant changes in neuron excitability and

responses to current injection. We further show that ketanserin applied in vivo can block the effects of 5-HT on behavioral responses. Thus, our results show that the previously observed effects of 5-HT on sensory processing within ELL and their consequences on behavior are mediated by 5-HT2 receptors.

1-D-126 Training induced dynamic filtering of auditory distractors in the rat primary auditory cortex

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Ignoring distracting sounds becomes increasingly difficult with aging even without significant hearing loss. We previously showed in aging rats that a reduced active inhibition of repetitive background sounds could explain this impairment. However, the cortical mechanisms involved in this dynamic contextual filtering of sound and the experiences that alter their specificity for complex sounds are still largely unknown. Here we tested the hypothesis that filtering spectrally complex sounds would be more robust and specific after training on an adaptive auditory task aiming to differentiate fine spectral features of complex sounds. We trained young adult (6-12 months old) and aging (20-24) rats on such a task until performance reached plateau and then densely mapped their primary auditory cortical (A1) responses to simple and complex trained and untrained sounds in the presence of background distractors. Similar recordings were performed in naïve untrained rats. Our results confirmed that aging associates with poorer A1 extraction of complex sounds in the presence of background distractors. We also found in both young and aged rats that expecting to hear a specific sound made A1 momentarily more sensitive to that sound and less sensitive to non-behaviourally relevant ones. This rapid expectation based shift in A1 tuning appeared to be mediated by increased cooperativity between A1 neurons sharing initial selectivity for the trained sound. Our findings indicate that distractor sensitivity in the aged brain can be improved by simple but targeted auditory training strategies.

1-D-127 Neural substrates involved in the integration of object properties and intended actions

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We tested seven right-handed subjects in an fMRI experiment to investigate the neural substrates involved in the integration of object properties and hand actions. Subjects viewed an object (visual cue: VC) and received auditory information about the action to be performed on the object (action cue: AC) in two successive phases. We manipulated the order of cue presentation (VC:AC and AC:VC), the action type (Grasp or Align) and the orientation of the object (two orientations). In the VC:AC conditions, an object was presented for 250ms followed by a delay of 8s. The audio cue was then provided and followed by a delay of 8s, after which a sound cued subjects to act on the object that was no longer visible. In the AC:VC conditions, the order of the cues was inverted. We hypothesized that areas involved in integrating object properties and action type would show higher response during the delay following the second cue because of the added integrative process of visual information into action planning. The posterior intraparietal sulcus (pIPS) and dorsal premotor area (dPM) in the left hemisphere showed higher response in the delay following object presentation when the action type had already been specified as opposed to when it had not. The pIPS also showed higher response in the delay following the audio cue about action type when the object had already been presented as opposed to when it had not. These results suggest that the fronto-parietal network is strongly involved in incorporating object properties, such as orientation, into context cues, such as action type.

1-D-128 The sensitivity of primary motor cortex to pre-perturbation muscle activity suggests knowledge of the inherent properties of motoneurons

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Following a mechanical perturbation, short-latency responses (25-45 ms) will increase proportional to the level of background muscle activity. This 'gain scaling' is thought to be due to the properties of motoneurons. Counteracting this property poses a challenge for the nervous system, which must ultimately counter the absolute change in load regardless of the initial muscle activity. We recently showed that this gain scaling diminishes throughout the long latency response (50-105 ms). Our hypothesis is

that this reduction reflects the increasing influence of transcortical feedback through primary motor cortex (M1), which possesses knowledge of the inherent properties of motoneurons. Thus, we predict that neurons in primary motor cortex are responsible for the reductions in gain scaling observed in the periphery. We examined this issue by quantifying the response of muscle activity and M1 neurons in monkeys to similar mechanical perturbations during a postural control task, with and without background loads. Similar to previous reports, we found that muscle activity exhibited gain scaling in the short latency response, but observed a rapid decrease in this scaling in the long latency epoch. Similarly, the perturbation response of M1 neurons initially scaled with the size of pre-perturbation neural activity. However, we found that the initial scaling was rapidly reversed at ~50ms post-perturbation. Critically, this reversal compensates for early gain scaling and highlights the sophistication of feedback control.

1-D-129 Retinotopic maps and functional properties of V1 in D2 dopamine receptor knockout mice

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In the retina, dopamine plays a critical role in the modulation of visual information through the activation of D1 and D2-like receptors. Interestingly, D2 receptors are also found in the primary visual cortex (V1) where their function is unknown. In this study, we evaluate the impact of D2 receptor deletion (D2r-KO) on the structure and visual processing of mouse V1 using optical imaging of intrinsic signals. Data from adult D2r-KO mice (n=12) and their wild-type (WT) littermates (n=9) were compared. Continuous visual stimulation and frequency-based analysis were used to obtain retinotopic maps and to calculate V1 shape, surface, ocular dominance and scatter. Sinusoidal gratings of varying spatial frequency (SF) and contrast values were used to evaluate population SF selectivity and contrast response function. No abnormalities were observed in D2r-KO retinotopic maps and no difference was observed between the various parameters calculated from the maps. Furthermore, no difference in the contrast response function was observed between the 2 groups. However, the

optimal SF was higher for D2r-KO mice when compared to the WT group ($0.031 \text{ cpd} \pm 0.002$ vs. $0.023 \text{ cpd} \pm 0.002$, $p < 0.05$, Student's t-test), consistent with a rightward shift of the SF selectivity curve and higher SF selectivity cut-off ($0.138 \text{ cpd} \pm 0.010$ in D2r-KO vs. $0.094 \text{ cpd} \pm 0.010$ WT, $p < 0.01$, Student's t-test). Our results suggest that D2 receptors do not contribute to the structure and connectivity of V1 but may modulate response properties such as spatial frequency selectivity of the comprising neurons.

1-D-130 Properties of synaptic inputs from hair cells and efferents onto the vestibular afferent calyx terminals

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Calyx afferent terminals completely cover the basolateral walls of hair cells and receive inputs from multiple hair cell ribbons as well as efferent fibers (mainly cholinergic). To investigate the synaptic properties of these inputs, in vitro whole-cell patch-clamp recordings were performed from calyces in cristae of 2-4 week old rats. AMPA-mediated postsynaptic currents showed an unusually wide range of decay time constants (< 5 to > 500 ms). Decay time constants increased (or decreased) in the presence of a glutamate transporter blocker (or a competitive glutamate receptor blocker), suggesting glutamate accumulation and spillover. Glutamate accumulation increased firing rates of calyces by slowly depolarizing them. To investigate acetylcholine (ACh) receptors mediating efferent inputs, 1 mM ACh was applied. About 56% of calyces ($n = 75$) showed an inward current at a negative holding potential, which reversed at ~ 0 mV ($n = 7$). Presence of 10 μM tubocurarine, 10 μM strychnine, 400 nM α -BTX ($n = 3$ for each) or 600 nM α -RglA ($n = 5$) resulted in an $\sim 80\%$ block. Thus, excitatory efferent inputs to the calyx are most likely mediated by $\alpha 9$ -containing receptors. Together, these findings suggests glutamate accumulation/spillover and cholinergic inputs function as gain control mechanisms in the majority of the calyces. Further studies are required to investigate whether other efferent fibers (e.g., glycinergic) also play a role in these and the calyces without any ACh response. This work was supported by NIDCD grants R01DC006476 and R01DC012957 to EG and a NOHR grant to SGS.

1-D-131 Investigating the transformation from a dense to a sparse neural code

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A fundamental operation performed by sensory systems is the transformation from a dense neural code in the periphery to a sparse code more centrally such that populations of 'dense coding' neurons which, encode a broad range of stimulus features project to populations of 'sparse coding' neurons that respond selectively to particular stimulus features. Previous investigations have established that such a transformation occurs at the level of the midbrain in the well characterized electrosensory system of the weakly electric fish *Apteronotus leptorhynchus*. Here we aimed to uncover the mechanisms underlying this transformation. Patch clamp recordings were made from neurons within the midbrain Torus Semicircularis (TS) in response to natural electrosensory stimuli and were filled with biocytin for post hoc identification of previously established cell types. We found that toral neurons form different classes based on their responses, some classes distinctly sparse and others dense. Additionally, we found that these classes correspond to distinct cell types. Cluster analysis revealed several groups of sparse coding and dense coding neurons based on response profiles to an ensemble of behaviourally relevant stimuli. Our results reveal that dense coding and sparse coding neurons correspond to different cell types within the TS.

1-D-132 Modulation of stimulus saliency on human pupil orienting response

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The sudden appearance of a salient stimulus initiates a series of responses to orient the body for appropriate actions, including not only shifts of gaze and attention, but also transient pupil dilation. Modulation of pupil dynamics by stimulus saliency is less understood, although its effects on other components of orienting have been extensively explored. Microstimulation of the superior colliculus (SC) evoked transient pupil dilation, and the initial component of pupil dilation evoked by microstimulation was similar to that elicited by the presentation of salient sensory stimuli, suggesting a coordinated role of the SC on this

behavior, although evidence in humans is yet to be established. To examine pupil orienting responses in humans, we presented visual stimuli while participants fixated on a central visual spot. Transient pupil dilation in humans was elicited after presentation of a salient visual stimulus in the periphery. The evoked pupil responses were modulated systematically by stimulus contrast, with faster and larger pupil responses triggered by higher contrast stimuli. The saliency modulation was pronounced under different levels of baseline pupil size. The pupil response onset latencies for high contrast stimuli were comparable to those produced by the light reflex and much faster than the darkness reflex, suggesting that the initial component of pupil dilation is more likely mediated by inhibition of the parasympathetic pathway. Together, our results suggest that the orienting pupil response in humans is modulated by stimulus saliency.

E - Homeostatic and Neuroendocrine Systems

1-E-133 Novel putative GOAT Inhibitor, CF801, Reduces Acylated Ghrelin & Body Weight in C57/BL6J Male Mice

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Ghrelin is a 28 amino-acid peptide implicated in the regulation of food intake and body weight. Ghrelin serves a wide range of physiological roles including the promotion of food intake and adiposity. Once secreted, ghrelin binds to the growth hormone secretagogue receptor (GHSR), to increase food intake and body weight. The ability of ghrelin to bind to the GHSR is dependent on a post-translation modification of the mature ghrelin protein, wherein an n-octanoic acid is added to the third serine residue on the ghrelin molecule through the activity of the ghrelin-O-acyl-transferase (GOAT) enzyme. Here we present a novel peptide, CF801 (patent pending), that decreases acylated ghrelin concentrations in vivo potentially through the inhibition of GOAT. Intraperitoneal administration of CF801 reduced circulating plasma acylated ghrelin levels, body mass and total adipose tissue mass in mice, relative to animals receiving vehicle-injections. Furthermore, CF801 administration reduced caloric intake following an overnight fast in a dose dependent fashion, relative to vehicle-injected controls. CF801 treated animals did not

show evidence for anxiety or depressive-like behaviors, as measured by social interaction and open field test. Thus, we propose a novel compound to be further investigated for the potential treatment of metabolic disorders associated with body weight gain and adiposity.

1-E-134 Role of Acyl-CoA Binding Protein (ACBP) in Hypothalamic Control of Energy Homeostasis

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The control of energy balance mainly relies on the hypothalamus and its capacity to detect nutritional signals including glucose and fatty acids (FA). FA act in the mediobasal hypothalamus (MBH) to inhibit food intake and glucose production, but the mechanisms and cell types involved have not been identified. ACBP binds fatty acyl-CoA with high affinity and regulates their metabolism in the periphery. In the central nervous system (CNS), ACBP is secreted by astrocytes and central administration of ACBP has anorectic effects and increases glucose tolerance. Our preliminary results suggest that ACBP modulates intracellular FA metabolism in cultured astrocytes. Altogether this suggests a role for ACBP in central FA action and control of energy homeostasis. First, we show that ACBP is mainly expressed in astrocytes and tanocytes in the MBH. Second, we generated an astrocyte-specific ACBP KO using the Cre-Lox strategy, ACBP-GFAP KO mice, and assessed their metabolic status and feeding behavior. Our results show that astrocyte-specific deletion of ACBP does not affect food intake, satiety, weight gain or glucose tolerance in male mice fed with a regular chow or high fat diet (HFD). Female mice however have a tendency for increased weight gain and fat mass when fed HFD without changes in food intake. Additional behavioral and metabolic phenotyping is ongoing to assess the impact of ACBP deletion in astrocytes on glucose and energy homeostasis. Our results suggest that ACBP deficiency in astrocytes increases the susceptibility to diet-induced obesity in a gender-specific manner.

1-E-135 Transgenerational transmission of maternal care via the regulation of estrogen receptor alpha

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Variations in maternal behavior influence the development of multiple biological systems in the offspring and such traits are transmitted from one generation to the next through genomic as well as nongenomic processes. Female rats will exhibit high levels of maternal behaviors towards their offspring when reared by mothers who display high levels of maternal licking and grooming (i.e. High LG). High LG offspring have increased mRNA and protein levels of estrogen receptor alpha (Esr1) in the medial preoptic area (mPOA) when compared to Low LG offspring. We aimed to assess the functional role of Esr1 expression in the mPOA on maternal behavior. Lentiviral shRNAs targeting Esr1 were injected into the mPOA of High and Low LG dams. After reducing protein and mRNA Esr1 expression in the mPOA of High LG dams, there was a significant decrease in maternal behavior such that their maternal LG resembled the levels seen in Low LG reared dams. Offspring of both groups had decreases in Esr1 expression in the mPOA and a decreased pup LG. Thus, we were able to disrupt the transgenerational transmission of both Esr1 expression and pup LG. We then analyzed histone modifications and histone binding proteins in the mPOA, and found significant differences in the Esr1 promoter region of High and Low LG offspring in acetylation and methylation determining histone marks. These studies demonstrate that expression of Esr1 in the mPOA in female offspring of High LG dams is critical for the transgenerational transmission of individual differences in LG and is epigenetically regulated.

1-E-136 Involvement of Reactive Oxygen Species (ROS) and Transient Receptor Potential Canonical (TRPC) in glucose sensitivity of hypothalamic neuron.

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The mediobasal hypothalamus (MBH) houses specific glucose-sensitive neurons known as high-glucose excited (HGE) neurons which

increase their electrical activity in response to increased glucose level. Despite their suggested role in the control of glucose homeostasis, molecular mechanisms involved in HGE neuron glucose response are unknown. Reactive Oxygen Species (ROS) are produced into the MBH in response to increased blood glucose level and necessary for glucose detection. Interestingly, some transient receptor potential canonical (TRPC) channels are ROS-sensitive. Thus, we hypothesized that HGE neuron detect glucose through a ROS-TRPC signaling pathway. Activity of freshly dissociated rat MBH cells was monitored by Fura-2 calcium imaging in response to increased extracellular glucose level from 2.5 to 10 mM. We found that ~100% of MBH HGE neurons responses to increased glucose are inhibited by antioxidants or the non-selective TRPC channel inhibitor (SKF96365). Interestingly, ~70% of HGE glucose responses are inhibited by the TRPC3 channel inhibitor Pyr3 or activated by the TRPC3 activator OAG, suggesting that this specific channel is involved in the majority of MBH HGE neurons glucose response. TRPC channel expression in HGE neurons is being explored by single-cell RT-PCR. Finally, pharmacological inhibition of TRPC3 channel into the MBH in vivo decreases insulin secretion in response to increased brain glucose level. Altogether, these data suggest that ROS-TRPC3 signaling is implicated in MBH HGE glucose sensitivity and cerebral control of glucose homeostasis.

1-E-137 A novel circuit for stress termination

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Responding appropriately to a stressor is necessary for survival; it requires rapid recruitment of neural and endocrine systems to meet an impending challenge. This response is well understood, but represents only the engagement component of the stress response. By contrast, very little is known about the neural circuits responsible for terminating the stress response. The termination is usually associated with a series of behaviours, such as grooming in rodents that serve a de-arousal function following stress. We hypothesized that the principal drivers of the visceral stress response, the corticotrophin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) may also contribute to dis-engagement following stress. We utilized a

transgenic CRH-Cre mouse strain and targeted PVN CRH neurons with recombinant adeno-associated virus carrying channelrhodopsin 2. In vivo stimulation of PVN CRH neurons with blue light induced rapid and robust grooming. There was an inverse relation between grooming and the anxiety state of the animal. In order to elucidate the neural circuit(s) responsible for stimulated grooming, we traced CRH fibers and identified neurons in the perifornical area/lateral hypothalamus. Using whole-cell patch clamp recordings, we determined that these cells receive glutamatergic input from PVN CRH neurons. Our data provide evidence that PVN CRH neurons, serve a dual function, contributing to both the launch of the visceral response to stress and driving behaviours that contribute to the termination of the stress response.

1-E-138 The monounsaturated fatty acid oleate in the ventral tegmental area inhibits food intake and dopamine neurotransmission

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Dopamine (DA) neurons of the ventral tegmental area (VTA) are critical for the control of motivation and reward-relevant behaviors. Evidence that DA neurons respond to hormones like leptin, ghrelin and insulin to modulate feeding, reward-relevant behavior and DA tone raises the possibility that, as in the hypothalamus, cells of the VTA act as metabolic sensors that integrate both hormonal and nutrient signals. The aim of the present work was to evaluate the impact of long-chain fatty acids (FA) in the VTA on feeding and DA neurotransmission. Methods: Following stereotaxic implantation of a double cannula into the VTA, male Wistar rats (n=12-15/group) received either vehicle (2-HydroxyPropyl- β -cyclodextrin (HPB) in ACSF; 500nl), oleate (12mM; monounsaturated FA) or palmitate (12mM; saturated FA). Patch clamp recordings were made in rat VTA slices preparations perfused with oleate (6 μ M, 2.5mM glucose; n=15) or oleate+phloretin (6 μ M; 2.5mM glucose; 100 μ M phloretin; n=6), to block fatty acid transport. Results and conclusion: Behavioral results show that a single injection of oleate, but not palmitate, in the VTA significantly decreased dark cycle chow intake. Oleate significantly inhibited firing in ~50% of DA neurons recorded

- an effect blocked by phloretin, and reduced the amplitude but not the frequency of mEPSCs. Together, the findings suggest that oleate in the VTA has anorectic actions that may involve intracellular transport of FA and inhibition of DA neurotransmission and offer a means whereby dietary FA may directly modulate brain reward circuitry. Supported by CIHR

1-E-139 Optogenetic dissection of the MCH system: implications for sleep-state modulation.

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The hypothalamus consists of intermingled inhibitory and excitatory neural circuits. Their activity correlates with one or more vigilance states, including wakefulness, non-Rapid Eye Movement (REM) sleep and REM sleep. Recent evidence suggests that neurons expressing Melanin-Concentrating Hormone (MCH) have a sleep-promoting action; however, their selective modulation of sleep states remains unclear. To investigate the specific role of MCH neurons, we genetically targeted the expression of activatory (ChETA, SSFO) and inhibitory (eNpHR3.0) optogenetic tools to MCH neurons to reliably control their activity in vitro and in vivo. Using real-time EEG/EMG detection of vigilance states, we found that optical activation of MCH neurons during NREM sleep increased the probability of NREM-to-REM sleep transitions, while MCH neuron activation during REM sleep extended its duration in ChETA animals compared to EYFP-expressing controls. These results were confirmed by SSFO activation of MCH neurons in vivo. In contrast, we showed that optogenetic silencing of MCH neurons during REM sleep significantly reduced theta rhythm amplitude concomitant to an increase of slow theta range amplitude (3-5 Hz). Finally, we demonstrated that optical activation of MCH terminals induced fast GABAA-mediated inhibitory currents in local wake-promoting histaminergic neurons, an effect that is partly mediated by the release of MCH peptide. Collectively, these results support a causal role for MCH neurons in the onset and maintenance of cortical REM sleep in the mammalian brain.

1-E-140 Alterations in Hypothalamic Feeding Circuitry and Leptin Response in CD-1 Mice Perinatally Exposed to the Endocrine Disruptor Bisphenol-A (BPA)

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Bisphenol-A (BPA) is a component of polycarbonate plastic, and is commonly found in food and drink containers. BPA has been characterized as an endocrine disruptor capable of acting as a xenoestrogen and an obesogen in a variety of experimental models. Given the sensitivity of the developing hypothalamus to the organizational effects of sex steroids, we hypothesized that early-life BPA exposure adversely affects the development of hypothalamic feeding circuitry in order to bring about an obesity-prone phenotype. To test this, we used male and female CD-1 mice exposed via maternal diet to either a control diet, a diet containing BPA (appx. 13.5 µg/kg/day), or the estrogenic diethylstilbestrol (DES) as a positive control (appx. 3 µg/kg/day) during pregnancy and lactation. Adult male BPA-exposed offspring weaned onto a control diet had impaired glucose tolerance and reduced pro-opiomelanocortin (POMC) innervation into the hypothalamic paraventricular nucleus (PVN) relative to control animals. Adult BPA exposed females became hyperphagic and obese when exposed to a high-fat diet, and showed no evidence of POMC up-regulation in the hypothalamus, nor a drop in body weight in response to exogenous leptin. Serum from pups was collected on PND2, 8, 10, 12, 16, and 21 for analysis of circulating leptin. Results from this study show that BPA and DES exposed pups have respectively delayed and blunted postnatal leptin surges - a state of affairs that points to a role for leptin in the organizational effects of early-life xenoestrogen exposure.

1-E-141 The effects of gonadectomy on hippocampal dendritic spine density and branching in adult male mice**Dallan McMahon¹, Sarah Atwi¹, Craig Bailey¹, Neil MacLusky¹**¹University of Guelph

The effects of androgens on hippocampal morphology and function are poorly understood. Gonadectomy (GDX) induces a small decline in dendritic spine density in CA1 and CA3 in adult male rats, accompanied by an outgrowth and increase in branching of apical dendrites of neurons located in the CA3 pyramidal cell layer. In mice, by contrast, previous work has suggested that androgen may have trophic

effects on hippocampal dendrites, suggesting a possible species difference. To test this hypothesis, we examined the effects of GDX on dendritic branching patterns and dendritic spine density in CA1 and CA3 pyramidal neurons of adult male mice. The brains of CD-1 mice were removed and stained using the Golgi-Cox method 21 days after either GDX or sham-operation. Sholl analysis was used to assess dendritic branching of 3-dimensionally traced pyramidal neurons in 300µm thick coronal sections. These same sections were used to measure dendritic spine density in the proximal, medial, and distal regions of the apical dendrites of CA1 and CA3. Results show a small overall decline in dendritic spine density in CA1 and CA3 following GDX, similar to what has been observed in the rat. However, Sholl analysis revealed a decline in dendritic branching in both CA1 and CA3, without an effect on dendritic length. This result is consistent with previously observed effects of manipulating androgen levels in mice, supporting the view that mice and rats may show very different hippocampal responses to changes in circulating androgen levels. [Supported by NSERC 197293-2007 and CFI 30381]

1-E-142 Infusions of ghrelin into the medial preoptic area inhibits appetitive sexual behaviour and shortens copulatory behaviours in the male rat**Stephanie Rosenbaum¹, Daniel Palacios², Matthew Graham², James Pfau², Alfonso Abizaid¹**¹Carleton University, ²Concordia University

Ghrelin, an orexigenic peptide, has been shown to play an inhibitory role on the hypothalamic pituitary gonadal axis as well as overall reproductive physiology. The role of ghrelin on rodent sexual behaviour, however, has not been investigated. The medial preoptic area (mPOA) has been shown to be an important region in mediating male sexual behaviour by integrating environmental and internal physiological stimuli and has been shown to contain receptors for ghrelin. Interestingly, no definitive role for ghrelin in the mPOA has been elucidated. Here we investigated the role of mPOA ghrelin receptors in mediating male rat sexual behaviours, both appetitive and consummatory. Sexually-experienced male Long-Evans rats were implanted with bilateral cannulae aimed at the mPOA and a week later received micro-infusions of ghrelin (1µg/ul) or saline (1µl) into the mPOA, prior to access to a sexually

receptive female. We observed that ghrelin-infused animals displayed less activity in anticipation to a receptive female, as measured by level changes in the bi-level chamber apparatus ($p < .05$). Further, males receiving ghrelin displayed shorter latencies to their first ejaculation compared to saline animals ($p < .05$). Finally, no significant difference in food intake was seen 1-, 2-, or 24-hours post-testing across all groups, suggesting that ghrelin in the mPOA has no effect on food consumption. Results from the current study suggest that ghrelin acts on the mPOA to inhibit sexual appetitive behaviours and shorten copulatory behaviours without influencing food consumption.

F - Cognition and Behavior

1-F-143 Acute Effects of Nabilone on Sensory Gating in Healthy Participants: A Brain Event-Related Potential Study

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Chronic cannabis use has been shown to produce schizophrenia (SZ)-like cognitive impairments (CI) in predisposed individuals. Evidence has shown that cannabis use suppresses sensory gating, a neuronal level cognitive process which facilitates the pre-attentive filtering out of unimportant/redundant sensory information. Though the precise mechanisms remain unclear, it has been hypothesized that neuroadaptive alterations in cannabinoid receptors, specifically increases in cannabinoid-1 (CB1) receptors, may be precipitated by cannabis use, facilitating the expression of sensory gating related CIs and possibly contributing to the pathology of SZ. An obstacle in clarifying the effects of chronic cannabis consumption is its frequent use in combination with tobacco, thus limiting our ability to relate CIs to the specific use of one drug. The primary goal of this study was to examine the independent effects and interactions between nicotine and the CB1 receptor agonist nabilone. Using the auditory P50 paired-clicks paradigm, sensory gating was analyzed in 20 male non-tobacco/cannabis users using a randomized, double-blind, placebo controlled design. Based on previous findings, nabilone was predicted to show P50 impairments while the nicotine dose produces a

pro-cognitive effect. The results of this study clarify the comorbidity between cannabis/tobacco use and the expression of psychotic symptoms as well as the possible role of cannabinoid-nicotinic receptor interactions in schizophrenia-like CIs.

1-F-144 Investigation of the role of insulin deficiency and loss of PI3K-AKT downstream regulators GSK3 β -CREB signaling in the pathogenesis of diabetic brain

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Canadians are subject to an epidemic of diabetes mellitus (DM). Our lab has developed a robust streptozotocin (STZ)-induced murine model with changes analogous to human diabetic brain, with evidence of brain atrophy, white matter disease, and cognitive decline and we have shown that replacement of insulin in the brain via intranasal delivery prevents DM-mediated neurodegeneration. Insulin is speculated to act through activation of PI3K-Akt signaling. In our proposed work, we examined downstream of PI3K-Akt, looking at phosphorylation of cAMP response element-binding protein (CREB)/ glycogen synthase kinase 3 beta (GSK3 β), critical neuronal signals in the insulin pathway. Intranasal insulin delivery avoids conventional subcutaneous routes to prevent hypoglycaemia at high dose. We also used transgenic models with downregulation of CREB to complement interventions to attempt to rescue the diabetic brain in the murine model of type1 DM. GSK3 β was inhibited by intranasal delivery of TDZD-8. Our model was used to study cognitive behavioral testing (Morris water maze, hole board, radial arm, object recognition), magnetic resonance imaging (MRI) and molecular testing (Western blot) to examine insulin-mediated pathways including CREB and GSK3 β . Knockout of CREB in forebrain blocked insulin's beneficial effect upon previously detected cognitive and MRI measures. Blocking GSK3 β activation had beneficial effects on cognition. Our results suggest that the beneficial effect of insulin on brain cognition and structure results from CREB activation and GSK3 β inactivation. (Supported by CIHR)

1-F-145 The Neurobiology of Adult Attachment

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Attachment style comprises behaviors, affects and cognitions that developed in response to variations of the caregiver's sensitivity to a child's biologically embedded signals for proximity. Adults who are comfortable and confident in close relationships are described as having a "secure" attachment style. Those who fear rejection and yearn for intimacy have an insecure "anxious" attachment style. Those who are uncomfortable with closeness and prefer self-sufficiency have an insecure "avoidant" attachment style. Adult attachment style has long been associated with many aspects of health, behavior and disease, but its neurobiological underpinnings remain poorly understood. In this study I will record resting electroencephalogram (EEG) in adults aged 18-25. Growing evidence shows an association between left frontal asymmetry measured at rest and approach motivation, whereas right frontal asymmetry is associated with withdrawal motivation. I will identify associations between asymmetry level and the attachment style of participants as measured by the Attachment Style Questionnaire. I will use sLORETA to estimate intracerebral electrical sources underlying alpha1 and alpha2 activity recorded at the scalp. I predict that those who are insecurely attached will have neural circuitry that is different from those who are securely attached. Specifically, I predict that relatively low resting-state activity in the left prefrontal cortex will predict avoidant attachment, while relatively high resting-state activity in the left prefrontal cortex will predict anxious attachment.

1-F-146 Genetic deletion of Akt3 in mice altered motor learning

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The serine/threonine protein kinase B (Akt) is suspected to be engaged in the molecular events leading to synaptic plasticity. However, whether it plays a role in motor learning and control is unknown. Here, we investigated the effect of a genetic deletion of Akt3 in the memorization processes associated with a variety of motor behavior tests in mice, such as the accelerating rotarod test for the acquisition of skilled behavior, a pole and stepping tests for sensorimotor functions, and a wire suspension test for motor abilities. We observed a significant reduction in the rotarod performances of Akt3 knockout mice that was reminiscent of impaired

consolidation processes. In fact, Akt3 knockout mice still learned the rotarod task, but to a much slower degree than the WT mice. On the other hand, notably, genetic deletion of Akt3 did not affect the capacity of mice to execute the wire suspension, the pole test as well as the stepping test. We hypothesized that the observed exclusive impairment in rotarod learning could be attributed to a reduction of Akt downstream targets activation. Our results proposed that Akt3 activity in mice was mainly controlling motor learning rather than motor abilities.

1-F-147 Changes in rat inhibitory control and response adjustment with aging and time-out penalty

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The stop task measures inhibitory control by examining the ability to withhold a response to a go stimulus when a stop signal is presented occasionally (Logan & Cowan, 1984). Human responding is slower relative to go trial-only tasks (Akerfelt et al., 2006) and task performance declines with age (Van de Laar et al., 2011). We investigated these aspects of the stop task in rats. We also explored whether post-error slowing observed for rats (Beuk et al., 2014) but not humans (Emeric et al., 2007) could be attributed to the 10-s post-error timeout that only rats received. Male Wistar rats were trained to respond to a visual stimulus by pressing a lever below an illuminated light for food reward, but to countermand the lever press (25% of trials) subsequent to a tone (stop signal) presented after a variable delay. Experiment 1: Rats (N=13) were tested at approximately 6 and 12 mo. of age with intermittent training between tests. Experiment 2: Rats (N=12) were tested for 3 consecutive sessions (counterbalanced) in a stop task with a 10- or 1-s timeout following errors or a go trial-only task. Rats at 12 mo. of age demonstrated slower responding and stopping and less post-error slowing than at 6 mo. Responses were slower for the stop task in comparison to the go trial-only task. Post-error slowing was observed in sessions with a 10-s, but not a 1-s timeout period; response speeding following consecutive go trials was also no longer observed. Thus, rat performance in the stop task declines with age and response times can be adjusted depending on task parameters. (Funded by NSERC)

1-F-148 Neural correlates of temporally and spatially predictive saccades**Benedict Chang¹, Donald Brien¹, Brian Coe¹, Douglas Munoz¹**¹Queen's University

Prediction is the process of using information from either the past or present to guide future behaviour, and is important for compensating for neural delays between sensory and motor outputs. We investigated the behavioural control and the neural correlates involved in temporal and spatial prediction by administering a task in the Magnetic Resonance Imaging scanner that involved both temporally and spatially (un)predictive saccades with 4 conditions: (a) spatially/temporally predictive (STP), (b) temporally predictive/spatially unpredictable (TP), (c) spatially predictive/ temporally unpredictable (SP), and (d) spatially/temporally unpredictable (NON). Participants (n=24; ages=19-25) displayed distinct behavioural differences between conditions. All participants showed primarily predictive saccades (saccadic reaction time (SRT) <100ms) in the STP condition, and elicited primarily reactive saccades (SRT>100ms) in the NON conditions. For the SP condition, SRTs fell between the average SRTs of the STP and the NON conditions. However, no significant difference in SRT was observed between the NON and TP conditions. Functional contrasts of predictive conditions isolating both spatially and temporally predictive areas show bilateral activation of the DLPFC and the PEF, while contrasts that isolate spatially predictive areas show activation of the pre-SMA and insular cortex. Overall, there were clear behavioural changes between select predictive conditions which allowed for the identification of neural correlates of spatial prediction as well as both spatial and temporal prediction.

1-F-149 Validity and application of the modulation index in assessing cross-frequency coupling**Anna Choutova¹, Jean-Philippe Thivierge¹**¹University of Ottawa

Increasing attention has been directed at investigating the functional role of oscillatory synchronisation between different frequency bands, a phenomenon termed cross-frequency coupling (CFC). Studies in this area rely upon accurate quantification of CFC, and require robust, sensitive, and statistically powerful

measures to extract meaningful information from electroencephalographic (EEG) data. Although a number of methods have been devised to measure CFC, no gold standard has been established--each measure provides its strengths and limitations, and must be carefully investigated before valid conclusions about the data can be drawn. In the present study, we evaluate the performance of one common measure of CFC, the modulation index (MI). Using artificial signals, we assess the measure's ability to correctly identify CFC and to discriminate between different strengths of CFC. We then assess the measure's performance on several signal-specific factors and offer theoretical insights into their influence on results. Finally, we relate our findings from artificial signals to resting-state EEG data and discuss the scope of conclusions that can be made between CFC and underlying cognitive processes, given the measure's computational constraints.

1-F-150 Premotor cortical activity reflects value and effort biases during reach decisions**Alexandre Pastor-Bernier¹, Marie-Claude Labonté², Paul Cisek²**¹Cambridge University, ²University of Montreal

The "affordance competition hypothesis" suggests that when the brain decides between actions, they are represented simultaneously and compete against each other within sensorimotor circuits, biased by any factors relevant to the choice. Consistent with this, we have shown that primate dorsal premotor cortex (PMd) can simultaneously represent multiple potential actions modulated by their relative expected value. However, the hypothesis further predicts that neural activity in sensorimotor regions will integrate all factors that influence choices, including the expected costs of actions. We tested this prediction by recording single-unit activity in PMd while monkeys performed delayed center-out reaching tasks with one or two potential targets whose stimulus features indicated the associated reward or the effort that would be needed to obtain that reward. Consistent with our hypothesis, we found that neural activity in PMd increased with value and decreased with effort associated with a cell's preferred target. The interactions between two simultaneous targets were stronger when the targets were further apart, consistent with the hypothesis that the biased competition takes place within a sensorimotor map of action

space. These results support the hypothesis that when deciding between actions, the factors relevant to the choice (reward, effort, estimates of success probability, subjective preferences) all converge to bias a competition evolving within the fronto-parietal sensorimotor circuits that guide the movements themselves.

1-F-151 Metabolic Imbalance, Default Mode Network Activity, White Matter Integrity and Cognitive Outcome in Stroke Patients

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After acute stroke, diaschisis leads to changes in cerebral blood flow, white matter integrity and metabolism in areas connected to the ischemic lesion. Our goal was to investigate whether certain metabolites in the contralesional hemisphere are altered and if these alterations are correlated with resting-state activity, white matter integrity and cognitive outcome. 11 survivors of a single right ischemic stroke and 17 healthy controls were included. At three months after stroke, principal metabolites, fractional anisotropy and resting-state activity values were extracted in the anterior cingulate. Stroke group showed lower levels of Myoinositol (mI), Glycerolphosphocholine Phosphocholine (Cho compounds), and Glutamate Glutamine (Glx) when compared to controls. Levels of mI and Glx were associated with the Rhythms subtest; Cho levels were related with percentage of line cancellation, with Mini mental State Examination (MMSE), and with the Barthel Scale. Functional activity at rest was greater for the stroke group when compared to controls. This greater activity was associated with levels of mI, N-Acetylaspartate (NAAG) and Glx and with the MMSE, TMT-part A and GPT for the stroke group. Stroke group showed less white matter integrity when compared to controls and it was associated with the GPT. Our preliminary data demonstrated persistent neurometabolic, resting-state activity and white matter alterations in the contralesional hemisphere of stroke survivors.

1-F-152 Neural response to social evaluation in previously depressed compared to healthy young women: Differential engagement of dorsal anterior cingulate over time

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Studies have shown that activation of dorsal anterior cingulate (dACC) to negative feedback is associated with feelings of distress and poor health outcomes. However, it remains unclear whether the recruitment of dACC dynamically changes over the course of being exposed to social evaluation in both healthy participants and those at-risk for mental health disorders. Here, healthy controls (HC) and previously depressed (PD) young women received socially evaluative feedback, in two bouts, from a confederate, regarding participants' pre-recorded interview; we examined participants' mood and neural responses. At the beginning, and at a halfway point, of the social evaluative session, participants saw short clips of their interview to remind them of what the confederate is watching and evaluating. Following this session, everyone reported increased feelings of being evaluated ($p < .001$); however, only PDs reported increased negative mood ($p = .05$). We ran a flexible factorial analysis of group x time (first- vs second-half) for negative > positive feedback. We observed that, in the first half of the evaluation, in response to negative > positive feedback, the HC group recruited dACC bilaterally, which then decreased in the second half; the PD group showed the opposite pattern over time ($p = .006$, 111 voxels, FDR small-volume corrected). Impact of the differential timing of recruitment of the dACC between the groups with respect to dACC connections to emotional regulatory neural networks are being assessed; these results will be presented at the conference.

1-F-153 The effect of memory reconsolidation blockade on functional connectivity

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Memory reconsolidation is a process where retrieved memories are re-stabilized. Conversely, propranolol, a β -adrenergic receptor antagonist drug, can attenuate the reconsolidation of emotional memory. Little data; however, is available on this effect in humans. Resting state functional magnetic resonance imaging (rs-fMRI) studies assess inter-regional correlations of brain activity (functional connectivity) and are ideal for studying phenomena across distributed brain regions. Hence, the objective of this study was to exploit novel multivariate functional connectivity methods to explore the distributed effects of propranolol on emotional memory consolidation. Fifty-two healthy individuals were assigned to one of four groups in a two-by-two design. Participants underwent two ten-minute rs-fMRI scans: propranolol vs placebo preceding the first scan and reactivation vs no reactivation was initiated during the second scan. A bootstrap analysis was applied to the data to identify group brain networks that formed the basis of the connectivity analysis. Under propranolol, in contrast with placebo, the cuneus and precuneus showed increased connectivity with the parahippocampal gyrus (PHG), while the precuneus also showed an increase with the superior frontal gyrus and the fusiform gyrus. Reduced connectivity was found between portions of the PHG-amygdala network and the inferior parietal lobule during memory reactivation under propranolol. The interaction between propranolol and reactivation in these regions suggest an important role in mediating emotional memory reconsolidation.

1-F-154 Behavioral anxiolysis without reduction of hippocampal theta frequency after histamine application in the lateral septum of rats

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Hippocampal theta activity is linked to various processes, including locomotion, learning and memory, and defense and affect (i.e., fear and anxiety). Interestingly, all classes of clinically effective anxiolytics, as well as experimental compounds that decrease anxiety in pre-clinical animal models of anxiety, reduce the frequency of hippocampal theta activity elicited by stimulation of the reticular formation in freely behaving or anesthetized animals. In the present experiments, we found that bilateral histamine infusions (0.5 μ g/hemisphere) into the

lateral septum (LS) of rats decreased anxiety-like responses in two models of anxiety, the elevated plus maze and novelty-induced suppression of feeding test. Surprisingly, these same infusions significantly increased hippocampal theta frequency elicited by reticular stimulation in urethane-anesthetized rats. In contrast to these findings, additional experiments showed that the clinically effective anxiolytic buspirone (40 mg/kg, i.p.) reduced theta frequency, confirming previous observations. Taken together, the dissociation of behavioural anxiolysis and theta frequency reduction noted here suggest that hippocampal theta frequency is not a direct index of anxiety levels in rodents. Further, the mechanisms underlying the behavioural and physiological effects elicited by histamine in the lateral septum require further study.

1-F-155 Dopamine "Plasticity" genes- A review of the theory and its potential relevance for understanding overeating and obesity

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The vast majority of genetic association studies, including those focused on obesity, are based on the classic notion that "risk alleles" lead to pathology by altering key biological pathways underlying a given disease state. An alternative approach, informed by Belsky's differential susceptibility hypothesis and the biological sensitivity to context model developed by (Boyce and Ellis, 2005), considers how genetic variation might contribute to one or more disease states by promoting individual differences in environmental susceptibility and developmental plasticity. In this scenario, a given genetic variant would be associated with an increased risk of pathology in negative environments but resilience in enriched ones. The goal of the current paper is to systematically review recent evidence in support of these genetic developmental plasticity models, with a particular emphasis on dopamine system gene variants such as the 7-repeat allele of DRD4 and the DRD2 Taq1 polymorphism. These variants have been associated with both an increased risk for psychopathology in the

presence of early adversity and with resilience in more nurturing environments. After having systematically reviewed the literature on the effect of DRD4 and DRD2 Taq1 polymorphism on eating and obesity that has had thus far an exclusive "risk allele" focus, the paper discusses how these particular gene variants might influence eating related phenotypes and obesity through one or more developmental plasticity effects.

1-F-156 Loss of Stat3 signalling in midbrain dopamine neurons enhances anxiety-like behaviour in female mice

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The adipocyte-derived hormone leptin targets the long form of its receptor (LepRb) in the CNS to produce several behavioural actions compatible with the maintenance of energy balance. Leptin activates a number of LepRb signalling pathways including janus-activated kinase 2 - signal transducer and activator of transcription-3 (Jak2-Stat3), MAPK, PI3-K and AMPK pathways. Jak2-Stat3 constitutes a key pathway by which leptin modulates gene expression. Leptin has anxiolytic effects which have been tied to LepRb signaling on mesolimbic dopamine (DA) neurons that project to the central nucleus of the amygdala (CeA), however, it remains to be resolved which LepRb signalling pathways are involved. To assess the contribution of Stat3 signalling in DA neurons on anxiety-related behaviour we generated a DA-specific Stat3 knockout by crossing dopamine transporter (DAT)Cre mice with Stat3lox/lox mice. Female knockout (KO) mice exhibited normal feeding, locomotion, body weight and behavioural despair (forced swim test) relative to littermate controls. However, KO mice showed increased anxiety-like behaviour in both the elevated plus maze and open field tests as well as increased plasma corticosterone levels. Blocking D1 DA receptors in the CeA by local microinjection of the D1 antagonist SCH23390 reversed the anxiogenic phenotype of KO mice. These results suggest that activation of Stat3 in midbrain DA neurons that project to the CeA mediates the anxiolytic effects of leptin. Supported by a CIHR grant to SF and doctoral awards to MFF from the Canadian Diabetes Association and UdeM

1-F-157 Amyloid-beta regulates memory stability

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Although it is well-established that the peptide amyloid beta (A β) plays a role in the pathogenesis of Alzheimer's disease (AD), there is accumulating evidence of its involvement in normal physiological function, neuronal plasticity, and memory. For instance, A β can be released during neuronal stimulation (Kamenetz et al., 2003, Cirrito et al., 2005) and acute stress (Kang et al., 2007), and may facilitate memory encoding at normal physiological levels (Garcia-Osta & Alberini, 2009). Elevated A β has been observed to enhance both the induction of synaptic depression via NMDA-receptors containing NR2B subunits, and the synaptic exchange of NR2B for NR2A (Kessels et al., 2013). Based on these findings we posited dual functions for endogenous A β in learning and memory. First, it may promote the induction of plasticity after a behavioural experience. Then it may facilitate the subsequent stabilization of this memory during the consolidation process. We report here that several A β -inhibiting treatments had effects on auditory fear memory reconsolidation that were largely consistent with these predictions. Molecular mechanisms mediating these effects (including alteration of NMDA-receptor expression and composition) will be discussed based on the results of several biochemical assays. Together our findings suggest that this process implicated in the onset of AD may initially serve to promote the adaptive stabilization and updating of memories. This may provide one explanation for why several clinical trials of A β -inhibiting drugs have caused worsening AD symptomatology.

1-F-158 Behavioral adverse effect profile of Brivaracetam versus Levetiracetam in the rat kainic acid (KA) model of temporal lobe epilepsy (TLE).

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Background Adverse effects of anticonvulsants in patients are among the main reasons for non-observance of medication. Levetiracetam (LEV), used since the year 2000, targets the synaptic vesicle SV2A in the brain and inhibits voltage-gated Na channels. Some studies report adverse effects such as aggressiveness,

drowsiness and loss of appetite. Brivaracetam (BRV) is a newly developed drug with the same target, but its affinity for the SV2A vesicle is about 10x greater. **Methods** Our current study characterises the adverse effects of LEV and BRV on rat behavior, using the kainic acid (KA) model of temporal lobe epilepsy (TLE). At P60, rats are injected with either KA (12mg/kg) or a saline solution intraperitoneally (i.p.). At P90, they receive an injection of LEV (300mg/kg), BRV (30mg/kg) or saline i.p., one hour before testing. **Behavioral tests include:** Open Field (OP), Elevated Plus Maze (EPM), Fear Conditioning (FC), Forced Swim Test (FST), Morris Water Maze (MWM) and Resident-Intruder (RI) test allowing us to measure anxiety, mobility, depression, spatial memory, social interaction and aggressiveness. **Results:** Our results indicate an increase of aggressiveness in control rats receiving LEV (N=10). We also see a rescue of exploration behavior in the OF of KA rats who receive LEV (N=9) or BRV (n=10) and a rescue of time spent in closed arms in EPM of KA rats who receive LEV (N=9) or BRV (N=10). Our findings confirm the aggressiveness caused by LEV, as seen in previous studies, and suggest that both anti-convulsants modify the anxiety behaviour in epileptic rat

1-F-159 Detection of bottom-up and top-down attentional control mechanisms using a vibro-tactile Brain-Computer Interface (BCI)

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Brain-computer interfaces (BCI) refer to a category of devices that use electrophysiological signals to modulate the activity of a computer. Non-communicative individuals, e.g., those with Disorders of Consciousness (DOC), can benefit from BCIs because these devices may provide a means to communicate without overt movement. BCIs can also provide an assessment of covert cognition not available with traditional behavioural tests. The current work focused on the development of a BCI that can detect bottom-up and top-down attentional control mechanisms. Healthy participants (n=16) directed their attention to infrequent vibrations on their wrists (10% of all stimuli per wrist) while regular vibrations occurred more frequently (80% of all stimuli) on their upper back. In blocks of about 60 s duration, participants counted the number of vibrations on one wrist of interest [explicitly attended] and ignored the infrequent vibrations on their other wrist [implicitly

attended]. Event-related potential markers of attentional control - i.e., the P3a and P3b - could be reliably detected across participants. Crucially for communication purposes, a machine-learning algorithm could reliably predict which wrist was being attended with high accuracy (Trials: M=70.43%; SE=±2.76%; Blocks: M=93.30%, SE=±0.031%). These results are promising for future work with DOC patients in that this BCI will allow for the identification of prognostic brain markers of attentional control, while simultaneously providing a communication device for those patients with top-down attention.

1-F-160 Local Morphology Predicts Functional Organization for the Cognitive Map Task within Parahippocampal Cortex in the Human Brain

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The relationship between local morphological features defining the parahippocampal region of the human brain and functional activation in this region measured during a navigation task with functional magnetic resonance imaging was examined on a subject-by-subject basis in 14 healthy participants. The location of the peaks of functional activation was examined in relation to the morphology of the collateral sulcus as described by Huntgeburth & Petrides (2012). There were three main clusters of activation in the parahippocampal cortex: two posterior and one middle parahippocampal cluster. One of the posterior parahippocampal peaks was located within the posterior portion of the collateral sulcus proper where it merges with the parahippocampal extension of the collateral sulcus. The second posterior peak was located within the parahippocampal extension of the collateral sulcus. The middle parahippocampal activation peak was located where the anterior segment of the collateral sulcus makes way for its posterior segment, when two segments are present. These results illustrate the importance of local morphology and how it may clarify the location of functional activity in the human brain. Functional neuroimaging studies using navigation tasks should take into consideration this differentiation in activation patterns within the parahippocampal cortex.

1-F-161 The effects of beta-amyloid administration in an animal model of diabetes

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Alzheimer's disease (AD) is a progressive neurodegenerative disease affecting primarily the elderly. Although AD research primarily examines the contributions of beta-amyloid accumulation and tau hyperphosphorylation, an additional theory concerning the etiology of AD has been postulated - namely the co-factor model of AD. This theory suggests that multiple combinations of factors, such as stroke, circadian rhythm disruption, stress and diabetes, lead to the development of AD. Indeed, there are many links between diabetes and AD, such that diabetics are more likely to develop AD, and have a worse disease prognosis. There is a complex interplay between diabetes and vascular events as well, such that diabetics are more likely to experience a cerebrovascular event, such as stroke. Stroke itself also acts as a risk factor for AD, as AD patients are at a higher risk for stroke as well as suffer a worse disease prognosis following a cerebrovascular accident. With these risk factors in mind, we used an animal model of obesity and type 2 diabetes, the Zucker obese rat either alone or in combination with an intracerebral infusion of beta-amyloid. Following surgery recovery, all rats were exposed to a stimulus-response version of radial arm maze, Morris water task and discriminative fear-conditioning to context. In addition to these behavioural tasks, fasting blood glucose and blood glucose in response to a glucose challenge were measured. The present study highlights the importance of diabetes in the etiology of AD.

1-F-162 Correlated spiking during spatial working memory in macaque prefrontal area 8r

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Neurons in the primate dorsolateral prefrontal cortex (dlPFC) are known to exhibit selective activity during the delay period of spatial working memory (SWM) tasks. It has been hypothesized that functional interactions between these units

may be involved in SWM maintenance, but whether and how these units interact with each other remains poorly understood. In order to investigate this issue, we recorded responses of multiple single units in dlPFC area 8r of two macaca fascicularis using microelectrode arrays. The task consisted of fixation on a central spot for 494-800ms, presentation of a circular sine wave grating at one of 16 randomly selected locations for 507ms, then offset of the grating followed by a delay period that could last between 494-1500ms, and ended with the offset of the central fixation point, cuing the animals to make a saccade to the remembered stimulus location. We recorded the activity of neurons in blocks of 32 channels and sorted spikes using Plexon software (Plexon Inc, TX). We isolated responses of 199 single units for a total of 1375 neuronal pairs. Neurons were classified as being selective (one-way ANOVA, $p < .05$) for the spatial location of the stimulus during the stimulus presentation period (visually-selective, $n = 128$, or 64%) and the delay period (memory-selective, $n = 145$, or 73%). We then computed spike-rate correlations and found that interactions between neurons in the dlPFC vary based on task epoch and neurons' selectivity.

1-F-163 The loss of a generation: the accelerating decline of hearing acuity among healthy adults

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According to the Canadian Association of Speech-Language Pathologists and Audiologists 20-40% of adults over 65 have a significant hearing problem. However, little is known about the manner in which hearing evolves throughout adulthood. Is hearing loss gradual or sudden? Does the rate of change accelerate after a certain age? This study aims to establish a detailed audiometric portrait of healthy adults. One hundred forty two healthy adults ranging in age from 19 to 93 years old participated in the study. Their pure tone hearing thresholds, speech reception threshold (SRT) and distortion product otoacoustic emissions (DPOAEs) were measured. Mean hearing losses across frequencies were compared to normative values for each age group. Hearing thresholds are significantly affected by age when controlling for gender and educational level ($F(6,133) = 53.54$, $p < .001$), as are DPOAEs amplitudes ($F(6,133) = 13.32$, $p < .001$). SRT increases with age ($r =$

.63, $F(1,140) = 93.71$, $p < .001$). Hearing loss begins around 50 to 59 years old and worsens with age. While higher frequencies are affected first, lower frequencies (including the conversational range) progressively weaken. Adults between 19-29 years old have a mean hearing loss of 18 dB, which is 17 dB worse than normative values published in 1949. In contrast, older adults (60 to 93 years old) exhibit only a 5 dB decline compared to the 1949 data. These results suggest that normal age-related hearing decline has changed over the past fifty years occurring earlier and being more extensive.

1-F-164 Glutamate presynaptic vesicular transporter and postsynaptic receptor levels correlate with spatial memory in aging rat models

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In human, memory capacities are generally affected in aging even in the absence of neurological disorders. Mechanisms underlying cognitive decline are still not well understood. Glutamate is the major excitatory transmitter in the brain and numerous data from the literature suggest major alteration of glutamatergic transmission during normal and pathological aging. Here, we examine whether or not post-synaptic glutamate receptor (GluR) levels and pre-synaptic vesicular glutamate transporters (VGLUTs) expression are affected by aging in 24-month-old Long-Evans rats, with intact (aged-unimpaired, AU) or impaired (AI) spatial memory. AU rats maintained similar levels of hippocampal ionotropic post-synaptic GluR levels as young animals. On the other hand, receptor expression was significantly reduced in AI animals. Conversely, VGLUT1 and VGLUT2 levels were enhanced in AI rats and inversely correlated with individual GluR levels. These data suggest that unbalanced expression of pre- and postsynaptic components is involved in age-related cognitive deficits. The obesity-resistant healthy aging Lou/C/Jall (LOU) rat strain does

not develop memory deficits with aging. Therefore, we investigated the status of glutamatergic receptors and VGLUTs in aging LOU rats. No differences were observed between 24-month-old LOU rats and their young counterparts. Correlation of spatial memory capacities with VGLUTs and postsynaptic GluR in these two rat strains suggests that intact coordination of the glutamatergic neuronal networks appears to be a prerequisite to healthy cognitive aging.

1-F-165 Increasing the speed of cocaine delivery augments the motivation to take the drug and promotes regulation of corticostriatal BDNF and TrkB mRNA

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The rapid delivery of drugs to the brain increases the risk and severity of addiction. We have shown that rats with a history of self-administering rapid cocaine infusions (delivered i.v. over 5 vs. 90 s) later show increased motivation to take the drug. BDNF-mediated signalling in corticostriatal regions mediates the development and expression of addiction-relevant patterns of drug taking. Thus, we hypothesized that the intake of rapid cocaine infusions involves increased regulation of brain-derived neurotrophic factor (BDNF) and TrkB mRNA in corticostriatal nuclei. Rats self-administered cocaine or saline delivered i.v. over 5 or 90 s under a fixed ratio schedule (FR) during nine 6-hour (h) sessions. Next, motivation for cocaine was assessed using a progressive ratio (PR) schedule. Finally, rats were tested under FR for 1h and decapitated 15 minutes later. BDNF and TrkB mRNAs were measured using in situ hybridization. Compared to 90-s rats, 5-s rats consumed more cocaine during FR sessions and showed greater motivation to obtain the drug during PR sessions. In parallel, only 5-s rats showed altered BDNF and TrkB mRNA levels. Relative to 90-s and control rats, 5-s rats had increased BDNF mRNA levels in several cortical regions coupled with decreased TrkB mRNA levels in both cortex and dorsal striatum. Thus, the intake of cocaine under conditions that lead to a rapid rise in brain drug levels facilitates the brain changes that promote excessive motivation to take the drug. The present data suggest that these neural changes could involve increased regulation of BDNF/TrkB.

1-F-166 Linking memory-related firing of prefrontal neurons to the strength of their entrainment to theta oscillations in the rhinal cortex and ventral hippocampus

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The lateral entorhinal cortex (LEC) and medial prefrontal cortex (mPFC) are necessary for the expression of consolidated memory (Takehara-Nishiuchi et al., 2006; Morrissey et al., 2012), and they closely communicate with one another as detected by synchronized theta oscillations (Takehara-Nishiuchi et al., 2011). However, precisely what information is transferred between these regions remains unclear. By examining the phase-locking to afferent oscillations of single neurons encoding specific information, we may reveal the source of this encoding. To this end, we simultaneously recorded single neuron activity in the prelimbic (PrL) mPFC and local field potentials in several afferent cortical regions, including the LEC, perirhinal, secondary visual, and ventral hippocampus while rats formed an association between two different conditioned stimuli (CS) and a single aversive outcome (US). Roughly 25% of PrL neurons exhibited significant phase-locking to theta oscillations in all cortical regions examined, but individual neurons preferentially phase-locked to one of the afferent regions. Neurons that were selective for the CS-US association were more numerous and robustly phase-locked to afferent theta oscillations than neurons that either did not respond to the CS or unselectively responded to the two CS. However, the degree of phase-locking of association selective neurons to the LEC was comparable to the other regions. Thus, the association-selective activity of prefrontal neurons may be shaped by converging inputs from the rhinal, sensory cortex and ventral hippocampus.

1-F-167 A brain network for strategic decision-making

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During competitive interactions, one's actions and their outcomes change dynamically based on the actions of other agents. A mixed-strategy is often used, choosing among actions unpredictably and stochastically, to avoid

exploitation from opponents. We have previously shown that mixed-strategy decision-making (DM) recruits a distributed brain network in humans; however, the role of motor structures remains unknown. The current study used fMRI to dissociate key regions of a strategic network from those involved in controlling specific motor effectors; the eye and hand. A colour-based version of Matching Pennies was played against a dynamic computer opponent that exploited biases in player's responses. Players chose one of two different coloured visual targets, and were rewarded if their choice matched the opponent's. Using a block design, we contrasted brain activation underlying choices made using a saccade with choices made using a button-press; differences in brain patterns should highlight effector-specific processes. Strategic DM, regardless of the effector, was associated with activation of the caudate nucleus (head), dorsolateral prefrontal, anterior cingulate, parietal, insular and orbitofrontal cortices, comprising the core elements of a strategic network. Conversely, we observed effector-specific activation of the frontal eye fields during saccade blocks, and the supplementary motor area (SMA) and pre-SMA during button press blocks. Results suggest that strategic DM activates a common brain network, distinct from regions involved in controlling specific effectors.

1-F-168 Analysis of the sleep - wake cycle in EphA4 knockout mice

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Introduction: Sleep is a vital function that can be disrupted by external and internal factors. Sleep regulation, and regulation of sleep intensity in particular, has been linked to changes in synaptic functioning. EphA4 is a receptor involved in synaptic plasticity that was shown to regulate glutamatergic transmission. Here we investigated the impact of its absence on sleep architecture. Methodology: Vigilance states (wakefulness, non-rapid eye movement [NREM] sleep and REM sleep) duration was quantified using continuous electrocorticography [ECoG] in mice lacking EphA4 (provided by K. Murai and bred on site). Male mice (ten-week-old) from three genotypes (15 wild-type [WT], 16 heterozygous [HET] and 13 homozygous mutants [KO]) were used. ECoG was recorded during 4 days: 2 baseline [BL] days, a 6h sleep deprivation and 42h of recovery. Preliminary

analyses have been performed on the second BL day. Results: We observed a significantly shorter REM sleep duration in KO mice than in WT mice during the 24h period. This observation was also specific to the 12h light period. KO showed significantly longer wake bouts duration compared to WT, which occurred, for the most part, in the 12h light. The duration of NREM sleep bouts was also significantly higher during the 12h light in KO mice. Discussion: These results suggest that EphA4 may regulate sleep as its absence seems to consolidate wake and NREM sleep occurrence, and lessen the duration of REM sleep. Current analyses are evaluating the impact of sleep deprivation and the spectral profile of vigilance states.

1-F-169 Hyperdopaminergia induced by DAT blockade alters synaptic plasticity, learning and memory in the temporal hippocampus.

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Endogenous levels of dopamine (DA) have been shown to modulate activity-dependent synaptic plasticity in the rodent hippocampus (HP). In dopamine transporter (DAT) knock-out mice, a model of persistent hyperdopaminergia, long-term depression (LTD) was impaired at Schaffer's collateral (SC) - CA1 synapses and mice exhibited impaired behavioral flexibility in the Morris water maze (MWM). To investigate further the effect of DA elevation on HP synaptic plasticity, we first tested the effect of the DAT blocker GBR12935 on SC-CA1 synapse LTP and LTD in vitro. Field recordings in CA1 of adult C57BL/6J mice in presence of GBR (30nM) resulted in no impairment of LTP after high frequency stimulation. Conversely, GBR application suppressed paired-pulse low frequency-induced LTD. Then we clarified how high DA levels affect HP dependent memory processes by implanting bilateral canulae in either septal or temporal HP of C57 mice and testing their performance in the MWM following chronic administration of GBR. Infusion of GBR (300nM) in the septal CA1 resulted in no significant impairment in the spatial MWM. Conversely, GBR infusion in the temporal CA1 resulted in a deficit in escape latency and success rate compared to controls. Mice treated with GBR in the temporal HP also exhibited a total deficit in the novel object recognition task. Altogether, this ongoing study reinforces the

belief that a modulation of DAT activity triggers important DA related changes in the mice HP and it should help us to better understand the role of endogenous DA levels in learning and memory processes.

1-F-170 The Role of Cholinergic Tone in Depression

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Major depression is one of the most prevalent psychiatric disorders worldwide. The cholinergic hypothesis of depression suggests that dysregulation of the cholinergic system may contribute to the etiology of major depression. In support of this hypothesis, antagonists of acetylcholine receptors (nAChR) have an antidepressive effect on animals as well as on humans. However, it is not clear which cholinergic nuclei in the brain control depression-like behaviour. We generated three novel mouse lines where the vesicular acetylcholine transporter (VACHT), a protein responsible for synaptic storage and release of ACh, was specifically deleted from the pedunculo pontine tegmental nucleus (PPT), the basal forebrain, or the striatum. We hypothesized that decreased cholinergic signaling from one or more of these cholinergic nuclei will cause an antidepressive-like phenotype, as measured by the tail suspension test (TST) and forced swimming test (FST). Interestingly, mice with a VACHT knockout in the basal forebrain are not different from controls in both the TST or the FST, but shown a resilient phenotype in these tasks after stress. Striatal VACHT knockout mice showed antidepressive-like phenotype in the FST but not in the TST. Consistent with the cholinergic hypothesis of depression, mice with a VACHT knockout in the PPT showed a significant antidepressive-like phenotype in both the FST and the TST, suggesting a role for the PPT cholinergic system in depression. Our experiments provide new insight regarding the role of altered cholinergic signaling in psychiatric disorders.

1-F-171 Role of dopamine signaling in electrotactic swimming behavior of the nematode *Caenorhabditis elegans*

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C. elegans (worm) is an established model organism for the study of the neuronal basis of behavior. The nervous system of worm consists of just 302 neurons and utilizes conserved neurotransmitter signaling such as dopamine (DA). DA modulates a range of behavior in *C. elegans* such as feeding and locomotion. We have recently shown that DA signaling is involved in the electric field-induced swimming behavior (termed "electrotaxis") of worms. In electrotaxis, *C. elegans* moves toward cathode in the presence of a DC electric field. To investigate the role of DA signaling in electrotactic behavior, we used a novel microfluidic device set-up wherein the worm movement is controlled by the electric field. The DA transporter mutant, *dat-1*, exhibits a slow electrotactic swimming speed. However, mutations in *AADC* and *VMAT* genes, *bas-1* and *cat-1*, respectively, cause worms to have faster speed. Furthermore, *bas-1* mutant phenotype is partially rescued by exogenous DA treatment. Similar to mammals, *C. elegans* DA mediates extrasynaptic signaling through DA receptors that are present in different cells including cholinergic motor neurons and muscle cells. Of the two D2-like receptors in worms (*DOP-2* and *DOP-3*), *dop-3* mutants have a faster speed whereas *dop-2* are normal. Thus, *DOP-3* appears to be the primary receptor through which DA acts to inhibit the electrotactic movement. The D1-like receptor mutants, *dop-1* and *dop-4*, exhibit a normal electrotactic response. These results provide first evidence for the role of DA signaling in modulating the electrotactic swimming behavior in worms.

1-F-172 Sex differences in olfactory discrimination learning in mice

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Mice have been shown to perform olfactory based tasks better than tasks requiring the use of other sensory modalities. We assessed sex differences in CD-1 mice using automated olfactometers. Male and female mice were tested on two-odour discrimination and reversal tasks. During the initial training phase male mice learned the task faster than female mice. The males also displayed greater performance than the females on the two-odour discrimination task. No sex differences were seen on the reversal task. Although the mice easily learned the initial discrimination task, both male and

female mice displayed difficulty learning the reversal, making a greater numbers of errors during the reversal than the discrimination task. These results of sex differences on a simple olfactory discrimination task show the importance of testing both sexes of mice when conducting experiments.

1-F-173 Trace eyeblink conditioning depends on neural activity, but not NMDA or muscarinic acetylcholine receptors, in the lateral entorhinal cortex

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The entorhinal cortex is necessary for Pavlovian trace conditioning, which requires animals to associate a neutral conditioned stimulus with a salient unconditioned stimulus across a temporal gap. Yet, it remains unknown what neurotransmitter receptor mechanism supports memory acquisition in the entorhinal cortex. Here, we examined whether NMDA receptors or muscarinic acetylcholine (mACh) receptors in the lateral entorhinal cortex are involved in memory acquisition in trace eyeblink conditioning. We first confirmed that the lateral entorhinal cortex is necessary for acquisition by inactivating this region during acquisition sessions with microinfusions of a GABAA receptor agonist, muscimol. Subsequent experiments kept the same infusion volume and location as the first experiment with muscimol, but infused a NMDA receptor antagonist, APV or mACh receptor antagonist, scopolamine. Contrary to expectations, pharmacological blockade of NMDA or mACh receptors did not have a significant effect on the acquisition or expression of the conditioned response. Thus, trace eyeblink conditioning requires normal activity of the lateral entorhinal cortex; however, it does not depend on local NMDA or mACh receptors. These results highlight a unique role of the lateral entorhinal cortex in trace eyeblink conditioning that does not include NMDA receptor-dependent synaptic plasticity or cholinergic-dependent persistent activity.

1-F-174 The hormone replacement therapy Premarin differentially affects spatial memory and hippocampal plasticity dependent on reproductive experience in middle age female rats.

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Hormonal replacement therapies are commonly prescribed during menopause and have been found to affect cognition, dependent on estrogen type (estradiol v. estrone) and subject age. Reproductive experience has persisting effects on the aging hippocampus, increasing cell proliferation in response to estrogens, altering performance on hippocampus-dependent tasks and varying the sensitivity of the female brain to estrogens long after offspring are weaned. Estrogen treatments may therefore affect brain health in aging females differently depending on reproductive experience and hormonal milieu. The current study aimed to elucidate the effects of reproductive experience and ovarian status on the cognitive and neuroplastic effects of Premarin, an estrone-based therapy, in middle-aged female rats. Primiparous (one time pregnant and mothering) or nulliparous (no reproductive experience) rats underwent ovariectomy or sham ovariectomy at 8 months. Seven months later rats received daily subcutaneous doses of Premarin or vehicle for 21 days. In the final week of hormone administration, rats were tested for hippocampus-dependent learning in the Morris Water Maze. Chronic Premarin administration enhanced performance in nulliparous rats but impaired performance in primiparous rats. Brains are being processed for immediate early gene and neurogenesis markers. Our findings suggest that Premarin has differential effects on the aging primiparous brain which has potential clinical implications for treatment during aging and for neurodegenerative disease. Funded by AD Society of Canada to LAMG.

1-F-175 A longitudinal study of stress-induced hippocampal volume changes in mice that are susceptible or resilient to chronic social defeat

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Hippocampal shrinkage is a commonly found neuroanatomical change in stress-related mood disorders such as depression and PTSD. Since the onset and severity of these disorders are closely related to stressful life events, and as

stress alone could reduce hippocampal volume in animal studies, vulnerability to mood disorders may be related to a susceptibility to stress-induced hippocampal shrinkage. However, a smaller hippocampal volume before stress exposure has also been suggested to confer vulnerability of stressed individuals to PTSD or depression. In this study, we examined the contribution of either innate hippocampal volume differences or hippocampal susceptibility to stress-induced shrinkage to the formation of stress-related psychopathology using longitudinal MRI measurements of hippocampal volume in inbred C57 mice before and after chronic social defeat stress. We found that only half of the stressed mice were susceptible to stress and developed psychopathological behaviors such as social avoidance and the other half were resilient to stress. Before exposure to stress, we observed a positive correlation between hippocampal volume and social avoidance. Exposure to chronic social defeat associated with an increase in the left hippocampal volume in resilient and nonstressed control mice. Intriguingly, this increase in hippocampal volume was not found in susceptible mice. Our findings suggest that both a larger hippocampus before stress exposure and a susceptibility to stress-induced hippocampal volume changes confer vulnerability to psychopathology after chronic stress.

1-F-176 Infusions of Neuropeptide Y into the ventral hippocampus decrease rats' defensive behaviour in the shock-probe burying test of anxiety

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The current study investigated whether Neuropeptide Y (NPY) in the ventral hippocampus contributes to anxiety regulation. Prior research implicates NPY in the regulation of rats' anxiety-related behaviors. The hippocampus is similarly implicated in anxiety and defensive behaviors - specifically, the ventral hippocampus regulates innate defensive behaviors, and contains a high concentration of NPY receptors as well as cells that produce NPY. I explored the role of ventral hippocampal NPY in anxiety by bilaterally implanting cannula guides and infusing either 1.5µg/side of NPY or physiological saline into the rat ventral hippocampus. This was followed by behavioural testing in animal models of anxiety - i.e., the

elevated plus-maze (nNPY = 13 nsal = 17) and the shock-probe burying task (nNPY = 12, nsal = 10). Although NPY-treated rats did not significantly differ in percentage of open arm entries, rats that received an NPY infusion displayed a significant reduction in burying without any drug-related changes in the duration of time spent immobile, the number of contact-induced probe shocks and mean shock reactivity scores. These results underscore the selective actions of intra-ventral hippocampal NPY infusions on defensive burying and support the role of NPY in the ventral hippocampus in regulation of anxiety-like behaviors.

G - Novel Methods and Technology Development

1-G-177 A bright genetically-encoded sensor to determine protein expression at the cell surface in live cells

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The process of signal transduction from the extracellular environment is largely mediated by membrane-bound proteins expressed at the cell surface. This important class of proteins includes ionotropic and metabotropic receptors including the glutamate and cys-loop family of ion channels as well as G-protein coupled receptors (GPCRs). Changes to the extent of surface expression are the basis for a multitude of physiological regulatory mechanisms including synaptic plasticity as well as GPCR desensitization. Here we describe a simple method of quantifying the extent of protein expression at the plasma membrane of individual live cells using a pH-sensitive superfolder GFP. The increased brightness of this genetically-encoded fluorophore coupled with total internal reflection fluorescence (TIRF) microscopy permits for improved detection sensitivity compared with existing biochemical methods. Additionally, this method avoids the pitfalls of inadvertent permeabilization due to fixation when employing immunohistochemical methods. To demonstrate its practicality, we have used this technique to examine differences in surface expression of heteromeric kainate receptor subtypes containing the GluK4 or GluK5 subunits. Additionally, we show how this technique can be used to quickly assess the surface expression profile of various iGluR mutants.

1-G-178 A simple procedure to improve immunohistochemistry co-labelings with BrdU

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5-bromo-2'-deoxyuridine (BrdU) is an analog of the nucleoside thymidine widely used in the study of cell proliferation as dividing cells incorporate BrdU into their DNA during the S-phase of the cellular cycle. Immunohistochemistry (IHC) is then used to label the cells, thereby providing visual evidence of cell division. Binding of the BrdU antibody requires denaturation of the DNA by heat or acid, which can considerably damage the tissue. We found that incubation for 30 minutes in 2N HCl at 37°C was optimal for both DNA denaturation and tissue preservation. However this protocol was not optimal for every antigen we wished to co-localize with BrdU. For example, the EYFP antigen found in NG2Cre/Rosa26S-EYFP transgenic mice, while incorporated into cells, appeared to be degraded by the acid treatment, thus preventing visual optimization with an anti-GFP antibody. While alternatives to BrdU do exist, such methods also present pitfalls which limit their usefulness in the context of proliferation and lineage tracing studies. Sample fixation is believed to greatly condition the denaturation step. We found that incorporating a second post-fixation step (overnight at 4°C in 4% paraformaldehyde-picric acid mixture) following the anti-GFP IHC but prior to HCl treatment and BrdU IHC conserved the fluorescent labellings even after DNA denaturation. This IHC protocol also improved visualization of doublecortin and other antigens of interest in the study of glio- and neurogenesis.

1-G-179 In vivo inhibition of subcutaneous glioma progression by oral 2,5-dihydroxyphenyl sulfonate in rats through reduction of tumoral angiogenesis and apoptosis induction

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Despite the use of multimodal therapies which includes surgery, radio and chemotherapy,

glioblastoma prognosis is very poor. Pathological angiogenesis combined with the aggressive invasion and rapid diffusion into the brain are characteristics of glioblastoma. The considerable evidences pointing to fibroblast growth factor (FGF) as promoter of tumor angiogenesis and progression, awake the scientists interest to look for an effective inhibitor to abolish the pro-angiogenic and anti-apoptotic activities of the FGF in vivo. Recently, the capacity to inhibit FGF-mediated biological actions by 2,5-dihydroxyphenyl sulfonate (DHPS) has been described. The aim of the present study was to evaluate the in vivo efficacy of a derivative of DHPS, 2,5-diacetyloxyphenyl sulfonate (DAPS) to inhibit tumor progression in a rat model of subcutaneous glioma induced by C6 cell implantation in immunocompetent rats. Oral DAPS (200 mg/kg/day) for 10 days resulted in inhibited progression of glioma as demonstrated by the reduction of size (235 ± 48 vs. 111 ± 27 mm³, $p < 0.05$) and weight (346 ± 49 vs. 129 ± 29 mg, $p < 0.01$) of subcutaneous gliomas obtained from DAPS-treated rats. This effect was accompanied by significant inhibition of angiogenesis (73.2 ± 16.5 vs. 39.6 ± 6.3 vessels/mm², $p < 0.05$) and increase of apoptosis (5.5 ± 1.1 vs. $24.4 \pm 3.3\%$, $p < 0.001$) in DAPS-treated tumors. These findings suggest that DAPS displays efficacy in inhibiting glioma progression by reducing angiogenesis and promoting apoptosis. DAPS represents a promising candidate for treatment of glioblastoma and other similar malignancies

1-G-180 Objective kinematic assessment of torticollis using motion sensors

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Visual assessment of cervical dystonia (CD) is challenging due to complex abnormal posturing and superimposed hyperkinetic movements like tremors. Sensor-based kinematic technology can measure multiple movements simultaneously, providing an objective, quantitative measure of dystonic movement. 11 patients were assessed at weeks 0, 6, 16, 22 and 32, receiving BoNT-A injections at week 0, 16 and 32. TWSTRS and UDRS were administered at each visit. Sensors were attached to the surface of the skin over spinal segments C2 and T2, above the right ear and on each shoulder. Sensors measured kinematic movements. Recorded positions included: rest

with eyes open/closed, 90° left/right rotation, and tilt up/down/left/right. Graphical representation was used to display angular deviation from neutral position in lateral, rotational and vertical degrees of freedom (DOF). These objective measures showed the unique positional composition of each patient's torticollis. At natural position, angular tremor amplitudes were plotted in all DOF. Range of motion was captured in all DOF at caput and collis positions. Kinematic measures distinguished dystonic posture, superimposed tremors and dynamic range of motion. Kinematics can quantitatively measure the biomechanics of neck movements in lateral, rotational and up/downward degrees of freedom. Since dystonic posturing, range of motion and superimposed tremor may all contribute to the abnormal neck movement, BoNT-A injections can be better tailored if these movements are separated. Kinematics is able to do this, as visual assessment cannot.

1-G-181 Characterization of a new red genetically encoded calcium indicator optimized for 2-photon imaging

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In recent years, there has been intensive development of calcium ion indicators that can be used to study the activity of neuronal populations in vivo. Much progress has been made in improving the sensitivity of green-emitting Genetically Encoded Calcium Indicators (GECIs) based on the jellyfish green fluorescent protein (GFP), allowing the measurement of calcium transients in specific cell types and even within subcellular compartments. A larger color palette of GECIs to facilitate the study of multiple intermingled and potentially interacting cells would greatly expand the potential applications for GECIs. In the current study, using high-speed multiphoton imaging, we characterize the sensitivity of REX-GECO, a novel red coral-derived GECI with a long Stokes shift that we expressed in the brains of *Xenopus laevis* tadpoles. The 2-photon excitation peak for REX-GECO is 910 nm, coinciding with the maximum excitation of EGFP, making it well suited for simultaneous imaging of both REX-GECO either EGFP or GCaMP. Using GCaMP6s as a gold standard, we electroporated GCaMP6s and REX-GECO

plasmids in tectal neurons and compared their responses to various stimulation intensities. We measured responses to visual stimuli in vivo and to pharmacological and electrical stimulation in an isolated brain preparation. Our results show that although this first variant of REX-GECO has properties that make it a promising red GECI for 2-photon imaging, there are still improvements to be made, regarding its sensitivity and toxicity to neurons. Funded by a CIHR grant to REC.

1-G-182 Neurointensivist as the main providers of care for Tele-stroke transfers: Our 4-year experience.

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Introduction Telestroke networks are crucial to providing early and effective care in areas that lack access to acute specialized stroke care services. Numerous studies have shown variable transfer rates from spoke to the hub facility, but little has been described about the disposition of the telestroke patients once admitted to the hub. **Objective** To retrospectively analyze all the Tele-stroke patients requiring transfer from the peripheral hospitals (spoke) to Oschner Medical Center (hub) from 2009 to 2013 and to determine the proportion of transferred patients requiring Neurocritical Care services based on admitting diagnosis. **Methods** We analyzed the Telestroke program database at Oschner Medical Center from the year 2009 to 2013. The patients were classified into the following diagnosis: ischemic stroke (IS), hemorrhagic stroke (HS), transient ischemic attack (TIA). **Results** The spoke hospitals requested 2060 Oschner Telestroke Program consults with 652 patients (31.65%) transferred to the hub. Of the patients transferred 523 (80.21%) required Neurocritical Care services. Out of these Neurocritical Care patients 425 (81.26%) were diagnosed with IS, 60 (11.47%) with ICH, and 38 (7.27%) were non-stroke diagnosis. **Conclusion** Neurointensivists provided the care to the majority of patients transferred to our facility due to their complexity. With the rapid expansion of tele-stroke centers, the expertise of neurointensivists will be increasingly needed and will translate to a need for more Neurocritical Care beds and trained Neurocritical Care intensivists.

1-G-183 Whole-brain mapping of direct inputs to serotonergic neurons of the dorsal and median raphe nucleus

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The serotonin system is proposed to regulate physiology, behaviour and to underlie mood disorders; nevertheless, circuits controlling serotonergic neurons remain uncharacterized. We therefore generated a comprehensive whole-brain atlas defining the monosynaptic inputs onto forebrain-projecting serotonergic neurons of dorsal versus median raphe based on a genetically-restricted transsynaptic retrograde tracing strategy. Using a genetically modified rabies virus in transgenic mice we have selectively mapped the monosynaptic inputs to serotonin transporter (SERT) expressing neurons. We identified discrete forebrain, midbrain and hindbrain neurons targeting serotonergic neurons and found specific inputs from hypothalamus, cortex, midbrain and basal ganglia, displaying a greater than anticipated complexity and diversity of the cell-type specific connectivity. Our results reinforce the importance of direct inputs to serotonergic neurons from prefrontal cortex and lateral habenula, while uncovering a prominent role for basal ganglia circuits, including inputs from striatum, globus pallidus as well as dopaminergic and non-dopaminergic substantia nigra neurons. Furthermore, we functionally probed selected input circuits by using rabies virus containing channelrhodopsin, thus selectively stimulating input populations to the dorsal raphe nucleus. Our data provide a whole-brain view of the diversity of circuits that directly control the activity of the serotonin system.

1-G-184 Lipid nanoparticle delivery of RNA for loss- and gain-of-function studies in neurons in vitro and in vivo.

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A lipid nanoparticle (LNP) technology was developed to deliver mRNA and siRNA into neurons in vitro and in vivo with high efficiency

and low toxicity: LNPs encapsulating RNA were prepared using the NanoAssemblr. LNPs mimic low-density-lipoproteins (LDL) which are taken up by cells through the LDL-receptor in presence of Apolipoprotein E4 (ApoE4). In vitro - More than 98% of primary neurons (PN) grown +/- astrocyte feeder layer incubated 1h with siRNA-LNP (3.3 µg/mL) showed LNP uptake in presence of ApoE4. PN incubated with 100 ng/mL of PTEN siRNA-LNP (siPTEN-LNP) for 72h showed > 90% knockdown (qPCR, WB). The knockdown sustained for 21 days after treatment (qPCR). Incubation of PN with 100 ng/mL siPTEN-LNP on DIV 6, 9, 13 and 16 showed > 80% knockdown 72h post-treatment (qPCR). PN incubated with 500 ng/mL GFP mRNA-LNP for 72h showed GFP expression (WB). Comparison of Neuro9Kit to contemporary transfection kits showed significantly higher transfection efficiency of 100 ng/mL siPTEN (qPCR). Neuro9Kit showed no toxicity (LDH). In vivo - An intracranial injection of siPTEN-LNP (500 nL at 5 mg/mL) showed > 85% knockdown of in cortical slices 5 days post-injection (WB). Solid-core LNP manufactured using the NanoAssemblr demonstrated rapid uptake by neurons which mediated effective and sustained silencing/expression of a target gene in vitro and in vivo with no detectable toxicity. This technology offers a simple and flexible alternative to viral vectors, electroporation and lipofection for loss- and gain-of-function studies in neural development, injury and degeneration.

H - History, Teaching , Public Awareness and Societal Impacts in Neuroscience

1-H-185 Canadian Media Discourse about Fetal Alcohol Spectrum Disorder

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Fetal Alcohol Spectrum Disorder (FASD), a range of neurodevelopmental disabilities caused by exposure to alcohol in utero, could affect as many as 1 in 100 Canadians. Its actual prevalence remains unknown due to confounds such as inaccessible diagnostic services, the effects of stigma on willingness to report drinking during pregnancy, and uncertainty about subtle alcohol-related neurodevelopmental effects. The Canadian news media has explored some of these challenges; however, given that public

understanding can impact public policy, comprehensive public communication is necessary. In order to identify and address informational gaps or contradictions in media discourse, we have conducted a content analysis of 189 Canadian news articles published between 2003 and 2011 found using the Factiva database. Major themes that emerged included 1) descriptions of FASD (e.g., primary and secondary disabilities, severity); 2) systemic concerns (e.g., inadequate social services, inadequate criminal justice accommodation); 3) medical concerns (e.g., access to - and efficacy of - diagnoses, preventions, treatments); and 4) social concerns (e.g., criminality, stigma, race and class myths). Preliminary results indicate a wide variability in reported safe amounts of alcohol while pregnant and descriptions of FASD. This likely reflects disagreements in the literature itself, where these questions remain heavily debated. Our final results will quantify the kind of information presented to the Canadian public so that we can better gauge public discourse about FASD.

1-H-186 For shame! Stigma against fetal alcohol spectrum disorder: Examining the ethical implications for healthcare practices and policies

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Fetal alcohol spectrum disorder (FASD) is the leading, non-genetic cause of developmental disability in North America. Although stigma is presumed to be an important factor of the experiences of individuals with FASD, we lack specific reflections about stigma or stigma process in this context. We conducted a review of social sciences and biomedical literatures about stigma and mental health, neurodevelopmental disorders, and disability as well as qualitative research about the experiences of individuals with FASD. A deliberative working group of experts (the authors) in the fields of FASD and neuroethics was convened. The following questions were used to guide discussion by the working group about stigma and FASD: Are there elements of stigma associated with FASD that are unique from other illnesses? What elements of stigma deserve special attention from an ethical

standpoint? What ways could stigma be impacted by healthcare or public policy decision-making? The individual with FASD faces complex sources of stigma due to the wide range of disabilities and deficits related to the disorder and the negative associations with their prenatal exposure to alcohol. Stigma experienced by other members of the family, especially the biological mother may also influence and impact the individual with FASD's own experience with stigma. From an ethical perspective, stigma not only undermines respect for the person and their equal access to opportunities, but also leads to important compromises in distributive justice across various health and social systems internationally.

1-H-187 The rising tide of transcranial direct current stimulation (tDCS) in the media and peer review literature

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TDCS has caused excitement in the lay public and academia as a "portable, painless, inexpensive and safe" therapeutic and enhancement device. This paper reports the results of a content analysis of print media coverage and academic papers on tDCS. Four broad areas of focus were identified: therapeutic, enhancement, investigative and technical aspects of tDCS use. The academic literature focused primarily on therapeutic uses of tDCS (N=427; 45%), with less emphasis on investigative uses (N=294; 31%), enhancement uses (N=120; 13%), and technical aspects (N=104; 11%). In contrast, the focus of the print media was on enhancement and therapeutic applications (N=92; 42% each), followed by investigative uses (N=23; 11%) and technical aspects (N=11; 5%). Print media articles focusing on tDCS for enhancement neglected reporting side effects. The headlines in print media were enthusiastic. The rising tide of academic and print media coverage of non-investigative uses of tDCS calls for urgent regulation.

1-H-188 Extending the neuroscience classroom: Approaches to enhance student learning and specific learning outcomes

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Several senior level neuroscience courses in the neuroscience program have undergone significant content and curriculum re-design to enhance transparent, active, student-centered learning. Specifically, the re-design sought to align undergraduate learning outcomes to match post-graduate expectations in neuroscience. These outcomes included independent literature research and critical review, experimental design, enhancing presentation skills and peer evaluation through a variety of online and in-class activities. Purpose: Course and curriculum re-design were intended to enhance the traditional classroom approach to learning. Elements of the re-design included technology-enhanced, extended classrooms and authentic, online assignments. Methodology: Students enrolled in two 3rd and 4th year courses were given different methods of learning outcomes assessments including several online publications, in-class peer reviews of presentations, in addition to classroom teaching. Results: Anonymous post-course surveys showed that students felt that online assignments have a beneficial impact and that peer review of assignments have the potential to become a useful pedagogical method.

1-H-189 The impact of a landmark paper on the concept of free will: Reconsidering the legacy of the Libet EEG experiments

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Neuroscientific findings impact the way in which individuals perceive themselves and others, especially when it comes to morality, responsibility, and self-control. Results generated by social and cognitive neuroscience often appear to generate fundamental insights into the workings of our own minds and, thus, their interpretations garner both academic and public attention. One landmark contribution are the EEG studies conducted by Benjamin Libet. In 1983, Libet reported the results of an experiment in which he was able to measure brain activity (or "readiness potentials") several seconds before participants reported being aware of their intention to act. This result was interpreted as limiting free will. We report the results of a historical meta-analysis of neuroscientific experiments using a Libet-like paradigm (N=40) in which we investigated (1) available scientific evidence that tries to confirm

or refute these claims, and (2) the authors' self-reported metaphysical and societal implications of this work. We found a large degree of variance within this body of literature both in terms of the methodology used and the purpose for conducting the work. Furthermore, despite the fact that the judgments that were measured vary from paper to paper, the results are often combined indiscriminately to refute the existence of free will. We recommend caution in making metaphysical claims based on neuroscience research given their societal impact.

1-H-190 "Everyday ethics" in neurodegenerative conditions: Examining salient challenges in Parkinson's disease research and health care

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Parkinson's disease (PD) is a common neurodegenerative condition that affects approximately 100,000 Canadians and involves progressive impairments in motor movement as well as psychiatric and cognitive co-morbidities. The majority of the ethics literature on PD has been focused on issues related to specific research and innovative interventions such as fetal tissue transplantation, gene transfer, and deep brain stimulation. While important topics, they do not reflect many of the ethical and social challenges that most PD patients will face in research and health care. Given the lack of literature on the "everyday" clinical and research experiences of PD patients, a review of such challenges is warranted. An expanded search of the available ethics and social science literature on the related challenges neurodegenerative populations experience can enable us to identify issues that are of particular relevance to PD. We undertake such a review by examining the current ethics-related literature discussing key challenges neurodegenerative populations and related healthcare providers and researchers face, and discuss how these issues relate to PD. We expect challenges are potentially related to autonomy, capacity, decision-making, communication of information, and obligations of researchers and healthcare providers, and are relevant for responsible conduct of research and the delivery of ethical care to PD patients. By calling attention to these everyday ethical issues, awareness can be enhanced and may contribute to a more patient-centered approach to research and health care.

1-IBRO-191 Duchenne Muscular Dystrophy in Probable Monozygotic Twins: Seventh Report Worldwide.

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Duchenne muscular dystrophy (DMD), an X-linked inherited disorder, is a rare incidence in monozygotic twins. We report a sporadic case of probable monozygotic twin boys (7yrs) clinically diagnosed with DMD. Sociodemographic & clinical data were documented using a standard questionnaire. The delivery of twin 1 and twin 2 was a normal vaginal & Emergency Lower Segment Caesarean Section with birth weights of 2.0 kg and 2.1 kg respectively where as twin 2 was in the special care baby unit for 3 days. Patients were having difficulties in walking & climbing stairs from the age of 4.5 years. Both demonstrated bilateral calf muscle hypertrophy with weakness of proximal muscles of lower & upper limbs, positive Gower's sign, elevated Serum creatinine kinase levels (>9500 U/L), myopathic EMG findings & no echocardiographic evidence of cardiomyopathy. Vignos & Brook scales scored 5 & 1 for functional status of lower & upper extremities respectively. Barthel Index demonstrated variation in the item "climbing stairs" scoring 0 and 5 by twin 1 and twin 2 respectively. Mutation detection in the dystrophin gene by multiplex PCR revealed deletion of exons 08,17 & 19 in twins. We are in the process of zygosity confirmation at the genetic level & analyzing the dystrophin gene mutations by Multiple Ligation Dependent Probe Amplification technique where the results will be discussed in the poster. The rare incidence of DMD in monozygotic twins provides an opportunity to study the extent of environmental, genetic & epigenetic factors that control the different manifestations of the disease

1-IBRO-192 Characterization of Wnt/b-catenin and BMP/Smad signaling pathways in an in vitro model of amyotrophic lateral sclerosis

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Different pathways activated by morphogens of the early embryonic development, such as the Wnt and the Bone Morphogenetic Protein (BMP) ligands, are involved in diverse physiological and pathological conditions of the nervous system, including neurodegeneration. In this work, we have analyzed the endogenous activity of the canonical Wnt/ β -catenin and BMP/Smad-dependent pathways in an in vitro model of amyotrophic lateral sclerosis (ALS), given by motor neuron-like NSC34 cells stably expressing wild-type or G93A mutated forms of human Cu/Zn superoxide dismutase-1 (SOD1). As ALS-derived motor neurons, NSC34 cells expressing mutated hSOD1 show a decreased proliferation rate, are more susceptible to oxidation-induced cell death and display Golgi fragmentation. In addition, they display an impaired ability to induce the expression of the motor neuronal marker Hb9 and, consistently, to morphologically differentiate into a motor neuronal phenotype. Regarding signaling, our data show that the transcriptional activity associated to the Wnt/ β -catenin pathway is decreased, a finding possibly associated to the cytosolic aggregation of β -catenin. In turn, the BMP-dependent phosphorylation of Smad1 and the transcriptional activation of the BMP/Smad pathway is increased in the pathologic model. Together, these findings suggest that Wnt/ β -catenin and the BMP-dependent pathways could play relevant roles in the neurodegeneration of motor neurons in the context of ALS

1-IBRO-193 Intracerebroventricular IGF-I gene therapy for cognitive deficit in the senile rat

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¹INIBIOLP

Ageing plays a key role in the development of neurodegenerative disorders. Although there is a substantial number of studies addressing the changes in the brain of old rats (24 mo), there is scarce information about neurological deficits in very old rats (28 mo or older). The use of neurotrophic factors, such as IGF-I, is emerging as a promising therapeutic tool to prevent neural damage and restore the function of the remaining population of neurons. In order to

gather more information on the age-related changes in very old rats, we undertook a study in 28 month-old female rats. A set of senile rats received an icv stereotaxic injection of either the therapeutic adenovector RAd-IGF-I, expressing the gene for IGF-I (experimental group) or the control adenovector RAd-DsRed2, expressing the gene for the red fluorescent protein. Both groups were tested in the Barnes Maze. Interestingly, the experimental group showed some improvement in spatial memory, whereas the control group displayed a poorer performance. Also, immunohistochemistry studies showed that the experimental group had a higher rate of neurogenesis in the Dentate Gyrus than the control counterparts. Also, the experimental animals showed less astrogliosis in the stratum radiatum of the hippocampus than the control group. These results suggest that short-term IGF-I gene therapy can ameliorate some age-associated functional and morphological deficits in rats and encourage us to undertake a long-term study, in order to assess the preventive capability of IGF-I gene therapy when the treatment is started at an earlier age

1-IBRO-194 Tectal EphA3 guides nasal retinal ganglion cells axons during retinotectal mapping by competing with axonal EphA4 for axonal ephrin-As binding.

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We demonstrated that tectal EphA3 stimulates axon growth of nasal retinal ganglion cells (RGC) toward the caudal tectum preventing them from branching in the rostral tectum. Now we postulated that activation of axonal EphA4 decreases axon growth and tectal EphA3 increases axon growth by reducing EphA4 activation throughout competing with axonal EphA4 for axonal ephrin-As binding. We used retinal explants treated with EphA3, PIPLC

(sheds ephrin-As) or KYL (EphA4 inhibitor). We electroporated retinas in vivo/in vitro with EphA4, KiEphA4 (dominant negative) or GFP. We showed that: -Nasal RGC axons present higher levels of ephrin-As, colocalization of ephrin-A2/EphA4, and EphA4P than temporal RGC axons. -Axonal response to EphA3 is associated to ephrin-A expression and EphA4-P. -The EphA3 and ephrin-A shedding both decrease the degree of EphA4-P. -Removal of axonal ephrin-As and inhibition of ephrin-As-mediated EphA4 signaling recapitulate the effects of EphA3 on RGC axon growth and branching. -In vitro overexpression of EphA4 produces neurons with shorter axons whereas neurons expressing KiEphA4 have longer axons than the control. In vivo overexpression of EphA4 produces nasal RGC axons with terminal zones closer to the rostral tectum than the control. Nasal RGCs expressing KiEphA4 form terminal zones closer to the caudal tectum. These results support the idea of a new molecular mechanism whereby tectal EphA3 increases axon growth toward the caudal tectum and collaborate to inhibit axon branching in the rostral tectum by decreasing ephrin-As-mediated EphA4 forward signaling.

POSTER SESSION 2**A - Development****2-A-1 Status epilepticus-induced precocious expression of KCC2 impairs excitatory synapse formation**

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Febrile seizures affect 5% of children. About 2% will develop atypical febrile seizures with an increased risk of developing epilepsy later in life. The presence of a cerebral malformation predisposes to the development of both atypical febrile seizures and temporal lobe epilepsy. We have established a rat model of dual pathology by combining a cortical freeze Lesion at post-natal day 1 (P1) and Hyperthermia-induced seizure at P10 (LH rats). 86% of LH males develop epilepsy and learning and memory deficits in adulthood. The cotransporter KCC2 is crucial for inhibitory GABA function and its dysregulation has been associated with several diseases. Whether alterations in KCC2 expression before the onset of spontaneous seizures are involved in neural circuit alterations in LH model is unknown. We found increased KCC2 expression exclusively in LH rats, as well as hyperpolarization of the reversal potential of GABA. We also found a significant reduction in spine density and mEPSC amplitude and frequency in CA1 pyramidal neurons. Interestingly, KCC2 has been implicated in spine development; we therefore mimicked KCC2 overexpression in hippocampal slice culture by biolistic transfection and in vivo by in utero electroporation. Both manipulations decreased spine density and maturity. In parallel, we found that shRNA-mediated KCC2 reduction in vivo reduced the time of seizure onset during hyperthermia in LH rats. Determining the role of KCC2 in spine development and seizure onset may help us understand the mechanisms underlying circuit alterations caused by atypical febrile seizure.

2-A-2 ER α immunoreactivity in the rat brain as a consequence of developmental exposure to nicotine

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The teratogenic effects of smoking during pregnancy have been discussed at length in the literature. For this reason, clinicians have been advocating the use of nicotine replacement therapy (NRT) as a safer alternative, despite animal and human studies questioning both its safety and efficacy. The mechanism and long-term impacts of nicotine treatment at doses mimicking NRT remain elusive. Therefore I propose a mechanism involving neuronal-glial interaction dependant upon estradiol homeostasis. By altering estradiol synthesis, this neurosteroid cannot act upon glial cell estrogen receptors, thus negating its ability to protect the brain from reactive astrocytes' proinflammatory response to nicotine, resulting in neuronal damage. Randomly assigned nulliparous female Wistar rats were injected subcutaneously with 1 mg/kg/day of nicotine bitartrate or saline for 2 weeks before mating until weaning (postnatal day 21). One cohort of pups (saline, n=6 and nicotine, n=6) was sacrificed at 26 weeks of age and GFAP (glial fibrillary acidic protein), NeuN (neuronal nuclei) and ER α (estrogen receptor α) immunohistochemistry were performed. Results suggest that low-level nicotine replacement during pregnancy alters programming of later life brain immunoreactivity in the offspring. However, further studies must be performed to further substantiate these findings throughout the lifespan.

2-A-3 Role of GPR55 in the development of the central nervous system.

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¹University of Montreal, ²St. Justine Hospital research center, ³University of Indiana

Guidance molecules, through their receptors expressed at growth cones (GCs), regulate the navigation of retinal ganglion cell (RGC) projections toward the visual thalamic nuclei. In this study, we demonstrate that GPR55 is expressed by the retinothalamic system during development and it regulates GC morphology and axon growth via the MAPK pathway. We observe that the GPR55 agonists LPI and O1602 induce a chemo-attractive effect on the GC, an increase of its size and the number of

filopodia present on the GC. Conversely, the GPR55 antagonist cannabidiol decreases these endpoints and induces chemo-repulsion. When these agents are added to neuronal cultures obtained from GPR55 knockout (GPR55^{-/-}) mouse embryos, no effects on growth cone morphology or on axon growth are observed. Hence, the effects of these pharmacological agents are mediated by GPR55. The MAPK pathway inhibitor CI1040 induces a decrease in the effects of the GPR55 ligands on the axon growth, the GC area and the number of filopodia. In vivo, a single intraocular injection of a GPR55 agonist (LPI) increases branching in the lateral terminal nucleus (LTN) in hamsters. Axon branching was decreased following treatment with cannabidiol. These results suggest that GPR55 modulates the navigation of retinal projections and may play an important role in the development of the neurovisual system. These findings highlight, for the first time, the important role played by the GPR55 in the formation of the central nervous system. A better understanding of the brain wiring mechanisms will lead to potential new therapies.

2-A-4 The molecular chaperone Hsc70 is a modulator of Trio Rac1 GEF activity, critical for netrin-1/DCC-dependent cortical axon outgrowth and guidance

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During development, neurons extend axons towards their associated targets in a mechanism mediated by extracellular guidance cues. Netrin-1 is one such cue that mediates attractive axon guidance through the receptor Deleted in Colorectal Cancer (DCC). Reorganization of the actin cytoskeleton, which underlies axon growth induced by netrin-1, requires the activity of Rho GTPases. The Rac1 guanine nucleotide exchange factor (GEF) Trio is essential for netrin-induced axon outgrowth, but the molecular mechanisms governing Trio activity have remained elusive. Here, we identify the molecular chaperone heat shock cognate protein 70 (Hsc70) as a novel Trio regulator. Hsc70 dynamically co-associated with both Trio and DCC in cortical neuron growth cones. The association of Hsc70 with Trio is accommodated by the N-terminal Sec14-containing and Rac1 GEF domain regions of Trio. Intriguingly,

exogenous expression of Hsc70 potentiated Trio Rac-GEF activity in cultured fibroblasts, while expression of ATPase-deficient Hsc70 (D10N) abolished both Trio Rac1 GEF activity, and netrin-1-induced Rac1 activation. Furthermore, depletion of Hsc70 abolished netrin-1-mediated cortical axon growth and turning responsiveness to a netrin-1 gradient. Taken together, we implicate the chaperone activity of Hsc70 in the regulation of Trio-dependent Rac1 activation in cortical neurons during netrin-1/DCC-mediated axon outgrowth.

2-A-5 The effects of reducing early life estradiol on Morris water maze performance in rats

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Estradiol (E2) is important for the development of the brain, specifically in areas that exhibit estrogen receptors (ER) such as the telencephalon, frontal cortex, amygdala, and hypothalamus. The cortical regions of the limbic area are imperative in development of cognitive skills such as memory and learning. The current study is interested in investigating the effects of reduced E2 levels on behaviour in rats with regard to specific tasks such as the Morris water maze (MWM). The model developed for diminishing E2 levels in the rats is through subcutaneous injections of the aromatase inhibitor formestane (FORM); aromatase is the enzyme responsible for converting androgens to estrogens. Prenatal administration was done through the pregnant dams, with either sesame oil (vehicle) or FORM given for the last 4-5 days of pregnancy. During the first four days of life, neonatal days, pups were treated with either vehicle or FORM, to construct the following treatment groups: prenatal/neonatal: oil/oil, oil/form, form/form, form/oil. Analysis of 12 trials of the MWM task revealed a significant four-way interaction between sex, testing block (a block consists of 4 consecutive trials), prenatal and neonatal treatment on the latency to find the submerged platform - females neonatally treated with FORM showed elevated latency scores in the second testing block of the MWM. Probe trial analysis is ongoing for time spent in each quadrant and thigmotaxis (swimming near the border).

2-A-6 Calcium imaging in the neural stem cell of the adult brain

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Neural stem cell (NSC) persists in the subventricular zone during adulthood. These NSC transit from the quiescent to the proliferative states to produce new neurons for the olfactory bulb. The mechanisms regulating the transition from quiescence state to proliferation states remains unclear. To address this issue we performed calcium imaging in the adult NSC at their different states. To distinguish different cellular populations, we used BrdU label-retaining protocol in GFAP-GFP mice and a proliferative marker Ki67 post hoc immunostaining to differentiate stem cells at their quiescent and proliferative states. Our data reveal that quiescent stem cells display a 2-fold higher frequency of calcium signal compared to proliferative NSC. Next, we aim to elucidate cellular and molecular mechanisms in the NSC leading to the changes in the calcium dynamic. To label NSC, we electroporated CAG-GFP plasmid into the postnatal brain and analyzed GFP-retaining cells in the adult brain. Characterisation of electroporated population in the adult brain reveals that a majority of GFP cells are also immunopositive for GFAP (expressed by NSC and astrocytes) and that among GFP /GFAP cells a substantial proportion is Ki67 and labels with BrdU-retaining protocol. We currently employ the pharmacological approach to dissect the mechanisms underlying different calcium signalling in NSC during their different states. Altogether, our data suggest that the mechanisms regulating the transition from quiescent to proliferative state are calcium dependant.

2-A-7 The translational regulators eIF4E and eIF4E-T form a repressive protein:mRNA complex that determines neural stem cell self-renewal versus differentiation**Guang Yang¹, David Kaplan¹, Freda Miller¹**¹Hospital for Sick Children and University of Toronto

eIF4E is genetically-perturbed in autism, raising the possibility that eIF4E-dependent translational regulation plays a key role in mammalian brain development. Here, we have asked if this is so, focusing upon stem cells of the embryonic murine cortex. We identify a

repressive protein:mRNA complex involving the translational regulator eIF4E and its binding partner eIF4ET that is essential to maintain neural stem cells in an undifferentiated state. This complex is localized to granules, and perturbations of eIF4E, eIF4ET, or the P-body proteins Lsm1 and Rck all cause premature neurogenesis and deplete stem cells. Analysis of the eIF4E-T complex shows that it is highly enriched in mRNAs encoding transcription factors, including many known to drive neurogenesis. Thus, embryonic neural precursors are primed to differentiate by expression of proneurogenic mRNAs, but an eIF4E/eIF4E-T complex sequesters these mRNAs in a repressive granule, thereby inhibiting their translation and maintaining the stem cell state until an appropriate developmental time point.

2-A-8 Anatomical study of the relationship between neurons releasing gonadotrophin hormone and the terminal nerve in neonatal opossum (Monodelphis domestica)**Naussicca Hour¹, Jean - Francois Pflieger¹, Therese Cabana¹**¹Université de Montréal

The newborn opossum is quite immature but crawls, unassisted, from the birth canal to a mother's nipple where it attaches for several weeks. The forebrain of the newborn is very immature; fibers which are probably the terminal nerve are the only ones expressing the 200kDA neurofilament NF200, an indicator of maturation of neuronal fibers. They form a thin fascicle from the olfactory bulbs to the lamina terminalis. It was shown in neonatal opossums that neurons expressing the gonadotropin luteinizing hormone releasing hormone (LHRH) migrate from the olfactory placode to the brain using an unidentified neural pathway. To determine if the NF200 fascicle is associated with the migration of gonadotrophin releasing hormone (GnRH) neurons, we have used NF200 and GnRH1 immunochemistry on forebrain slices from neonatal opossums. Our results reveal NF200 labeled fibers coursing from the nasal septum, along the olfactory bulb near the midline and reaching the hypothalamus. GnRH1 labeled cells were seen along the nasal septum but were more numerous in the olfactory bulbs. The NF200 fascicle clearly overlapped GnRH1 fibers as well as GnRH1 cells between the nasal septum and the olfactory bulbs. At 15 days, the NF200 fascicle is no longer detected and

GnRH1 fibers were observed in the hypothalamus down to the nucleus of the diagonal band. These results suggest that NF200 labeled fibers observed in the forebrain of neonatal opossums are the terminal nerve which serves as substrate for the migration of GnRH1 cells at least to the olfactory bulbs and of GnRH1 fibers to the hypothalamus.

2-A-9 14-3-3 proteins regulate commissural neuron responses to netrin

Andrew Kaplan¹, Ricardo Alchini¹, Christopher Kent¹, Timothy Kennedy¹, Alyson Fournier¹

¹McGill University

The ability of neurons to interpret a wide array of environmental cues as they project axons to their targets is critical to establish correct neuronal connections during development. We previously identified 14-3-3 proteins, a family of phospho-serine and -threonine binding adaptor proteins, as major constituents of the growth cone that are important for axon guidance in vitro and in vivo, in part through regulation of protein kinase A (PKA). Here, we show that inhibition of 14-3-3s with the R18 peptide abolishes netrin-dependent attraction of pre-crossing spinal commissural axons in the Dunn Chamber turning assay and blocks netrin-induced growth cone expansion. Analysis of cell signaling reveals that R18 blocks netrin-induced engagement of pathways that are crucial for axon attraction. Evaluation of DCC dimerization reveals that R18 impairs netrin-induced receptor dimerization, suggesting that 14-3-3 proteins may function to facilitate DCC dimerization, thereby allowing for appropriate signal transduction. In a co-immunoprecipitation screen for 14-3-3 isoforms that interact with DCC, we show that 14-3-3zeta, specifically co-precipitates with DCC in a netrin-dependent manner. Using a GST pull-down assay, we further show that this interaction is direct. We therefore suggest a novel role for 14-3-3 proteins in modulating signal transduction by regulating receptor dimerization. Future work will continue to address the role of 14-3-3zeta association with DCC in promoting attractive responses to netrin.

2-A-10 Neuronal activity drives vascular network formation in layer IV of the mouse somatosensory cortex

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In the central nervous system, the substrate for interactions between vascular and neuronal modules is known as the "neurovascular unit". However, the basic principles orchestrating the development and plasticity of the neurovascular unit remain to be elucidated. To address this, we developed an approach integrating mouse genetics, 3D imaging and computational analysis. First, we characterized the formation of vascular networks in the developing somatosensory (S1) cortex. We constructed a compound transgenic mouse to simultaneously visualize both neuronal and vascular components and identified two phases of vascular maturation in layer IV of the S1 cortex: i) an early growth period starting at birth with a peak of complexity at postnatal day 14 (P14), ii) a subsequent remodeling period where vascular complexity decreases and then stabilizes. Then, using a loss of function approach, we investigated whether the vascular maturation observed in the S1 cortex is driven by neuronal activity. We tested the effects of various sensory deprivation paradigms, including follicular lesions and whiskers removal, which all resulted in a reduction of vascular complexity in layer IV of the barrel cortex, thus identifying a direct role of neuronal activity in vascular development. Finally, using a mutant mouse in which neurotransmitter release is dramatically reduced at thalamocortical synapses, we further demonstrated genetically that neuronal activity drives vascular maturation. These results deepen our understanding of neurovascular development and plasticity.

2-A-11 Spinal Neuron Identity and Survival in the Absence of Neurosecretion

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Spinal cord development is critically dependent upon intercellular communication via the regulated secretion of morphogens, neurotrophins and neurotransmitters. Electrical activity influences neural circuit development by affecting gene transcription, neural tube patterning and axon guidance, though

understanding of these effects has been hampered by the imprecise nature of pharmacologically-induced electrical activity manipulations. The Munc18-1 protein is critical to neurosecretion and regulates docking of vesicles containing neurotrophins or neurotransmitters. We examined development of the spinal cord of mice lacking Munc18-1 to probe the role of patterned activity and neurally-derived neurotrophin secretion in spinal neuron patterning and survival. Mice lacking Munc18-1 lack patterned activity, though numbers of molecularly-defined motor- and interneurons are normal, demonstrating that patterned electrical activity is not critical for neuronal specification. However, in Munc18-1 mutants there is an increase in cell death both in vivo and in vitro. As we also observe a dysregulation of neurotrophin receptor localisation, we hypothesise that the observed apoptosis is due to inhibited neurotrophin signalling. When Munc18 is ablated only in motor neurons, they are spared from apoptosis at E13.5, suggesting that it is a non-cell autonomous phenotype. Given that Munc18 mutants have a normal response to peripheral limb-derived neurotrophins, our hypothesis is that there is a remarkable dependence of motor neurons and interneurons on central neurotrophic signals.

2-A-12 The Transcriptional Co-Regulator Cited2 functions at Two Distinct Stages of Precise Neocortical Callosal Projection Neuron Development

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The neocortex contains thousands of distinct types of precisely connected neurons, allowing it to perform remarkably complex tasks of high-level cognition. The broad population of interhemispheric callosal projection neurons (CPN) plays a key role in complex associative cognition, connecting the cerebral hemispheres via the corpus callosum (CC), and integrating cortical information. Disruptions in CPN / CC development are correlated with cognitive and behavioural deficits in multiple neurodevelopmental disorders, including agenesis of the CC, autism spectrum disorders, and schizophrenia. Currently, however, little is known about the molecular development and heterogeneity of CPN, and even less is known about subpopulations of CPN with distinct, and likely important associative functions. We have identified that the transcriptional co-regulator

Cited2 regulates two distinct stages of precise CPN development in mouse: Cited2 first functions broadly in embryonic basal progenitors of the SVZ to regulate generation of superficial layer CPN throughout the neocortex; and, next, functions in an areally-restricted manner postnatally to refine the distinct identity and development of somatosensory CPN. This identification of a postnatal disruption in a specific areal subpopulation of CPN with distinct connectivity and function, following a global reduction in progenitors, highlights not only the diversity of CPN, but also the fine precision and refinement necessary for the functional development of this population of neurons that are centrally-implicated in high-level cognition.

2-A-13 High-Fat Diet-Induced Obesity Disrupts Hippocampal Synaptic Plasticity in both Female Rats and their Offspring

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¹University of Waterloo

The notable increase in obesity prevalence has been accompanied by a rise in the number of women of reproductive age who are either overweight, or obese. Maternal obesity can place offspring at a higher risk of developing metabolic disease, and mounting evidence suggests that brain development may also be disrupted. As a result, the current study sought to investigate the effects of diet-induced maternal obesity on development of the hippocampus (an area important for learning and memory). Female rats were fed either a control-diet (CD), or a high-fat diet (HFD) for 16 weeks, and various biometric measures were taken to establish an obese phenotype. Synaptic plasticity (long-term potentiation; LTP) was examined in the hippocampal CA1 subfield by recording field potentials from acutely prepared slices. In addition, a subset of animals were bred, and kept on their respective diets throughout gestation and lactation. Pups were weaned onto the CD, and sacrificed at young-adulthood to permit biometric and electrophysiological analyses. The HFD induced an obese phenotype in the maternal generation, but did not affect offspring body weight, or glucose metabolism. In each generation, baseline synaptic transmission was unaffected, but a reduction in the magnitude of LTP was observed in the HFD-fed dams, as well as in both male and female offspring. Taken together, our data confirm that a high-fat diet can affect plasticity within the maternal generation, and illustrate that maternal obesity can alter the

development of cellular processes responsible for hippocampal plasticity.

2-A-14 The effects of environmental enrichment on transcriptional regulation in the hippocampus are associated with early life maternal care in rats.

Carine Parent¹, Xianglan Wen¹, Josie Diorio¹, Michael Meaney¹, Tieyuan Zhang¹

¹Douglas Mental Health University Institute

Maternal care leads to individual differences in physiology and behavior within rat offspring. The offspring of High-licking/Grooming (High-LG) mothers show increased learning in the Morris Water Maze (MWM) and decreased corticosterone responses to acute stress. Environmental enrichment (EE) rescues learning abilities on the MWM in low-LG offspring but does not ameliorate performance in High-LG offspring. We used a genome-wide array to characterize the transcriptional changes associated with EE in the hippocampus of High- and Low-LG offspring. We performed microarray analyses using Affymetrix Gene 1.0 ST to examine whole genome hippocampal transcriptional regulation in adult High- and Low-LG offspring exposed to standard housing or EE. Ingenuity IPA software analysis revealed that variations in maternal care differentially regulated gene expression. In High-LG offspring, EE regulated genes related to endocrine system disorders, cell-to-cell signaling and hematological system development. In Low-LG offspring, EE regulated genes related to metabolic disease, cell signaling and connective tissue development. Variations in maternal care regulated gene expression within the central nervous system of offspring. In high-LG offspring, EE influenced inflammatory and immune functions and affected the expression of genes in a network regulated by NFκB. In Low-LG offspring, EE influenced metabolic and endocrine function and affected the expression of genes in a network regulated by TNF. Thus, the effects of EE are differentially influenced by variations in early life maternal care.

2-A-15 Defining a novel subset of mesencephalic derived cerebellar nuclei neurons

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The cerebellum functions in motor coordination and also implicated in non-motor behaviors including emotion and cognition. The basic circuitry of the cerebellum is well understood. Purkinje cells (Pcs) are the sole output of the cerebellar cortex and they project to the cerebellar nuclei (CN). The CN provide the main output of the cerebellum. During cerebellar development the CN neurons and Pcs are the earliest born among the different neuronal subtypes. However, they are generated from two distinct germinal zones: the ventrally located ventricular zone, which produces Pcs and the dorsally located rhombic lip, which produces large CN neurons. This study utilized whole mount/section IHC, and primary dissociated cerebellar and embryonic cultures to examine the origin and organization of a new subset of CN neurons. The results showed that a subset of CN neurons, which are immunopositive for SNCA, originate from the mesencephalon and migrate to the rostral end of nuclear transitory zone. SNCA and p75 neurotrophin receptor double immunostaining suggests that these cells are derived from the neural crest and they form a combination of neurons and nerve fibers that terminate near the pial surface of lobules VI/VII. Interestingly, the SNCA /p75 cells divide the cerebellar primordium into rostradorsal and caudoventral compartments, which correspond to the mature anterior and posterior cerebellum. The position and direction of mesencephalic derived early CN neurons suggest an important role as an intrinsic organizer that divides the cerebellum into anterior and posterior regions.

2-A-16 Medial preoptic morphology in the lactating rat and the effects of pCREB

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The medial preoptic area (mPOA) is an important brain region involved in mediating maternal behavior. In this study, we examined the expression of phosphorylated cyclic adenosine monophosphate responsive element-binding protein (pCREB) in the mPOA of lactating rat dams at 5 days post parturition and mPOA neuronal morphology at 10 days post parturition. We found significantly higher levels of pCREB immunoreactive cells in the mPOA of high-licking/grooming (LG) mothers compared to low-LG mothers after an LG bout on post parturition day 5. Morphological analysis using Golgi staining also revealed significant

differences in the mPOA between high-LG and low-LG dams at 10 days post parturition. Low-LG mothers displayed a significant increase in number of neurons with greater dendritic complexity in the mPOA compared to high-LG mothers. A significant negative correlation was found between the number of neurons with greater dendritic complexity, and dam's LG and arched-back nursing scores. We also found that over-expressing CREB in mPOA cell cultures significantly reduced the number of more dendritically complex neurons present. These findings suggest that the pruning of neurons in the mPOA through CREB expression may be associated with the morphological differences we observed in the mPOA and may in part account for individual differences observed in the maternal behavior of these dams.

2-A-17 Brain-derived neurotrophic factor (BDNF) in the nucleus accumbens mediates individual differences in behavioral responses to a natural, social reward

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BDNF regulates behavioral responses to psychostimulants as well as natural rewards. Oxytocin is critical for maternal behavior and facilitates behavioral sensitization to psychostimulants. Since the neural systems that mediate the expression of maternal behavior in mammals overlap considerably with the reward circuitry, we wondered whether oxytocin might act to regulate BDNF expression, and whether such effects would in turn mediate the expression of maternal behavior. In situ hybridization and qRT PCR results revealed increased BDNF mRNA in the medial prefrontal cortex (mPFC), hippocampus, and ventral tegmental area (VTA) of high LG mothers compared to low LG mothers on post partum day 4 (PP4). ELISA and western blot results revealed increased BDNF and phospho-TrkB expression in the nAcc of high LG dams compared to low LG dams on PP4. In order to examine the effect of mesolimbic BDNF on maternal behavior, we immunoneutralized BDNF using bilateral anti-BDNF infusions in the nAcc and were able to eliminate the differences in pup LG between high and low LG mothers. In addition, we bilaterally infused an oxytocin antagonist (OTA) into the ventral subiculum of PP4 dams, the major input of the hippocampus

to the nAcc and an area rich in oxytocin receptors, and found that OTA infusions significantly reduce maternal LG. Finally, we used hippocampal cell cultures to show that oxytocin increases BDNF expression through MAP kinase signaling. We suggest that oxytocin-induced regulation of BDNF provides a neuroendocrine basis for individual differences in maternal behavior.

2-A-18 Exposure to water immersion stress in mid-adolescence increases risk-taking behaviour and sensitivity to amphetamine in adult rats.

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We previously found that exposing rats to intermittent physical stress (IPS) during mid-adolescence increased their exploration of the normally avoided open-arms of the elevated plus-maze (Wilkin et al., 2012). Others have linked individual differences in rats' open-arm exploration with individual differences in sensitivity to drugs of abuse, such as amphetamine (e.g., Watt et al., 2009). Thus, the primary goal of the current study was to determine whether mid-adolescent stress-induced increases in open-arm exploration are associated with increases in sensitivity to amphetamine in adulthood. The secondary goal was to examine the generality of our prior findings by using a modified stress regimen. In the current experiment, male rats were randomly exposed to a 45 min water immersion, 6 times randomly, across the mid-adolescent (PD35-46) period. The rats were later tested in adulthood (~PD72), using the EPM and amphetamine-induced locomotion tests. In line with our previous work, we observed trend-like increases in open-arm activity in adult rats, previously exposed to water immersion stress in mid-adolescence, relative to age-matched no-stress controls. Additionally, the mid-adolescent stress rats also displayed increases in locomotor activity, in response to low dose (1mg/kg) amphetamine, in adulthood. Together these results suggest that stress during the mid-adolescent period might increase an organism's propensity to engage in risk-taking behaviour as well as increase their sensitivity to psychostimulants in adulthood.

2-A-19 Nemo kinase modulates bmp signaling in synaptic growth

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Retrograde Bone Morphogenetic Protein signaling is essential for the coordinated growth of neuromuscular junction synapses during *Drosophila* larval development: postsynaptic release of a BMP ligand triggers a retrograde cascade, which culminates in the phosphorylation and nuclear translocation of the transcription factor Mad in presynaptic motor neurons. Previously, we reported that Nemo (Nmo) kinase phosphorylates Mad at a site distinct from that phosphorylated by BMP receptors, thereby modulating the ability of Mad to accumulate in the nucleus. This modulation influences synaptic structural growth but not function and neurotransmitter release. We find that overactivation of Nmo affects synaptic function but not structure. Combining fly genetics, electrophysiology and imaging, we set out to investigate how these two regulatory actions of BMP signaling are alternatively regulated by Nmo. Our live imaging analysis suggest that activation of Nmo reduces the residency of nuclear Mad, most likely through an increase in the rate of nuclear export of Mad. This accelerated nuclear export is correlated with a significant reduction in synaptic release, which can be restored when a non-phosphorylatable Mad transgene is expressed in motor neurons. Interestingly, quantitative PCR as well as in vivo reporter assays suggest that transcription of Nmo mRNA is regulated by BMP signaling in motor neurons. We propose a model in which BMP signaling self-regulates its action in motor neurons through transcriptional regulation of its own modulator, ensuring normal synaptic growth and function.

B - Neural Excitability, Synapses, and Glia: Cellular Mechanisms**2-B-20 GABAergic transmission in cerebellar granule cells is regulated through mitochondrial metabolism****Michael Accardi¹, Beverley Orser², Derek Bowie¹**¹McGill University, ²University of Toronto

Reactive oxygen species (ROS) are important signaling molecules in the CNS found to play both physiological and pathophysiological roles. ROS are particularly important in the context of synaptic function since recent evidence has demonstrated that mitochondrial-derived ROS (mROS) can regulate the strength of GABAergic

synapses in cerebellar stellate cells. However, it remains unclear whether this regulation is specific to all GABAergic synapses. Thus, we set out to address whether mROS can influence GABAergic neurotransmission in another cell type, specifically, cerebellar granule cells. To do this we used whole cell patch-clamp electrophysiology and a mitochondrial poison (e.g. antimycin-A) to elevate mROS. Similar to stellate cells, mROS increased the frequency of miniature inhibitory postsynaptic currents (mIPSCs) in granule cells in a time dependent manner which was attenuated in the presence of an antioxidant, N-acetylcysteine. Interestingly, there was a gradual reduction in tonic current over time in the presence of antimycin-A. The presence of the δ -subunit was essential for the increase in mIPSC frequency since δ -KO mice did not possess an mROS-induced time-dependent mIPSC frequency increase. Taken together, our data indicates that mROS plays a more global regulatory role at several GABAergic synapses since, like stellate cells, granule cells possess GABAergic synapses which are modulated in a subunit-specific manner via mROS.

2-B-21 EphA7 in adult rodent brain: regional distribution and ultrastructural localization in hippocampus and cerebellum**Akofa Amegandjin¹, Wafaa Jammow¹, Sylvie Laforest², Mustapha Riad¹, Moogeh Baharnoori¹, Frédérique Badeaux¹, Luc DesGroseillers¹, Elena Pasquale³, Guy Drolet², Guy Doucet¹**¹Université de Montréal, ²Centre de recherche du CHU de Québec, Université Laval, ³Sanford Burnham Medical Research Institute

EphA7 is widely expressed in CNS during embryogenesis. We mapped its distribution in adult rat and mouse CNS using in situ hybridization (ISH), immunohistochemistry (IHC) and electron microscopy (EM). By ISH, the strongest signal was observed in the hippocampal pyramidal and granule cell layers. Moderate levels were detected in the habenula, striatum, amygdala, the cingulate, piriform and entorhinal cortex, and in the cerebellum, notably Purkinje cells. By IHC, the general distribution was consistent with ISH results, considering the transport of the EphA7 protein to neuronal processes, as exemplified in the strongly labeled neuropil layers and weakly stained pyramidal layers of hippocampus. In contrast, in cerebellum, the protein remained in the Purkinje cell bodies. In EM, EphA7 was localized

essentially in dendritic spines and astrocyte processes, often perisynaptic, in hippocampus. Immunopositive axon terminals were rarely observed. In the cerebellum, EphA7 immunoreactivity was prominent in Purkinje cell somata, with much weaker staining in the granule cell layer. Ultrastructural examination showed EphA7 mostly intracellularly, associated with vesicles, within Purkinje cell somata. In the granule cell layer, the immunolabel was detected in mainly axons, some axon terminals, and in dendrites. The preferential localization of EphA7 in dendritic spines and perisynaptic astrocytic processes of the hippocampus is consistent with a role in synaptic plasticity. Further examination of cerebellar deep nuclei should help clarify its potential roles in this region.

2-B-22 Brain hemodynamic response to somatosensory stimulation in Neuroligin-1 knockout mice

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Introduction: Neuroligin 1 (NLG1) is a postsynaptic adhesion molecule that determines N-methyl-D-aspartate receptor (NMDAR) function and localization. Our recent work showed that Nlg1 knockout (KO) mice cannot sustain neuronal activity occurring during wakefulness for a prolonged period. Since neuronal activity (and NMDAR activity) drives a vascular response, we used multispectral optical imaging to determine if the observed sleep/wake modifications were associated with changes in the cerebrovascular response to neuronal stimulation in Nlg1 mutant mice. Methodology: Wild-type (WT), heterozygous (HET) and homozygous Nlg1 KO male mice (B6; 129-Nlgn1^{tm1Bros}/J) were studied. The brain hemodynamic response was recorded (12 bit CCD camera), under deep ketamine/xylazine anesthesia following stimulation of the left forepaw with two different intensities (twofold- and fourfold-threshold). Results: Nlg1 KO mice showed a 10% lower response rate to twofold-threshold stimulation compared to both WT and HET on both the ipsi- and contralateral sides of

the somatosensory cortex but no genotype differences existed for the response to fourfold-threshold. Preliminary analyses revealed that KO mice tend to show a reduced contralateral oxyhemoglobin increase after stimulation compared to WT and HET, but no differences for deoxyhemoglobin were observed. Discussion: These preliminary results may suggest that NLG1 is involved in the cerebrovascular response to neuronal activity. Current analyses are being performed on specific response parameters (e.g., amplitude, peak time, response duration).

2-B-23 GABAA receptor antagonist promotes oligodendrocyte precursor cell proliferation in adult mice

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Oligodendrocyte progenitor cells (OPCs) give rise to myelinating oligodendrocytes during embryogenesis and early stages of post-natal life. A large number of OPCs maintain their undifferentiated state after these initial developmental stages making them abundant in the adult brain. While their proliferative activity does decline with age, they continue to undergo cell division throughout adulthood, representing the most active population of cycling cells within the adult brain. Some studies have also shown that they may be pluripotent in later stages of life but little is known about the conditions promoting OPC proliferation and differentiation in the healthy adult brain. OPCs contain receptors for both glutamate and GABA which, when activated, induce depolarization of their membrane. We examined if activity-induced changes in OPC dynamics could be modulated through synaptic transmission. We measured the effects of GABAergic agonists and antagonists on OPC proliferation and differentiation in vivo. Muscimol (GABAA receptor agonist), baclofen (GABAB receptor agonist), picrotoxin (GABAA receptor antagonist), and saclofen (GABAB receptor antagonist) were administered acutely to NG2Cre/R26S-EYFP mice to study OPC proliferation and also over 6 days to study OPC differentiation. Preliminary results suggest that GABAA receptor antagonist picrotoxin promotes OPC proliferation in healthy adult rodents. Supported by NSERC

2-B-24 Ion channel regulation and internal calcium flux of retinal horizontal

cells under hypoxic conditions in the goldfish (*Carassius auratus*)

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Mammals are intolerant to prolonged periods of hypoxia or oxygen-glucose deprivation (OGD) whereas some ectotherms like goldfish (*Carassius auratus*), can survive weeks under anoxic conditions. Neurons are especially susceptible to anoxic damage from pathologically disrupted ion regulation due to reduced energy availability; Therefore protective strategies that minimise anaerobic energy demand are key. Of these, "channel arrest", or the down-regulation of ion channel abundance or function is critical to anoxic survival. Glutamate receptors (GluRs), major contributors to excitotoxicity, undergo channel arrest in the anoxic turtle brain, and channel arrest has also been demonstrated in the goldfish brain. Recently, hemichannels too have been implicated in the generation of OGD-mediated damage in mammals. The retina serves as a model of sensory physiology as well as of central nervous system circuitry. Horizontal cells (HCs) are second order neurons of the retina which are chronically depolarised by photoreceptor-released glutamate under light conditions, and utilise GluRs and hemichannels to mediate formation of the visual field. Patch-clamp electrophysiology and calcium imaging techniques can be used to examine the activity of HC ion channels and induced changes in intracellular calcium. Preliminary results indicate that glutamate-elicited increases in intracellular calcium are reduced under conditions of OGD. Further study will elucidate if goldfish HCs retain a neuroprotective mechanism to preserve retinal function during low oxygen conditions.

2-B-25 Interplay between synchronization of multivesicular release and recruitment of additional release sites support short-term facilitation at hippocampal mossy fiber to CA3 pyramidal cells synapses

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Synaptic short-term plasticity is a key regulator of neuronal communication and is controlled via various mechanisms. A well-demonstrated property of mossy fiber (MF) to CA3 pyramidal cell synapses is the extensive short-term

facilitation they demonstrate during high frequency bursts. We addressed the mechanisms governing facilitation using whole-cell electrophysiological recordings, minimal stimulation and two-photon microscopy in acute hippocampal slices. Two presynaptic mechanisms were involved in short-term facilitation, with their relative contribution dependent on extracellular calcium concentration. First, synchronization of multivesicular release was observed during trains of EPSCs recorded in 1.2 mM Ca²⁺. Indeed, covariance analysis revealed an augmentation in quantal size during trains of EPSCs and a low-affinity glutamate receptor antagonist showed an increase in cleft glutamate concentration during paired-pulsed stimulation. Whereas synchronization of multivesicular release contributed to the facilitation in 1.2 mM Ca²⁺, variance-mean analysis showed that recruitment of more release sites (N) was likely to account for the facilitation observed in 2.5 mM Ca²⁺. Furthermore, this increase in N could be promoted by calcium microdomains of heterogeneous sizes observed in single MF boutons. Overall, our findings reveal that the combination of multivesicular release synchronization and the recruitment of additional release sites supported by the spatial profile of calcium elevations in boutons expands the dynamic range over which MF reliably transmit information.

2-B-26 Imaging synaptic vesicle tethers at the presynaptic terminal

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Neurotransmitter is released from presynaptic terminals by calcium-gated fusion and discharge of synaptic vesicles (SVs) at the active zone (AZ). Based on single-channel gated fusion we predicted that SVs are attached to the calcium channels by a molecular tether (Stanley 1993). Recent studies have implicated a link via the channel C terminal (Kaesler et al 2011, Wong et al. 2013). Previous studies have imaged structures that link SVs to the AZ but these methods required complex or analysis-intensive processing. We reasoned that tethered SVs might be visible if unrelated contents of the terminal could be removed. CNS synaptosomes (isolated presynaptic terminals) were ruptured by osmotic shock; a standard step used to separate cytoplasm and SVs from the nerve terminal membranes. When imaged by EM,

most of the resulting "synaptosomal ghosts" (SSMg) were empty of contents but some retained a few SVs either attached directly to the AZ surface membrane or within the SSMg interior. Fibrous projections that linked SVs to the AZ were identified as tethers. SVs within <~45 nm of the surface membrane often exhibited multiple tethers whereas more distant SVs exhibited only single tethers, with a maximum length (99% percentile) of 175 nm. Based on structural predictions, we estimate that channel C-termini could extend as far as ~200 nm into the presynaptic interior and hence, may correspond to the observed long single tethers. Biochemical analysis supports this interpretation (see: Gardezi et al. CAN 2014 poster).

2-B-27 Mechanisms of septin 5-mediated inhibition of neurotransmitter release

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Neurons communicate at chemical synapses via exocytosis of synaptic vesicles containing neurotransmitter. Exocytosis occurs when vesicle and plasma membranes fuse, a process mediated by the bundling of the SNARE proteins syntaxin 1A (STX1A), SNAP25, and VAMP2. Protein interactions with SNAREs can therefore influence exocytosis. Septin 5 (Sept5), a synaptic vesicle-associated protein, inhibits exocytosis and also binds to the SNARE STX1A. Sept5 is a member of the septin family of filamentous cytoskeletal proteins and is expressed predominantly in the brain, where it also prevents close docking of synaptic vesicles at the plasma membrane. However, the specific mechanism underlying the inhibition of exocytosis by Sept5 is unknown. Intriguingly, two sequences found within Sept5 contain residues found in the STX1A-binding region of VAMP2. Thus, Sept5 could compete with VAMP2 for STX1A binding. Using GST pull-down and yeast two-hybrid assays, the current study maps the region of Sept5 responsible for STX1A binding and aims to determine if mutating the VAMP2-like regions affects STX1A binding. This study thereby aims to advance our understanding of the mechanisms regulating exocytosis and neurotransmitter release.

2-B-28 PKC and Ca²⁺ suppress electrical signaling between neuroendocrine cells of Aplysia

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The bag cell neurons of *Aplysia* initiate egg-laying behavior by secreting egg-laying hormone during a prolonged period of synchronous and repetitive firing known as the afterdischarge. These neurons function as an electrical syncytium, which is the result of gap junctions. The generation of an afterdischarge is accompanied by an increase in intracellular calcium and upregulation of protein kinase C (PKC). Here, dual whole-cell recording of paired cultured bag cell neurons shows that electrical coupling is modulated by calcium and PKC. Elevating calcium with a train of voltage steps which mimics the onset of the afterdischarge decreased junctional conductance for up to 30 min. The inhibition was most effective when calcium entry occurred in both electrically coupled neurons. Depletion of calcium from the mitochondria, but not the endoplasmic reticulum, also attenuated junctional communication. Buffering calcium with high intracellular EGTA prevented uncoupling, as did inhibition of calmodulin kinase. Application of PMA, a PKC activator, to coupled bag cell neurons slightly decreased junctional current, while elevation of intracellular calcium in PMA-treated neurons inhibited electrical synapses to an even greater extent. Our results demonstrate that calcium-dependent activation of calmodulin kinase and PKC inhibit electrical signaling. This may contribute to an increase in bursting frequency and enhancement of neuronal excitability which results in the secretion of reproductive hormone.

2-B-29 PKA-GluA1 coupling via AKAP5 controls AMPA receptor phosphorylation and cell-surface targeting during bidirectional homeostatic plasticity

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Bidirectional synaptic plasticity occurs locally at individual synapses during LTP or LTD, or globally during homeostatic scaling. LTP, LTD, and homeostatic scaling alter synaptic strength through changes in post-synaptic AMPARs, suggesting the existence of overlapping molecular mechanisms. Phosphorylation is critical for controlling AMPAR trafficking during LTP/LTD. Here we addressed the role of AMPAR phosphorylation during homeostatic scaling. We observed bidirectional changes in

PKA phosphorylation of GluA1 S845, during scaling, resulting from a loss of PKA from the synapse during scaling-down and enhanced activity of PKA in the synapse during scaling-up. Altered synaptic PKA signaling, requiring the scaffold AKAP5, alters the effectiveness of neuromodulators and NMDAR activation. Increased phosphorylation of S845 could drive scaling-up while loss of S845 blocked scaling-up. Finally we show that AMPARs scale differentially based on their phosphorylation status at S845. These results show that rearrangement in PKA signaling controls AMPAR phosphorylation and surface targeting during homeostatic plasticity.

2-B-30 Role of calpain-mediated cleavage of NMDA receptor GluN2B subunit on synaptic potentiation

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¹CRIUSMQ

Long-term potentiation (LTP) of excitatory synaptic transmission involves Ca²⁺ influx through NMDA receptors (NMDAR), followed by a multitude of signaling processes in spines. The cytoplasmic tail (Ctail) of NMDAR subunit GluN2B acts as a central hub for several protein interactions in synaptic plasticity. Previous work indicated that CaMKII interaction with GluN2B Ctail plays a central role in LTP. We found that this interaction is required for activation of the ERK/MAPK pathway, critical in LTP. Another enzyme activated by Ca²⁺ influx through the NMDAR is calpain, which cleaves several substrates including the Ctails of GluN2 subunits. However, it is unknown whether the cleavage of NMDAR subunits occurs at synapses during synaptic transmission and whether it plays a role in synaptic signaling and remodeling. By blocking calpain activation, genetically or pharmacologically, we found that it prevented NMDAR-dependent ERK activation. We showed by immunoblotting from cortical synaptic fractions that GluN2B is cleaved upon NMDAR activation, releasing a Ctail fragment, still bound to CaMKII. Also, over-expression of a GluN2B Ctail fragment, not mutated in the CaMKII binding site, could support NMDAR-dependent ERK phosphorylation in presence of calpain inhibitor. We also mutated a putative calpain cleavage site on GluN2B and found that the over-expression of this molecular construct could block ERK phosphorylation. Our experiments suggest that NMDAR synaptic activation leads

calpain to generate a signaling complex containing CaMKII and GluN2B Ctail supporting synaptic potentiation

2-B-31 Characterization of drg sandwich synapse neuron pairs: involvement of TRPV1-POSITIVE sensory neuron types

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The dorsal root ganglion (DRG) encases the neuronal somata (NS) of the peripheral somatic sensory system, which projects a short bifurcating axon that branches to the sense organ and the spinal cord. Most of the NS are enveloped by a satellite glial cell (SGC) and isolated from each other and there is no evidence to suggest that direct synaptic connections occur between DRG NS. However, a subset of NS are enclosed in a common SGC capsule separated only by a thin SGC membrane, forming an NS-glial cell-NS (NGIN) trimer, termed "Sandwich Synapse" (SS) (Rozanski et al., 2012). Our laboratory has shown, through the use of electrophysiology and immunocytochemistry, interneuronal transmission between NS pairs via transglial-signaling pathway (Rozanski et al., 2013a,b). However, it is not known if SS contacts form randomly or involve specific DRG soma types. In this study we test whether SS neurons belong to the subtypes characterized by the presence of TRPV1 family of channels which include pain-sensing neurons. DRGs were dissociated by our standard gentle enzymatic method and were immunostained with an antibody against TRPV1. Approximately 60% of the dissociated neurons were TRPV1 sensitive. However, our initial results indicate that staining for this marker is random for the subset of neurons that form SS pairs. Thus, SS contacts are not formed preferentially between this class of sensory neurons in the normal ganglion.

2-B-32 The role of CAV2.2 DISTAL C-Terminus in synaptic vesicle tethering

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Voltage gated neuronal calcium channels (Cav2.2) gate synaptic vesicle (SVs) fusion and

discharge at transmitter release sites. Findings that a single Ca²⁺ channel can gate transmitter release (Stanley, 1993) suggested that the SVs are 'tethered' within 25 nm of the channel. Recent studies (Kaeser et al, 2011; Wong et al, 2013) have proposed that SVs interact with the channel distal C-terminus. Kaeser et al proposed that the SV is tethered to the C-terminus by RIM via two links. The first, a direct link to a 4 amino acid PDZ ligand domain within the C-terminus, and the second, an indirect link to a PxxP motif. We tested these hypotheses using a novel in vitro SV-binding assay termed SV-PD (Wong et al, 2013) and Cav2.2 distal C-terminus (C3 region) fusion proteins. SVs were captured by the normal C3 fusion protein. However, SV-PD was not observed with a truncated C3 fusion protein (C3prox) comprising the PxxP motif, narrowing down the SV attachment site to the last 58 amino acids. However, data that SV-PD persisted with C3 mutant fusion proteins with a defective PDZ ligand domain or in the presence of a mimetic peptide blocker argues against a PDZ-dependent mechanism. We conclude that the unidentified SV tethering site is within the terminal 49 amino acid residues proximal to the PDZ-ligand domain. Further, we predict that the channel C-terminal could extend as far as 200 nm from the surface membrane (see: Chen et al. CAN 2014 poster) and, therefore tethering may involve additional short range molecular links to bring the SV within gating range of the calcium channel.

2-B-33 ProBDNF and p75NTR regulate excitability and firing of pyramidal neurons

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Persistent activity of entorhinal cortex (EC) pyramidal neurons, regulated by cholinergic inputs from basal forebrain through activation of muscarinic receptors, has emerged as a key element in working memory. We are examining the role of neurotrophins and their receptors in modulating persistent activity within the EC. Here, we report on the role of p75NTR and proBDNF in this function. Electrophysiological recordings were performed on layer V pyramidal neurons in acute EC slices. In this preparation, persistent firing is induced by addition of carbachol, a cholinergic agonist. We observed

that the concentration of carbachol required to elicit persistent activity was significantly less in slices from p75NTR^{-/-} animals. To determine if this effect reflected a loss of neuronal or glial p75NTR, we used a *Talpha1:cre* driver line to selectively ablate p75NTR in neurons of p75NTR^{fl/fl} animals. To further determine if this effect reflected a developmental defect in p75NTR null mice, we generated p75NTR^{fl/fl}:CMV-ERcre animals. In both cases, deletion of p75NTR reduced the threshold for persistent activity in the EC, indicating that p75NTR expressed within adult neurons negatively regulates this property. Bathing EC slices with a p75NTR-blocking antibody also reduced the threshold for persistent activity whereas application of the p75NTR ligand proBDNF rapidly and reversibly blocked persistent firing induced by carbachol. Together, these results showed that the p75NTR controls neuronal excitability, acting as a negative regulator of persistent firing in pyramidal neurons of EC.

2-B-34 The characterization and role of mitochondrial Ca²⁺ dynamics in Aplysia neuroendocrine cells

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In many types of neurons, mitochondria regulate intracellular Ca²⁺. As such, these organelles can modulate Ca²⁺-dependent processes. To study this, we characterized mitochondrial Ca²⁺ dynamics in the bag cell neurons of the mollusc, *Aplysia californica*. Upon stimulation, these neuroendocrine cells undergo an afterdischarge during which elevated intracellular Ca²⁺ increases excitability and hormone secretion to initiate reproduction. Cultured neurons were fura-loaded with whole-cell recording, to monitor intracellular Ca²⁺ under voltage-clamp in response to a train stimulus. Secretory output and endocytosis were determined by measuring changes in membrane capacitance post-train. Stimulation increased cytosolic Ca²⁺, followed by clearance and mitochondrial Ca²⁺-induced Ca²⁺-release (CICR). Ca²⁺ recovery was slowed by the protonophore, FCCP, or double stranded RNA interference of the mitochondrial Ca²⁺ uniporter or H⁺/Ca²⁺ exchanger, LetM1. Conversely, CICR was sensitive to intracellular Na⁺, EGTA, or TPP (an inhibitor of mitochondrial Ca²⁺ exchangers). Furthermore, CICR was regulated by the plasma membrane Ca²⁺ ATPase, which, when inhibited by carboxyeosin, potentiated CICR. Lastly, while CICR did not

alter secretion magnitude, it did promote membrane endocytosis, as shown by the acceleration of membrane capacitance recovery. Thus, we find a contribution of mitochondria to both bag cell neuron Ca²⁺ dynamics and endocytosis. These results expand the known physiological roles for mitochondria and reinforce their importance for fundamental processes of the nervous system.

2-B-35 Effects of a KCC2 blocker on network activity in piriform and entorhinal cortices

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The efficacy of inhibitory transmission mediated by GABA_A relies on low levels of [Cl⁻]_i that is controlled by cation-chloride co-transporters such as KCC2. Here, we investigated the effects induced by blocking KCC2 activity with either VU0240551 (10 μ M) or bumetanide (50 μ M) on the epileptiform activity generated by piriform and entorhinal cortices during 4AP (50 μ M) application in an in vitro brain slice preparation. Field potential recordings revealed interictal- and ictal- like discharges along with high-frequency oscillations (HFOs, ripples: 80-200 Hz, fast ripples: 250-500 Hz) in these two regions during 4AP application. In addition, ictal discharges in piriform and entorhinal cortices occurred either synchronously or independently of each other; duration and interval of occurrence of independent ictal discharges was longer when compared to synchronized ictal discharges. We also found that ictal discharges were abolished in both areas during VU0240551 or bumetanide application; these pharmacological procedures decreased interictal discharge duration and their interval of occurrence. Finally, blocking KCC2 activity increased HFOs occurring in the piriform cortex during interictal activity. Our data demonstrate that ictogenesis can be abolished by inhibiting KCC2 activity. We propose that these effects result from the reduction of GABA_A receptor-dependent increases in [K⁺]_o that are known to rest on KCC2 function.

2-B-36 Post-synaptic Long-Term Potentiation of GABA Synapses in the Oval Bed Nucleus Stria Terminalis

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Neural circuits consist of highly dynamic networks of excitatory and inhibitory neurons. While synaptic plasticity at excitatory synapses throughout the brain is well-established, synaptic plasticity of inhibitory synapses is a far less characterized phenomenon. The bed nucleus of the stria terminalis (BNST) is a structure known to be an interface between homeostatic neuro-regulation and circuits mediating higher cognitive processes. We have recently shown that changes in synaptic plasticity in the oval nucleus (ovBNST) is significantly correlated with drug taking behaviors. Thus, the ovBNST serves as a region of interest for studying the role of plasticity, specifically, that of plasticity at inhibitory synapses. Using whole-cell patch clamping in the slice, low frequency stimulation (LFS; 1 Hz 900 pulses) at inhibitory synapses in the ovBNST produced long-term potentiation (LTP) of GABA_A inhibitory post-synaptic currents (IPSC). Twenty-minutes following LFS, IPSCs increased on average 173% \pm 77% (SE) above baseline values and was sustained and sometimes continued to increase for up to 60 minutes post-induction (n=9/8). This effect appears to be post-synaptically mediated as there was no change in paired-pulse ratios (0.9 \pm 0.04: 1.1 \pm 0.09, pre \pm SE: post \pm SE) and no significant change in the coefficient of variation (1/CV² pre \pm SE: post \pm SE, 26.8 \pm 8.2: 24.3 \pm 6.4). The ubiquity of this phenomenon in the brain has yet to be determined.

2-B-37 Neurosteroids modulate interictal activity and high frequency oscillations in the CA3 subfield

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Two types of spontaneous interictal discharges, identified as fast or slow events, are recorded from the hippocampal CA3 subfield in rodent brain slices during application of 4-aminopyridine (4AP, 50 μ M). Here, we addressed how neurosteroids modulate the occurrence of these interictal events and their associated high frequency oscillations (HFOs, ripples: 80-200 Hz, fast ripples: 250-500 Hz). Allotetrahydrodeoxycorticosterone (THDOC; 100 nM and 5 μ M) was applied during continuous 4AP application. Local field potentials were recorded from CA3 with glass micropipettes. Under control conditions (i.e., during 4AP application), ripples and fast ripples occurred with 12.3 % and 17.5 % of fast interictal

discharges, respectively. In contrast, ripples and fast ripples co-occurred with less than 1% of slow interictal discharges. Application of 0.1 to 5 μ M THDOC to 4AP-treated slices caused a dose-dependent decrease in the duration of the fast events while that of slow events increased. THDOC also led to an increase in the proportion of fast interictal events coinciding with ripples (15%) but there was no change in fast ripples co-occurrence. Finally, blocking glutamatergic transmission abolished the occurrence of ripples and fast ripples while blocking GABAA receptor signaling increased the occurrence of fast ripples associated with robust interictal activity. Our data show that neurosteroids differentially modulate fast and slow interictal discharges, and HFOs occurrence in the CA3 subfield. These effects are presumably due to the potentiation of GABAA receptor mediated activity.

2-B-38 Residues Important for Ca²⁺ Transport in the Neuronal Na⁺ /Ca²⁺ and K⁺ Exchanger (NCKX2)

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Na /Ca²⁺ K Exchangers (NCKX) belong to the SLC24 Solute Carrier gene family of membrane transporters. NCKXs play an important role in calcium homeostasis in excitable tissues. Five different gene products (NCKX1-5) of the exchanger have been identified in humans and they play a role in many biological processes including vision in cone and rod photoreceptors, olfaction and skin pigmentation. NCKXs are also widely expressed throughout the brain. NCKXs are bi-directional plasma membrane Ca²⁺ transporters which utilize the inward Na and outward K gradients to extrude Ca²⁺ from the cytosol (4Na :1Ca²⁺ 1K⁺). Here, we examined residues important for Ca²⁺ transport using a fluo4-based assay. Scanning mutagenesis was carried out in the two regions with the highest degree of homology between different NCKX isoforms (the α 1 and α 2 repeats). 13 residues were found to be important as their substitution in the human NCKX2 gene decreased the affinity of NCKX2 for Ca²⁺. Interestingly, most of these 13 residues are conserved in the distantly related archaeobacterial exchanger NCX_Mj for which a crystal structure was recently obtained. Location of these residues within the crystal structure of the NCX_Mj revealed that they are either in direct contact with the Ca²⁺ ion or lining a Ca²⁺ transport pathway at the center of the exchanger. Supported by CIHR MOP-81327.

Key Terms: NCKX, SLC24A gene family, calcium homeostasis, sodium-calcium exchange, fluorescent based assay, crystal structure of NCX_Mj.

2-B-39 Cholinergic regulation of cognitive function and underlying molecular mechanisms

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Cholinergic vulnerability, characterized by loss of acetylcholine (ACh), is one of the hallmarks of Alzheimer's disease (AD). Recent work has suggested that decreased ACh activity in AD may contribute to pathological changes through global alterations in alternative splicing. This occurs via the regulation of the expression of a critical protein family in RNA processing, hnRNP A/B proteins. Changes in pre-mRNA processing may underlie dysfunction of neurons; impairing plasticity, metabolism, the inflammatory response and promoting neurodegeneration. To assess the role of ACh in cognitive function we targeted the expression of the Vesicular Acetylcholine Transporter (VACHT), the rate limiting step in ACh release. To test the hypothesis that cholinergic tone regulates alternative splicing in neurons by controlling the fate of hnRNPA2/B1 expression, thereby influencing cognition, we employed a combination of genetic in vivo and in vitro techniques to alter cholinergic tone and evaluate expression of hnRNPA2/B1. Decreasing cholinergic tone reduced levels hnRNPA2/B1 and alternative splicing patterns mirroring those seen in Alzheimer's disease, while increasing cholinergic signalling in vivo increased expression of hnRNPA2/B1. This effect is not due to decreased hnRNP mRNA expression or increased degradation of the protein. Cell culture experiments demonstrated that muscarinic signalling may underlie cholinergic control of hnRNPA2/B1 expression. Finally we evaluated long term changes in APP processing and other key AD related transcripts in aged forebrain VACHT deficient mice.

2-B-40 Hydrogen sulfide influences sodium channels in subfornical organ neurons

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Hydrogen Sulfide (H₂S) has been shown to act as a gasotransmitter in the central nervous system to control cardiovascular function. Our previous microarray analyses and RT-PCR have shown that enzymes responsible for production of H₂S are expressed in the subfornical organ (SFO), and our previous intracerebroventricular (ICV) injections of H₂S into SFO resulted in increases in blood pressure. This led us to hypothesize that H₂S may alter the excitability of SFO neurons. We used RT-PCR to confirm the presence of the voltage gated sodium channels Nav1.1, Nav1.2, Nav1.3, and Nav1.6, which are responsible for producing the transient and resurgent Na currents. We then used whole cell patch clamp recordings in voltage clamp to investigate the influence of H₂S on these Na currents. We first used a voltage step protocol and observed a depolarizing shift in the activation curve of the transient Na current. We next used a voltage step protocol designed to isolate the resurgent sodium current. 60% of neurons tested passed a resurgent sodium current measuring on average $23.0 \pm 13.0\%$ of the peak transient sodium current. The current decreased by an average of 97.4 ± 8.7 pA in response to H₂S in all neurons passing the current. This study has therefore identified a potential mechanism whereby H₂S alters the excitability of SFO neurons by influencing the transient and resurgent voltage gated sodium currents. Supported by the Canadian Institutes for Health Research

2-B-41 Roles of alpha and betaCaMKII in exocytosis and synaptic trapping of AMPA receptors**Simon Labrecque¹, Benoit Audet¹, Christian Tardif¹, Paul De Koninck¹**¹Universite Laval

AMPA receptors (AMPA) and Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) are important mediators of synaptic plasticity. The synaptic delivery of AMPAR in the postsynaptic sites results from interplay between rates of exocytosis at extra/peri-synaptic sites and diffusional trapping at synapses. Little is known about the specific roles that α/β CaMKII isoforms play in these mechanisms. In this study, we are using cultured hippocampal neurons, gene transfer of recombinant SEP-GluA1 (AMPA receptor subunit fused to pH-sensitive GFP), TIRF microscopy and single particle tracking of

quantum-dot-labelled GluA1 to monitor both the exocytosis and lateral mobility of AMPARs in the plasma membrane. To assess the roles of α or β -CaMKII, we knocked down their expression with specific shRNAs (with or without rescue transfections) or overexpression of CaMKII natural inhibitor (CKIIN). Our experiments show that knocking down either subunits lower the frequency, the amplitude and accelerate the decay of exocytosis events of AMPAR-containing vesicles. Meanwhile, genetic knock down of either α or β -CaMKII increased AMPAR synaptic diffusion. However, upon chemical LTP induction, we found that the isoforms had distinct impacts on AMPAR diffusion at synapses. Our results suggest that both major isoforms of CaMKII in the brain promote the accumulation of AMPARs at synapses via a combined increase in their exocytosis and diffusional trapping, albeit through different mechanisms. We are currently examining the roles of AMPAR phosphorylation in these processes.

2-B-42 hERG and hEAG1 K⁺ channels are regulated by Src kinase and by SHP-1 tyrosine phosphatase via an active ITIM region in the cyclic nucleotide binding domain**Lyanne Schlichter¹, Jiahua Jiang¹, John Wang¹, Evan Newell², Florence Tsui³, Doris Lam⁴**¹Krembil Discovery Tower, Toronto Western Hospital, ²Singapore Immunology Network (SigN), ³University of Toronto, ⁴Krembil Discovery Tower, Toronto Western Hospital/ University of Toronto

Members of the EAG superfamily of K channels (EAG/Kv10.x, ERG/Kv11.x, ELK/Kv12.x subfamilies) are expressed in many cells and tissues. In microglia, innate immune cells of the CNS, we published the first studies demonstrating that endogenous ERG-like currents were regulated by Src, a protein tyrosine kinase. While the native current was very similar to ERG1, we could not rule out the possibility that it was produced by heteromultimeric channels; e.g., ERG1 and ERG2. Given the importance of EAG and ERG channels to human pathology, it is crucial to address regulation of the identified human channels, hERG and hEAG1 by tyrosine phosphorylation. In the present study, we demonstrate that tyrosine kinase inhibitor, PP1, and the selective Src inhibitory peptide, Src40-58, reduce the hERG current amplitude, without

altering its voltage dependence or kinetics. PP1 similarly reduces the hEAG1 current. Surprisingly, we discovered an 'immuno-receptor tyrosine inhibitory motif' (ITIM) within the cyclic nucleotide binding domain of all EAG-superfamily members, which is conserved in the human, rat and mouse sequences. We found that when tyrosine phosphorylated, this ITIM can directly bind to and transactivate SHP-1, a tyrosine phosphatase predominantly expressed in hematopoietic cells. We show that hERG and hEAG1 currents are regulated by activated SHP-1, in a manner opposite to their regulation by Src. Given the widespread distribution of these channels, Src and SHP-1, this work has broad implications in ERG and EAG functions in many cell types, including microglia.

2-B-43 Identification of gene targets for Nur77, a transcription factor associated with dopamine-related neuroadaptation

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Nur77 (NGFI-B, Nr4a1) is an orphan nuclear receptor of the transcription factor family expressed in brain structures innervated by dopamine neurons. Nur77 has been linked to L-dopa and antipsychotic drug-induced dyskinesias in animal models and Nur77 knockout (KO) mice and rats showed changes in these dopamine-related behaviors. These data converge to an implication of Nur77 in regulation and homeostasis of dopamine neurotransmission in the striatal complex. However, Nur77 gene targets implicated in these processes have not been identified yet. Using *in situ* hybridization, we compared basal and haloperidol-induced mRNA expression of several genes associated with striatal plasticity and functions, including neurotrophic tyrosine kinase receptor 2, glutamic acid decarboxylase 1, neurotensin and proenkephalin in wild type and Nur77 KO mice. We then proceed to a bioinformatic analysis to identify putative Nur77 response element in those gene promoters using MEME Suite platform. The NBRE consensus AAAGGTCA was used to find putative binding site for Nur77. Gene reporter assay, electromobility shift assay and chromatin immunoprecipitation were performed to confirm Nur77 regulatory action on these genes. Altogether, these results support Nur77 regulatory role of gene related to striatal adaptation and plasticity. Supported by the CIHR (MOP-300152). OP holds fellowships from

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2-B-44 Developmental regulation of synaptic function by hydrogen peroxide at developing *Xenopus* neuromuscular synapse.

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Successful synaptic transmission at the neuromuscular junction depends on the precise alignment of the nerve terminals with the postsynaptic specialization of the muscle fiber. We have previously demonstrated that muscle-derived IGF-1 is important in the development of neuromuscular synapse and hydrogen peroxide (H₂O₂) plays an important role in the release of IGF-1. Here we further test the role of H₂O₂ in the development of neuromuscular synapse of *Xenopus laevis* by using the recording of whole-cell patch clamp. Bath application of H₂O₂ dose-dependently potentiates the frequency of spontaneous ACh release in Day-1 *Xenopus* neuromuscular junction. The dose-response curve shift to left while the facilitation effect of H₂O₂ on SSC frequency was test in Day-3 cultures, suggesting the sensitivity of H₂O₂-induced facilitating effect reduced as synapse matured. Pretreatment of the culture with H₂O₂ scavengers both N-Acetylcysteine or sodium pyruvate for 1 day significantly hampered the development of synapse as the SSC frequency and amplitude both significant reduced in treated Day-1 synapses. We next test the effect of H₂O₂ on Day-1 N-Acetylcysteine/sodium pyruvate-pretreated synapse was test after extensively washed the culture to removed N-Acetylcysteine/sodium pyruvate. The SSC frequency facilitating effect induced by H₂O₂ was significantly enhanced in treated synapse, which supports the notion that H₂O₂ is involved in the development of synapse. Furthermore, co-pretreatment the culture with insulin in N-Acetylcysteine/sodium pyruvate group reversed N-Acetylcysteine/sodium

2-B-45 Sex differences in the spinal mechanisms underlying neuropathic pain in mice

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Chronic neuropathic pain is characterized by mild to severe pain and results in significant human suffering and economic burden. The spinal mechanisms underlying neuropathic pain have been extensively investigated in male rodents, indicating an integral role for spinal microglia-released brain-derived neurotrophic factor (BDNF) in the maintenance of pain hypersensitivity. However, investigation of these mechanisms in female rodents is nearly nonexistent. Consequently, we examined the spinal mechanisms underlying neuropathic pain in mice of both sexes. First, we demonstrated that mechanical pain hypersensitivity resulting from Spared Nerve Injury (SNI) was reversed after inhibiting (via intrathecal minocycline) or lesioning (via intrathecal MAC-1-saporin) microglia in male but not female mice. These findings indicate that pain hypersensitivity in female mice is mediated by a microglia-independent mechanism. Second, we demonstrated that pain hypersensitivity post-SNI reverts to a microglia-dependent system in immune deficient female mice. Furthermore, adoptive transfer of T-cells rescued the microglia-independent system in immune deficient female mice. Finally, we demonstrated a role for BDNF in mediating pain hypersensitivity post SNI in both sexes. Our experiments indicate that female and male mice use distinct spinal mechanisms in the mediation of neuropathic pain hypersensitivity. Taking into consideration sex differences in the spinal mediation of chronic pain may greatly improve future treatment development.

2-B-46 Christianson Syndrome-Linked Mutation in the Na⁺/H⁺ Exchanger SLC9A6 Disrupts Recycling Endosomes and Synaptic Structures

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The Na⁺/H⁺ exchanger SLC9A6/NHE6 is a recycling endosomal pH-regulating transporter that is abundant in the CNS. Within hippocampal CA1 pyramidal neurons, NHE6-containing vesicles are distributed throughout the soma and dendrites, with noticeable accumulation at dendritic spines and presynaptic terminals. A number of mutations in NHE6 have been

identified in different families with neurodevelopmental disorders, including a 6 base-pair deletion that results in the loss of amino acids E287 and S288 (Δ ES) located near predicted membrane-spanning segment 7. To better understand the nature of this defect, Δ ES as well as single (E287Q, E287A and S288A) and double (E287Q-S288A) mutations were engineered in wild-type (WT) NHE6, and the effects on its biosynthesis, post-translational oligosaccharide processing, membrane trafficking and function were assessed in transfected AP-1 cells as well as cultured hippocampal neurons. We found that only constructs containing mutations of E287 displayed impaired glycosylation and decreased half-life compared to WT. The mutants were still able to traffic to the plasma membrane, though their cell surface levels and rates of endocytosis were significantly diminished. Further examination of the Δ ES mutant revealed a pronounced impairment of recycling endosomal pH and cargo trafficking. Transient expression of the WT and Δ ES mutant in cultured hippocampal neurons also revealed aberrant trafficking of NHE6 Δ ES-containing vesicles that accumulated within the soma, were poorly sorted along the dendritic processes and lead to a reduction in synapses.

2-B-47 Endocannabinoid signaling enhances visual responses through modulation of intracellular chloride levels in retinal ganglion cells.

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Type I cannabinoid receptors (CB1R) are found throughout the retinae of all vertebrates, from human to fish, but the functional role of endocannabinoids in vision is not clear. Here we demonstrate that CB1R activation markedly improves contrast sensitivity, using a dot avoidance assay in freely swimming *Xenopus* tadpoles. We examined multiple levels of the visual system from the outer retina to the optic tectum, and identified a CB1R-mediated increase in the intrinsic excitability of retinal ganglion cells (RGCs) that paradoxically requires tonic inhibition through glycine

receptors. CB1R activation reduced intracellular chloride and negatively shifted the chloride equilibrium potential, an effect that could be mimicked and occluded by NKCC1 inhibition. Consistent with this, hyperpolarizing current injections increased RGC excitability, suggesting that tonic inhibition may enhance RGC responsiveness by de-inactivating voltage-gated channels. These results highlight a critical role for endocannabinoids in modulating vision, and present a novel mechanism for endocannabinoid regulation of neuronal activity.

2-B-48 Beta-Arrestin2 modulates the signalling complexes formed by the inward rectifying potassium channels KIR3 and delta opioid receptors

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Monitoring of G-protein coupled receptor (GPCRs) interactions has revealed that receptors, G proteins and downstream effectors reach the membrane as a signalling unit. These signalling complexes maintain their integrity during early stages of receptor activation implying that proteins responsible for receptor regulation are most probably recruited to the complex rather than to isolated receptors. Here we were interested in the regulation of DOR-Kir3 signalling complexes. In a first series of experiments, we used BRET and co-immunopurification assays, to establish if Kir3 channels constitutively associate with heterotrimeric G protein subunits and delta opioid receptors (DORs). We then showed that all complex components remained associated after sustained activation (30 min) of the receptor with SNC-80 (1 μ M). We further observed that DOR stimulation (SNC-80 1 μ M; 30 min) induced Barr2 recruitment towards DORs, Gbg subunits and Kir3 channels, and established that receptors and channels expressed in primary neuronal culture were both internalized by SNC-80 and morphine. Moreover, receptors and Kir3 channels colocalized with each other after stimulation with SNC-80 and morphine agonists but only SNC-80 induced colocalization of Barr2 with DORs and Kir3 channel subunits. Taken together, these data show that DORs and Kir3 channels form constitutive complexes that remain associated during late stages of receptor activation and indicate that regulatory proteins such as Barr2 recognize these complexes as a

unit, causing simultaneous internalization of the receptor and the effector.

2-B-49 Examination of TrkB-receptor signalling and accumbal dendritic spine density in the sensitization response to ethanol

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Repeated exposure to ethanol (EtOH) in mice produces behavioural sensitization. We have recently found that mice resistant to developing EtOH sensitization have decreased levels of trkB mRNA throughout the brain, suggesting a critical involvement for TrkB receptor signaling in this behavior. We have also found that sensitized mice show greater levels of pCREB in the nucleus accumbens (NAc) compared to resistant mice and saline controls. Given that pCREB changes in the NAc induced by other sensitization-producing drugs has been associated with structural changes in accumbal medium spiny neurons (MSNs), our finding raises the possibility that EtOH sensitization may also involve structural changes in this neuronal population. Therefore, the overall goal of the present study was to determine whether sensitization to EtOH is like that of other drugs, requiring TrkB signalling and involving NAc MSN spine density changes. To this end, male DBA mice received 5 biweekly EtOH (2.2g/kg, i.p.) or saline injections, after pretreatment with the TrkB receptor antagonist ANA-12 (0 and 0.5mg/kg, i.p.). In a separate experiment, brains were removed for the analysis NAc MSN spine density using diolistic labeling. Results showed that ANA-12 did not block the development of EtOH sensitization. Furthermore, sensitization to EtOH was not associated with changes in NAc MSN dendritic spine density. These results suggest that the neurobiology underlying EtOH sensitization is distinct from that of other sensitization-inducing drugs.

2-B-50 Activity-dependent localization and turnover of Argonaute proteins in hippocampal neurons.

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Localized protein synthesis in the dendrites of neurons is a cellular process critical for synaptic plasticity. This process is modulated by the selective transport of specific messenger ribonucleic acids (RNAs) to synapses and the

activity-dependent regulation of their translation into proteins. Argonaute (AGO) proteins loaded with microRNAs (miRNAs), a class of small non-coding RNAs, target messenger RNAs for storage, silencing and/or degradation. As such, AGO proteins are important actors in the control of local protein synthesis. However, the cellular pathways that direct AGO proteins function in neurons during synaptic plasticity remain poorly defined. We are observing by confocal microscopy the distribution of AGO proteins in hippocampal neurons. More specifically, we monitor the localization of AGO proteins in dendrites and their spines and changes following selective activation or inhibition of different membrane receptors involved in excitatory neurotransmission and synaptic plasticity. Using immunofluorescence labelling, we analyze the occurrence of endogenous AGO proteins alongside dendritic and synaptic structures. We also express ectopically different forms of AGO, some of which have mutations altering their affinity for RNA and their tendency to aggregate. We find that AGO proteins are degraded in an activity-dependent manner and that their ability to bind RNA and their targeting to RNA granules affects their localization and their turnover in the dendrites of hippocampal neurons.

2-B-51 Quantification of the frequency of spontaneous synaptic currents in the dorsal horn of the spinal cord using non stationary analyses.

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Primary afferent fibers carry somatosensory information from the periphery to the central nervous system. The complex network of interneurons in the dorsal horn of the spinal cord serves as the first integrator of sensory information before transmitting it to projection neurons. The temporal integration of inhibitory synaptic inputs (IPSCs) is central to the processing of somatosensory information in the dorsal horn. Using a voltage-clamp recording of spontaneous IPSCs (sIPSCs) from rodent dorsal horn neurons, we present a method of sIPSC time-frequency analysis based on non-stationary variance. The sIPSC sequence is represented as a time function. Using multi-linear regressions, we observed significant distributions that correlated with different states of synaptic activity. Based on the shape of the distribution, two types of sIPSCs synaptic activities were extracted. The first fit is linear

and suggests a constant synaptic activity, whereas the second fit is staircase-like and suggests a burst-like activity. These markers of synaptic activity enable us to better characterize the integration of somatosensory information in the dorsal horn.

2-B-52 Homeostatic synaptic plasticity at GABAergic synapses requires dystroglycan

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Dystroglycan (DG), a cell adhesion molecule well known to be essential for skeletal muscle integrity and formation of neuromuscular synapses, is also localized to inhibitory synapses in the central nervous system. Mutations that affect DG function not only result in muscular dystrophies, but also in severe cognitive deficits and epilepsy. Here we investigate the role of DG during activity-dependent homeostatic regulation of hippocampal inhibitory synapses. Prolonged elevation of neuronal activity increases DG expression as well as clustering of DG colocalized with GABA(A)Rs. In contrast, inhibition of protein synthesis prevents this activity-dependent accumulation of synaptic DG and GABA(A)Rs, and blocks scaling up of inhibitory neurotransmission. RNAi-mediated knockdown of DG also blocks scaling up of inhibitory synapses, as does knockdown of LARGE - a glycosyltransferase critical for DG function. The DG ligand agrin rapidly increases GABA(A)R clustering and mimics inhibitory scaling up induced by prolonged increased activity, indicating that activation of this pathway alone is sufficient to regulate GABA(A)R trafficking. These data demonstrate for the first time that DG is regulated in a physiologically relevant manner in neurons, and that DG and its glycosylation are essential for homeostatic synaptic plasticity at inhibitory synapses.

2-B-53 Optogenetic activation of glutamatergic neurons in the medial septum drive activity within the septum and across the hippocampal network.

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Neurons in the medial septum diagonal band of Broca (MS-DBB) are well known to provide important connections to the hippocampus, and critical for spatial learning and memory. Three main neuronal populations have been identified in this region: cholinergic, GABAergic and glutamatergic. Glutamate neurons were shown (by Williams's lab) to project to hippocampus, release glutamate and to discharge rhythmically at theta frequency. To further explore the role of MS-DBB glutamatergic neurons we have used optogenetics to target these neurons. We have targeted glutamatergic VGLUT2 neurons of the MS-DBB with the light-sensitive protein, ChETA. The VGLUT2 CRE-mouse line are injected with a ChETA Cre-recombinase adeno-associated virus to specifically target glutamate neurons of the MS-DBB. Using this model we aim to determine how the activation of glutamatergic neurons from MS-DBB modulate postsynaptic targets both within the septum and across the hippocampal network. By selectively activating these neurons, we have obtained postsynaptic excitatory potentials onto both neurons within the septum and to interneurons in the hippocampus. We will further determine how glutamatergic MS-DBB neurons can modulate hippocampal theta rhythm in the whole septo-hippocampal network in vitro and how this population affects hippocampal theta rhythms in vivo. These experiments will help to understand how MS-DBB glutamatergic neurons contribute to learning and memory in freely behaving mice.

2-B-54 Low voltage-activated calcium channels gate transmitter release at the dorsal root ganglion Sandwich Synapse

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The dorsal root ganglion (DRG) contains a subset of closely-apposed neuronal somata (NS) that are separated solely by a thin glial cell membrane septum. Stimulation of one NS leads to transglial activation of its neighbour via a bisynaptic purinergic/glutamatergic pathway, a signaling mechanism that we term Sandwich Synapse (SS) transmission. ATP release from a stimulated NS can be attributed to classical calcium (Ca²⁺)-dependent exocytosis but involves an undetermined voltage-gated Ca²⁺ channel (Cav) type. Specific blockers and toxins ruled out gating by the more typical high voltage-activated (HVA) Cav1, 2.1 and 2.2.

Transmission was, however, blocked by a moderate depolarization (-50 mV) or low-concentration Ni²⁺ (0.1 mM). Transmission persisted using low voltage pulses to -40 mV (holding potential -80 mV) and in the presence of the HVA current blocker ω -Agatoxin IIIA, confirming the involvement of a low voltage-activated (LVA) channel. This result limited the candidate channel type to either Cav3.2 or an inactivation- and Ni²⁺-sensitive Cav2.3 channel subtype. SNX482 had no significant effect on the NS Ca²⁺ current or SS transmission, arguing against the latter. We conclude that inter-somatic transmission at the DRG SS is gated by Cav3.2 type channels. DRG neurons exhibit sub-threshold membrane potential oscillations (Amir et al. J.Neurosci 1999) that are enhanced after nerve injury (Liu et al. J.Physiol 2000). Hence, gating of ATP release by LVA channels may serve a trophic or homeostatic function in the normal DRG or play a role in the etiology of neuropathic pain.

2-B-55 Identifying the interacting regions between GluN1 & ND2 in the Src-NMDAR pathway.

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Upregulation of NMDA receptors (NMDARs) by the tyrosine kinase Src is critical for chronic pain hypersensitivity in the spinal cord & hippocampal LTP. Src is anchored in the NMDAR complex by an adaptor protein, ND2, (NADH dehydrogenase subunit 2). The primary sequence requirements for the interaction between Src & ND2 have been determined, but the interacting regions between ND2 & the NMDAR complex have remained elusive until the present study. To elucidate the basis for this interaction, we transfected HEK293 cells with GFP-tagged ND2 or with one of a systematically generated series of GFP-tagged fragments of ND2, with NMDAR subunits or receptor controls. The NMDAR subunits/controls were fluorescently labeled & confocal images captured. Thresholded Pearson's Correlation Coefficient (PCC) was used as measure of colocalization. GFP-ND2 differentially colocalized with both GluN1 alone (0.61 ± 0.03) & a GluN1-C-terminal & Amino Terminal Domain (ATD) deletion mutant (0.61 ± 0.03), but the additional deletion of the TM4 region led to a loss of colocalization (0.09 ± 0.04). Swapping in a GluN2A TM4 region into GluN1 recapitulated this colocalization (0.78 ± 0.06), but neither the

GluN1 nor the GluN2A TM4 alone colocalized with ND2, illustrating that TM4 is necessary but not sufficient for this interaction. The ND2 fragment 151-223 (0.71 ± 0.02), also colocalized with GluN1 alone, but smaller ND2 fragments did not. Thus, we have determined the minimal interacting region of ND2 required in the ND2-NMDAR complex, & identified GluN1 TM4 as a critical GluN1-ND2 interacting region.

2-B-56 Neurosteroid modulation of synchronous activity in the piriform and entorhinal cortices of pilocarpine-treated and non-epileptic control rats

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We employed field potential recordings in brain slices obtained from pilocarpine-treated and from age-matched, non-epileptic control (NEC) rats to examine the effects of the neurosteroid allotetrahydrodeoxycorticosterone (THDOC) on the epileptiform activity induced by 4-aminopyridine (4AP) in the piriform (PC) and entorhinal (EC) cortices. Both structures are highly susceptible to generate seizures and may also be involved in epileptogenesis. Status epilepticus (SE) was induced by i.p. injections of pilocarpine (380 mg/kg) in adult Sprague Dawley rats. SE was terminated after one hour using diazepam (5 mg/kg) and ketamine (50 mg/kg). The in vitro electrophysiology experiments were carried out four-five weeks following SE. Transverse brain slices were obtained and bathed in 4AP (50 μ M) to induce epileptiform activity. THDOC (5 μ M) was bath applied and local field potential (LFP) recordings were obtained from the PC and EC networks. We found that THDOC: (i) decreased interictal discharge frequency in PC of pilocarpine-treated and NEC brain slices while increasing it in EC of pilocarpine-treated rats; (ii) reduced the duration of ictal discharges in NEC PC as well as in pilocarpine-treated EC; (iii) increased the occurrence of ripples and fast ripples during interictal events in pilocarpine-treated PC and EC; (iv) changed the timing of HFO occurrence during ictal discharges in pilocarpine-treated PC and EC. Our results demonstrate that neurosteroids maintain their ability to control epileptiform synchronization in pilocarpine-treated epileptic rats.

2-B-57 Seizure patterns induced by cortical deafferentation in adult mice

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Traumatic brain injury is a major risk factor for epileptogenesis. Understanding and preventing trauma-induced epileptogenesis (TIE) will prevent epilepsy and therefore significantly increase the quality of life of patients. We aimed to test the age-dependency of TIE in a mouse model of cortical undercut. Because the efficacy of homeostatic plasticity processes decreases with age, we hypothesized that cortical trauma will induce epilepsy in adult, but not young animals. We performed undercut in the somatosensory area in C57/BL6 young (3 months) and adult (12-14 months) mice and implanted LFP electrodes in diverse cortical areas and EMG electrodes for chronic recordings. The electrographic activities were recorded continuously for at least two months. Almost all animals generated acute seizures of variable morphology within the first 10 hours from lesion. In young animals only isolated interictal spikes were recorded afterwards. In the following weeks, all but one old mouse revealed recurrent seizure activities of different types. The most common type was 8-16 Hz spindle-like oscillation in frontal cortex accompanied with an increase in the muscle tone and either body freezing or rhythmic contractions. The lower frequency (3-6Hz) seizures were generalized and accompanied by behavioral freezing and low muscle tone or by rhythmic muscle and body contractions. The low frequency (1.5-3Hz) seizures were accompanied with rhythmic muscle contractions. We conclude that TIE is age-dependent and is likely due to an uncontrolled homeostatic up-regulation of excitation in adult animals.

2-B-58 Spreading depression in the brain of *Drosophila melanogaster*

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Spreading depression (SD) is characterized by a massive redistribution of ions which is accompanied with an arrest in electrical activity that propagates through neural tissue. It is important to understand the cellular mechanisms underlying SD due to its association with human pathologies such as stroke, migraine, and traumatic brain injury. We have recently designed an experimental protocol

where repetitive SD can be reliably triggered in the fly brain by inhibition of the sodium/potassium-ATPase with ouabain. Small volumes (~5nL) of ouabain were administered directly into the head using a pressure injection system and SD was monitored by recording the extracellular potassium concentration in the brain with potassium-sensitive microelectrodes. We administered increasing concentrations of ouabain (10-4M, 2x10-4M, and 5x10-4M) to individual preparations and found that ouabain exerts its effect in a concentration-dependent manner. Additionally, we show that hypotonic saline exacerbated ouabain-induced SD suggesting an effect of cell swelling. Furthermore, we found that flies lacking the white gene (w1118 mutants), which encodes for an ATP-binding cassette transporter, were more susceptible to ouabain-induced SD compared to wild-type flies (Canton S strain). Lastly, by measuring direct current (DC) potential with two microelectrodes placed at increased distances from the ouabain injection site we have demonstrated that these events propagated throughout the fly brain at rates similar to that observed in mammalian cortex.

2-B-59 Structural Insight into the Uncoupled Conformation of the Nicotinic Acetylcholine Receptor

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Nicotinic acetylcholine receptors (nAChRs) are members of the superfamily of pentameric ligand-gated cation channels (pLGICs). They are ubiquitous in mammalian nervous systems and are implicated in a range of neurological diseases, including Alzheimer's disease, etc. nAChR activity is regulated by its lipid microenvironment, which can differentially stabilize activatable resting versus non-activatable desensitized or uncoupled conformations. Interestingly, neuronal nAChRs expressed in heterologous systems often adopt an uncoupled state, leading to the hypothesis that uncoupled nAChRs serve as a reserve population that can be "awakened" to potentiate the post-synaptic response. Our working model proposes that uncoupling results from a tilting-away of the lipid-facing transmembrane α -helix M4 from the body of the receptor, leading to weakened interactions between the agonist-binding domain and channel gating domains, and a collapsing of the channel pore. However, little direct structural insight into this conformation has been obtained. Here, we

investigate the hypothesis that the nAChR channel pore collapses in the uncoupled conformation by characterizing the pore binding of several non-competitive antagonists. Binding of several of these compounds was characterized for both the resting and desensitized conformations, but no (or only very weak) binding was observed to the uncoupled nAChR. These studies shed light on the structural changes that underlie the uncoupling of binding and gating in a eukaryotic nAChR.

2-B-60 Selective post-synaptic deletion of DCC from CA1 pyramidal neurons alters dendritic spine morphology and impairs spatial memory in aging mice

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Little is known regarding the contribution of the netrin-1 receptor DCC to synapse function in adulthood. We have recently demonstrated that DCC is expressed by neurons in the adult brain and regulates synapse function and plasticity in the mature nervous system. DCC is enriched along both axons and dendrites during development, however the precise pre- and post-synaptic functions of DCC at synapses in the adult brain remain unclear. We therefore generated R4ag11::Cre/DCC^{f/f} mice in which DCC expression is selectively eliminated from CA1 pyramidal neurons but not in neurons in the adjacent CA3 region of the hippocampus. This conditional loss of DCC from the CA3-CA1 post-synaptic neuron resulted in aberrant spine morphology in CA1 neurons in aged mice, while maintaining overall topographical organization. We have also shown that the loss of DCC in these transgenic animals results in significant deficits in spatial memory and novel location recognition tasks, but fails to disrupt novel object recognition memory, suggesting that selective deletion of DCC in CA1 neurons results in disturbances in the neural circuits underlying contextual spatial memory. Together, these findings provide evidence for a critical post-synaptic role for DCC in adult mice and that DCC contributes to memory consolidation during spatial navigation.

2-B-61 Predicting brain-wide electrophysiological diversity of mammalian neurons from genome-wide expression atlases

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Brains achieve efficient function through implementing a division of labor, in which different neurons serve distinct computational roles. Neuronal computations are established through the expression of combinations of ion channels and associated proteins which define neuronal electrophysiological properties. Despite numerous studies characterizing neuronal physiological properties and gene expression patterns, linking neuron genomics to neuron function has been challenging due to the complexity of these heterogeneous data. Here, employing data integration and machine-learning approaches, we combine published reports of neuronal physiological properties with public genome-wide expression atlases. Specifically, we integrate NeuroElectro (www.neuroelectro.org), a database of literature-mined neuron-type physiological diversity, with the Allen Brain Atlas. We demonstrate that relative differences among the genes that neurons express are significantly predictive of neuronal biophysical parameters (such as resting potential and input resistance; $R^2 = .65, .41$). Our approach allows us to ask which genes, of the 20,000 in the genome, are most predictive of electrophysiological diversity. In addition to ion channel genes (such as inward rectifying K channels, e.g. *Kcnj4*), genes related to synaptic plasticity and neuronal differentiation (e.g. *Slit3*) were also surprisingly correlated with neuronal physiology. Moreover, cross-referencing these gene lists with those implicated in mental disorders, we find that these genes are also disrupted in epilepsy, schizophrenia, and autism.

2-B-62 The importance of D-serine in circuit refinement in the developing visual system of the *Xenopus* tadpole**Marion Van Horn¹, Edward Ruthazer¹**¹Montreal Neurological Institute

D-serine is an endogenous co-agonist for synaptic N-methyl-D-aspartate receptors (NMDAR). Here we examined the neurophysiological significance of D-serine, a known gliotransmitter, in regulating neuronal activity and axonal remodeling in the developing visual system of the *Xenopus* tadpole. Acute D-serine (100 μ M) wash-on significantly enhanced the NMDAR currents of optic tectal neurons indicating that the NMDAR glycine site is not

saturated under physiological conditions in vivo. Rearing tadpoles in elevated D-serine (100 μ M) for 2 days greatly increased the frequency of miniature excitatory postsynaptic AMPAR currents and resulted in higher retinotectal synaptic AMPA/NMDA ratios compared to control animals. To investigate pathways involved in modulating the release of endogenous D-serine we used D-serine-sensitive amperometric biosensors and found that glutamate receptor activation results in an increase in D-serine release. To examine the effects of D-serine on axonal development, retinal ganglion cells were electroporated to express EGFP and 2-photon images were collected daily over 4 days. We find that increasing available D-serine during early developmental periods results in less complex retinal axons arbors consistent with the hypothesis that D-serine promotes synaptic maturation and leads to stabilization of axonal branches. These results suggest that D-serine levels are modulated by glutamatergic neurotransmission in vivo and can influence the maturation of retinotectal synapses and axonal refinement. Supported by a CIHR grant to ESR and a Banting Fellowship to MVH.

2-B-63 δ GABAA receptors: A novel target for gabapentin actions**Jieying Yu¹, Robert Bonin², Beverley Orser¹**¹University of Toronto, ²Centre de Recherche Université

Gabapentin (GBP) is a γ -aminobutyric acid (GABA) analogue that is widely used to treat neuropathic pain and epilepsy. GBP has multiple side-effects including anxiolysis, sedation and ataxia. The mechanisms underlying the varied effects of GBP are poorly understood. The $\alpha 2\delta$ subunit of Ca² channels is a known target of GBP; however, the interaction at this site fails to fully account for the multiple effects of GBP. We previously showed that GBP increased a tonic inhibitory conductance generated by extrasynaptic GABA type A (GABAA) receptors in hippocampal neurons (Cheng et al 2006). GABAA receptors that contain the δ subunit (δ GABAA) generate tonic inhibitory currents in the CNS. We test the hypothesis that an increase in the activity of δ GABAA receptors contributes to the behavioural properties of GBP. To test this postulate, wild-type (WT) and δ GABAA receptor null mutant (*Gabrd*^{-/-}) mice were treated with GBP or vehicle (i.p.) 2 hours before testing. The antinociceptive, anxiolytic and ataxic effects

were studied with the formalin assay, elevated plus maze and rotarod, respectively. The results showed GBP had no effect on phase 1 of the formalin assay but inhibited responses in phase 2 to a similar extent in both genotypes. GBP treated WT mice, but not *Gabrd*^{-/-} mice spent more time in the open arms. GBP decreased time on the rotarod in a dose-dependent manner in WT but not *Gabrd*^{-/-} mice. The expression of δ GABAA receptors is necessary for the anxiolytic and ataxic, but not the anti-nociceptive effects of GBP. Thus, δ GABAA receptors appear to be a novel target for GBP.

C - Disorders of the Nervous System

2-C-64 Activation of the mammalian target of rapamycin promotes dendritic regeneration after axonal injury.

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Loss of vision in glaucoma is caused by the death of retinal ganglion cells (RGCs). Emerging data indicate that early retraction of RGC dendrites plays a prominent role in neurodegeneration. Our recent data demonstrate that: 1) the activity of the mammalian target of rapamycin (mTOR), a key regulator of cell growth and protein synthesis, is markedly reduced soon after axonal injury; and 2) that mTOR is a key mediator of dendritic stability in adult RGCs. Here, we sought to determine whether mTOR upregulation promotes dendrite regeneration. Acute axonal injury was induced by optic nerve axotomy in transgenic mice that express yellow fluorescent protein (YFP) in RGCs under Thy1 promoter control. In this strain, only ~1% of RGCs are YFP-labeled, allowing visualization of individual dendritic trees. Two approaches were used to activate mTOR: intraocular injection of a short interference RNA (siRNA) against the mTOR inhibitor *Redd2* (siRedd2), or systemic administration of insulin. Treatment with siRedd2 or insulin was performed three days after axotomy, a time when there is substantial dendritic retraction, and analysis of dendritic length and field area was assessed at one week post-injury. Our data show that mTOR activation promotes robust dendrite regeneration and restores dendritic length and field area to values similar to those found in intact RGCs. Rapamycin administration abrogated the regenerative effect of mTOR activation, confirming that dendritic regrowth was mTOR-dependent. Our data demonstrate that mTOR

activation promotes dendritic regeneration in injured neurons.

2-C-65 Persistent individual differences in stress susceptibility and antidepressant treatment response following chronic social stress in mice

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Identifying the determinants of individual differences in stress susceptibility and antidepressant treatment response will be crucial for our understanding of depression pathogenesis and for the development of novel antidepressant therapies. Here we used social defeat stress, followed by chronic antidepressant treatment, to model stress susceptibility and antidepressant treatment response in mice. Adult C57/Bl6 mice were physically defeated by a new CD1 aggressor mouse for 5 min every day, and subsequently housed across a partition in the same cage with the aggressor for 24 hours. After 10 days, mice were segregated into 'susceptible' or 'resilient' phenotypes depending on their social interaction (SI) score. Susceptible mice were socially avoidant (SI score < 1) and showed decreased sucrose preference, while resilient mice were not socially avoidant (SI score > 1) and showed similar sucrose preference as controls. Fluoxetine treatment (10 mg/kg/day, ad libitum) for 28 days reversed social avoidance behavior in 53% of susceptible mice, while 47% of mice did not respond to fluoxetine treatment. Fluoxetine had no effects on social avoidance behavior in control mice. We show that social defeat stress, followed by chronic fluoxetine treatment, allows segregation of stress susceptible and resilient phenotypes, as well as of responders and non-responders to antidepressant treatment. This model therefore provides a powerful paradigm to investigate persistent neurobiological mechanisms that are crucial for the pathogenesis and treatment of depression.

2-C-66 Aberrant glutamatergic synaptic connectivity with motoneurons in the spinal cord of zebrafish expressing mutant human TARDBP (TDP-43) with a mutation causing ALS and FTLD.

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Mutations in the TARDBP gene encoding TDP-43 have been found in patients with Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD). Although several animal models have been developed to studying pathologies associated with mutant TDP-43 expression very few have characterized the pathophysiological abnormalities that arise following their expression. To advance our understanding of the pathogenicity of these mutations we used a zebrafish model previously described by our lab in which we transiently expressed mutant human TARDBPG348C as well as wild type TARDBPWT mRNA in zebrafish larvae. We examined glutamatergic interneuron synaptic connectivity with primary motoneurons by performing paired recordings by evoking action potentials (APs) while monitoring excitatory post-synaptic currents (EPSCs). We observed that glutamatergic EPSCs in larvae over-expressing mutant TARDBPG348C but not wild type TARDBPWT mRNA were significantly larger in amplitude when compared to controls. We next examined glutamatergic miniature excitatory post-synaptic currents (mEPSCs) in CaP motoneurons. Two striking features were observed: the amplitude of mEPSCs were significantly larger and occurred at a higher frequency in fish expressing mutant (but not WT) TARDBPG348C. These data represent the first electrophysiological description of abnormal synaptic connectivity in the spinal cord following expression of a mutant TDP-43 and suggests that a significant excitotoxic component may occur in individuals harbouring mutations in TDP-43.

2-C-67 Acute and chronic increases in neuronal sodium concentrations during post-traumatic epileptogenesis

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Post-traumatic accumulation of intracellular Cl⁻ results in GABA becoming depolarizing, which reduces inhibition, enhances propagation of neuronal firing, and may contribute to early post-traumatic seizures. Charge balance dictates that increases in [Cl⁻]_i may be accompanied by increases in cations, which could underlie cytotoxic edema and accompany epilepsy. We tested for changes in intracellular Na⁺ concentration using acute hippocampal slices and organotypic hippocampal slice cultures from wild-type C57BL/6J mice, imaged with the Na⁺-

sensitive dye SBFI. In acute slices hippocampal neurons had significantly higher [Na⁺]_i than has been reported in undamaged neurons, particularly near the cut surface of the slice. In organotypic slice cultures, [Na⁺]_i returned to low levels ~2 days after slice trauma. At longer incubation times, during which slices become epileptic, [Na⁺]_i increased to levels seen immediately post-trauma. By 20 days after trauma, [Na⁺]_i returned to low levels. The Na⁺/K⁺ ATPase inhibitor ouabain and the KCC2/NKCC1 antagonist furosemide increased [Na⁺]_i. The NKCC1 antagonist bumetanide increased [Na⁺]_i during the first ~10 days after trauma, and thereafter decreased [Na⁺]_i; thus, in the days after trauma NKCC1 operates in reverse by exporting Na⁺. There was a correlation between [Na⁺]_i and propidium iodide staining, suggesting that [Na⁺]_i may serve as an early biomarker of neuronal death. Increases in intracellular [Na⁺]_i could engender cerebral edema, cause depolarizing shifts in the reversal potential for GABA, and thus promote early epileptic seizures.

2-C-68 Beta-amyloid inhibits PDGFβ receptor activation and prevents PDGF-BB-induced neuroprotection

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When applied exogenously, PDGF-BB is neuroprotective against ischemia, excitotoxicity, and HIV proteins. Given its ability to protect neurons against such a wide variety of insults, we wished to determine if PDGF-BB is neuroprotective against beta-amyloid (1-42), one of the putative pathological agents in Alzheimer's disease. In both primary hippocampal neurons and the human-derived neuroblastoma cell line SH-SY5Y, beta-amyloid treatment for 24 h dose-dependently decreased cell viability. Pretreatment with PDGF-BB did not provide any neuroprotection against beta-amyloid in primary neurons. In SH-SY5Y cells, PDGF-BB pretreatment significantly, but only partially, prevented beta-amyloid-induced cell death. PDGF promotes cell growth and division in several systems, and the application of PDGF-BB to SH-SY5Y cells resulted in an increase in cell number. Beta-amyloid attenuated the trophic effects of PDGF-BB. Upon closer examination, we determined that beta-amyloid decreased PDGF-BB-induced PDGFβ receptor phosphorylation. Despite

the ability of beta-amyloid to inhibit PDGFbeta receptor activation, immunoprecipitation experiments failed to identify a physical between beta-amyloid and the PDGF-BB ligand or the PDGFbeta receptor. The ability of beta-amyloid to block ligand activation of the PDGFbeta receptor may explain the lack of neuroprotective effects of PDGF-BB. Furthermore, as the PDGF system is upregulated upon neuronal damage, the ability of beta-amyloid to inhibit this endogenous neuroprotective system may contribute to the pathology of Alzheimer's disease.

2-C-69 Characterization of aldehyde dehydrogenase 2 (ALDH2) null mice as a model of age-related cognitive impairment with Alzheimer's Disease-like pathologies

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Most animal models of AD rely on the overexpression of genes related to familial AD. However, there is a paucity of animal models that mirror late-onset/age-related AD (95% of AD patients). Oxidative stress is considered a causative factor in age-related AD, and ALDH2 is important for the catabolism of toxic aldehydes associated with oxidative stress. For example, 4-hydroxynonenal (HNE) adducts accumulate in AD brain and are associated with AD pathology. Given this linkage, we followed changes in relevant AD markers in Aldh2 null mice and their wild type littermates over a 1 year period. Marked increases in HNE adducts arise in hippocampi from Aldh2 null mice, as well as age-related increases in A-beta, p-tau, p-cofilin and activated caspases. Also observed are age-related decreases in PSD95, synaptophysin, CREB and pCREB. Age-related memory deficits begin at 3 months. These mice exhibit endothelial dysfunction, A-beta deposition in cerebral microvessels, decreases in carbachol-induced pCREB formation, and also dystrophic neurites, dendritic spine loss, and brain atrophy. These AD-associated pathological changes are rarely observed as a constellation in current AD animal models. Chronic administration of GT1061, a neuroprotectant that targets synaptic failure via activation of NO/cGMP/pCREB signaling, reverses the progressive memory deficits and biochemical changes that occur in these mice, suggesting that this new model will prove useful for assessing the efficacy of therapeutic agents for improving memory and

for slowing, preventing, or reversing AD progression.

2-C-70 It's time to move: a dopaminergic oscillator driving rhythms of arousal.

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Ultradian (~4hrs) rhythms of locomotor activity that do not depend on the master circadian pacemaker in the suprachiasmatic nucleus (SCN) have been observed across mammalian species, including humans, yet not a single study has attempted to identify the neural substrate driving these rhythms. We observed that distinct alteration of the dopaminergic system - by either genetic ablation of the dopamine transporter gene or pharmacological manipulation with psychostimulants - leads to ultradian locomotor rhythm lengthening from approximately 4h to greater than 24 hours. Importantly, the antipsychotic haloperidol had the opposite effect; shortening periodicity in naïve, methamphetamine-treated, and DAT-/- animals. Finally, we observed that striatal dopamine levels fluctuate in synchrony with ultradian activity rhythms and that the period of these ultradian rhythms strongly predicts dopaminergic tone in both naïve and methamphetamine treated mice. Our data support the existence of a dopaminergic ultradian oscillator (DUO) operating in the mammalian brain that controls arousal in concert with the circadian clock. The striking similarity between the aberrant sleep/wake patterns observed here and in human subjects afflicted with either bipolar disorder or schizophrenia suggests that this clock is similarly perturbed in psychiatric conditions associated with hyperdopaminergia and that locomotor activity patterns may provide a new biomarker for altered dopaminergic tone and associated mental illnesses.

2-C-71 Systems Neurobiology of Autism Spectrum Disorders (ASD)

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ASDs are a heterogeneous group of disorders characterized by symptoms such as impaired and disordered language, decreased social interactions, extreme fixation on certain objects or activities and impaired fine and gross motor movements. We used Lurcher (Lc) mutants and Lc chimeras as mouse models of autism-like phenotypes to investigate the relationship between cerebellar pathology, behavioural deficits and differences in functional cortical activity at rest or following sensory-evoked cortical activation. We predict that cerebellar pathology in phenotypically autistic-like mouse models (including Purkinje cell (PC) and Granule cell (GC) death) causes a shift in the excitability of surviving cerebellar neurons and leads to changes in the activity of structurally and functionally connected brain regions. Using cFos as a marker of recent neuronal activity, we found that there is an inverse relationship between the number of surviving PCs and the density of cFos expression in surviving GCs. In addition, we tested our Lc chimeras on a higher order form of behavioural flexibility to study the relationship between PCs and attentional set shifting. If PCs are involved in attentional set shifting then we would expect to see a negative relationship between PC number and performance on learning measures. Finally, since the cerebellum has many connections with the cortex we used Voltage-sensitive Dye (VSD) imaging to look at differences in cortical activity between Lc mutants and wildtype mice to explore the relationship between the loss of PCs and changes in cortical activation.

2-C-72 Characterizing Motor Performance in the 3xTg-AD Mouse Model of Alzheimer's Disease

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The 3xTg-AD mouse model of Alzheimer's disease has three transgenes including a mutated mouse presenilin-1 gene (PS1M1461), a human amyloid precursor protein gene (APPswe), and a gene associated with human tau pathologies (Tau301L). We examined the motor performance of 3xTg-AD mice (15M, 15F) and their wildtype controls (B6129SF2; 15M, 15F) using a behavioural motor test battery at 6 months of age. Mice were tested on the Rotarod, wire hang test, grid suspension test and balance beam. 3xTg-AD transgenic mice had significantly better performance than

wildtype mice on the Rotarod. Conversely, wildtype mice had better performance than 3xTg-AD mice on both the grid suspension and wire hang tasks of grip strength. On the balance beam, wildtype mice had significantly fewer foot slips, indicating better performance than transgenic mice but no significant difference was found between genotypes in speed or number of turns. These findings suggest that the mechanisms underlying the improved motor performance of 3xTg-AD mice on the Rotarod may not be due to improved grip, balance, or speed but rather may be due to cognitive differences in endurance, motivation or anxiety. The improved Rotarod performance, deficits in grip strength and balance of 3xTg-AD mice should be considered when performing behavioural tasks on this strain. We are currently examining motivation and endurance using a voluntary wheel running task and performing gait analysis to identify any other motor abnormalities in these mice.

2-C-73 Increased expression of retinal Tau in experimental glaucoma is neurotoxic

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Retinal ganglion cell (RGC) death is the primary cause of vision loss in most optic neuropathies, including glaucoma. There is a high occurrence of glaucoma amongst Alzheimer's disease (AD) patients. Indeed, preferential loss of large diameter RGCs has been documented in AD patients. Moreover, AD and glaucoma share a number of pathological features such as the presence of amyloid beta plaques and Tau aggregates. In this study, we examined changes in retinal Tau expression and the effect of Tau on RGC survival using an experimental rat glaucoma model. Ocular hypertension (OHT) was induced in rats by injection of hypertonic saline into an episcleral vein. Retinal Tau expression and phosphorylation were assessed by western blot analysis using antibodies against phosphorylated and total Tau. The cellular localization of Tau was investigated by retinal and optic nerve immunohistochemistry using cell-specific markers. Our data demonstrate that OHT leads to a rapid accumulation of retinal Tau characterized by a complex phosphorylation profile. Both hyperphosphorylated and hypophosphorylated Tau were detected in glaucomatous retinas compared to intact controls. We show that soon after OHT induction, Tau accumulates within RGCs, primarily in their dendritic compartment.

Importantly, Tau deletion using siRNA led to substantial protection of RGC soma and axons from glaucomatous damage compared to retinas treated with control siRNA. In conclusion, OHT glaucoma displays features of a taupathy namely compartmental redistribution of Tau, altered phosphorylation and neurotoxicity.

2-C-74 Translocation breakpoint sequencing for the identification of pathogenic genes in psychiatry

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Numerous studies show high heritability of psychiatric disorders such as schizophrenia or bipolar disorder, but revealing the genes involved has proven difficult due to a combination of study cohort heterogeneity and imprecise phenotypic boundaries. One way to complement and support large cohort association or linkage studies is to identify exact breakpoint boundaries of balanced chromosomal rearrangements in subjects with psychopathology, thereby pointing to directly disrupted genes that may be associated with disease. To 151 with unipolar depression, anorexia nervosa, panic disorder, or autism spectrum disorders. High-throughput whole-genome sequencing localized the translocation breakpoint to intron 3 of LRRC4C, encoding the Netrin-G1 ligand (NGL1), a gene important in axon guidance during brain development. We then assessed 24,277 schizophrenia and neurodevelopmental disorder cases and 27,896 controls with Copy Number Variation (CNV) calls; as well as exome sequencing from 41 unrelated families (N=189) with a history of episodic mood disorders for supporting evidence of pathogenic variation in this gene. These results highlight the utility of studying balanced chromosomal rearrangements in families with high rates of psychiatric disorders and support the need for multi-disciplinary approaches to build the case for genetic risk factors in complex traits.

2-C-75 LKB1-regulated adaptive mechanisms are essential for clearance of protein aggregates and neuronal survival following mitochondrial dysfunction

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Mitochondrial dysfunction has emerged in recent years as an important factor contributing to the aetiology of neurodegenerative diseases. To study the impact of mitochondrial activity on neuronal function, we deleted the essential mitochondrial protein AIF in the mouse forebrain. The loss of mitochondrial function caused by AIF deletion activated several compensatory mechanisms and triggered the appearance of dysfunctional lysosomes and the accumulation of ubiquitinated protein aggregates, indicating that mitochondrial activity regulates the appearance of these important features of neurodegenerative diseases. Furthermore, similar dysfunctional lysosomes were also observed in a cellular model of mitochondrial dysfunction where OPA1, a key regulator of mitochondrial cristae structure, is deleted. This cross talk between mitochondria was dependent on the presence of LKB1, a kinase regulating changes in cellular metabolism following a decrease in cellular energy. Importantly, the changes in lysosomal function caused by the loss of mitochondrial function were independent of autophagy, but required reactive oxygen species. Since loss of mitochondrial function is a central mechanism implicated in neurodegenerative diseases, modulation of LKB1-dependent pathways may therefore represent an important strategy to preserve neuronal survival and function.

2-C-76 Neurophysiological traces of L-DOPA-induced dyskinesia in the bed nucleus of the stria terminalis of 6-OHDA-lesioned rats

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The most used treatment for Parkinson's disease (PD), L-3,4-dihydroxyphenylalanine (L-DOPA), results in involuntary movements referred to as L-DOPA-induced dyskinesia (LID). The mechanisms of LIDs are largely unknown and there is no current strategy to prevent or control LIDs. Recent evidence has found that the expression of several immediate early genes is positively correlated with LID severity in a basal forebrain structure, the bed nucleus of the stria terminalis (BNST). Thus, this study aims to identify potential neurophysiological traces of LIDs in the BNST of 6-OHDA-lesioned rats. Male Sprague Dawley rats (N=25) surgically received unilateral 6-OHDA lesions (2.5 µl at

3µg/µl). Three weeks post-op if the lesions produce PD symptoms measured by a stepping test, rats received daily injections of the vehicle Benserazide (15mg/kg, i.p.) or L-DOPA (6mg/kg, i.p.) in Benserazide. On the 10th injection day, rats were assessed for the severity of three subtypes of dyskinesia. Rats were then euthanized one hour post injection and whole cell patch clamping was done in both the juxtacapsular (jx) and oval (ov) areas of the BNST. L-DOPA treatment was not found to alter strength at excitatory synapses in the BNSTov or jx as measured by AMPA/NMDA ratios, nor was it found to alter DAergic modulations of glutamate transmission in these two areas. However, preliminary results suggest an alteration of GABA transmission in ov and jxBNST. If further investigation confirms this, we would have a non-striatal mechanism associated with LID that could be a target for treatment in the future.

2-C-77 Role of exogenous oxytocin on heroin self-administration in male Long-Evans rats: A time factor effect

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Recent studies reported that exogenous oxytocin (OXT) has some potential for treating individuals suffering from substance use disorders. To date, the involvement of OXT has been mostly reported in relation to psychostimulant drugs. However, earlier studies reported that OXT attenuated the development of tolerance to the analgesic effects of opiates and opiate withdrawal, and inhibited heroin self-administration (SA). Thus, we examined the effects of OXT on heroin SA in male rats and hypothesized that central administration of OXT will be effective in reducing heroin SA. Moreover, considering the very short half-life of exogenous OXT, we analyzed the effects of OXT on SA over time, under different types of injection and training conditions. Male Long-Evans rats were trained to SA heroin for 66 days under fixed and progressive ratio schedules of reinforcement to assess their motivation for heroin taking. Once heroin SA stabilized, rats were administered OXT (0.0, 0.5 and 2.5 µg/rat; i.c.v. or 1 mg/ml; i.p.; counterbalanced), before the SA session. The results suggest a strong initial sedative effect of OXT at the beginning of the SA session. However, a clear augmentation of responses for heroin was observed for the

rest of the session. Lastly, central administration of OXT occasionally resulted in severe seizures.

2-C-78 Clustering autism - using neuroanatomical differences in 27 mouse models to gain insight into the heterogeneity.

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Autism is heritable, with 250+ associated genes (Banerjee-Basu and Packer, 2010), yet no single gene accounts for more than 1-2% of autistic cases (Abrahams and Geschwind, 2010). The clinical presentation, behavioural symptoms, imaging, and histopathology findings are strikingly heterogeneous. We propose that examining 27 different mouse models related to autism using MRI based neuroanatomical phenotyping can provide a better understanding of autism. Imaging was performed ex-vivo using an optimized anatomical sequence. In total, 553 individual brains were scanned and analyzed with previously described methods (Lerch et al. 2012). The volumes of 62 different regions (Dorr et al. 2008) were calculated. Group differences across the models were then used to cluster both regions and the models together. The clustering of models revealed 3 large groups (Figure 1). Group 1 (En2, Fmr1, Nrnx1α, and Shank3) had increases in the cortex and white matter structures and decreases in the cerebellar cortex. Conversely, in group 2 (AndR, BTBR, Gtf2i (+/-), Itgβ3, and Nlgn3 KI) white matter structures were found to be smaller.

Group 3 (15q11-13, 16p11, BALB/c, Cntnap2 (-/-), Gtf2i (dp/dp), Mecp2, Nf1, Slc6A4 KI (129), and XO) had a mixture of increases and decreases (Figure 1). This all suggests that the autistic phenotype not only recapitulates the heterogeneity seen in human autism, but also affects key brain regions, and divides into clusters based on directionality and localization of the differences. This work can lead to an increased diagnostic specificity and targeted treatment.

2-C-79 Sustained Ischemia/Hypoxia Follows Cessation of Seizures and Results in Todd's Paralysis

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Seizures are frequently accompanied by behavioural impairments that occur after the seizure terminates and can last for several minutes to hours. Todd's Paralysis, for example, is specific to seizures that affect motor cortex and is marked by severe muscle weakness on the contralateral side. Although recognized first in 1849, we still do not have an explanation for its occurrence. Given the striking similarities to stroke, we hypothesized that there is a severe ischemic/hypoxic event that follows seizures and gives rise to specific behavioural impairments. We first approached this by recording local tissue oxygenation in the hippocampus of awake, freely moving rats. Following brief seizures, we discovered that hippocampal tissue oxygenation dropped below 10mmHg (severe hypoxia) and remained below this level for over an hour. Hypoxia was also confirmed with an immunohistochemical marker of hypoxic cells (HypoxyprobeTM-1). We then showed that hippocampal blood flow was reduced during the entire duration of hypoxia and pretreatment with nifedipine, a potent anti-vasoconstrictor, restored blood perfusion and tissue oxygenation to the hippocampus. Lastly, we employed nifedipine as a tool to determine the effect of hypoxia on forelimb motor weakness following neocortical seizures. Forelimb weakness was observed at 40 minutes post-seizure only in rats that experienced hypoxia after a seizure (vehicle). In sum, we have discovered a novel phenomenon, which we have termed, Seizure-Induced Severe Ischemic/Hypoxic Episode (SISIHE), which is responsible for Todd's Paralysis.

2-C-80 Optimization of Deep Brain Stimulation Parameters for PD Patients Using Objective Measures

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Background: Deep Brain Stimulation (DBS) is a complex surgical therapy that seeks to reduce the symptoms of advanced, L-DOPA responsive PD patients who are not adequately controlled by medication. DBS is a relatively new technique and as such its full potential remains elusive. The programming of the device is still a trial and error process. Methods: 24 DBS patients will be monitored during programming sessions for 6 months. Whole-body mobility, gait and speech are assessed. We use a gait analysis system (PKMAS) to measure a variety of gait parameters including: walking speed, stride length, cadence and functional ambulation profile. We also use a motion capture body suit (Animazoo) to measure mobility parameters such as: rest and action tremor, bradykinesia, arm swing, range of motion, balance and postural stability. Once the data has been collected and analyzed, we will establish a standardized DBS stimulation program for future DBS patients. Pre-operation assessments of future patients will be carried out. These will then be entered into the program and the patient's optimal DBS set-point will be computed. Results: Preliminary data has shown fluctuations in various programming parameters. We have documented that some programming parameters are more efficacious than others. Conclusion: Future clinicians will be able to use the system as a set point for their patients DBS device. As a result of this study DBS patients will have a significantly reduced DBS programming time, optimal personalized DBS settings and an overall improved quality of life.

2-C-81 Age-related changes in brain region weights are associated with caspase activation in wild type C57Bl6 mice.

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The prevalence of neurodegenerative disorders is increasing dramatically as people are living longer and now affects millions of people. Activation of caspases is a key event in the

pathogenesis of several neurodegenerative diseases. Caspase-6 (casp6) in particular has emerged as an important player in the neuronal degeneration in Alzheimer and Huntington disease where it is activated early in the disease. We undertook a study to assess the effects of aging on organ size in wild type C57Bl6 mice and to determine the role of apoptotic mechanisms in the pathophysiology of aging. Peripheral organs, body and brain region weights were collected from young (3-4m), mid (12m) and old (>23m) mice. Significant alterations in peripheral organ and brain weights are observed with aging (body ($p<0.0001$), liver ($p<0.0001$), kidney ($p<0.0001$), spleen ($p=0.0004$), heart ($p=0.0004$), testes ($p=0.0002$) and brain ($p<0.05$). Within the brain, hippocampi ($p=0.03$), striata ($p=0.02$) and olfactory bulbs ($p=0.04$) weight decreases with age. A significant increase in casp6 activity is detected in C57Bl6 cortex corresponding with the time point where brain weight is starting to trend downwards with age. Furthermore, there is an increase in full-length ($p=0.004$) and fragment levels ($p<0.0001$) of the casp6 substrate, STK3, in the aging cortex. Increases in cortical casp8 expression were also detected. These data further our understanding of the anatomical and biological changes that occur with aging and suggest that caspase activation may be an important event in the neurodegeneration observed in the aging

2-C-82 Mitochondrial Processing Peptidase Regulates PINK1 Processing, Import and Parkin Recruitment : Insights into mechanism

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Several lines of evidence implicate mitochondrial dysfunction in PD, including the role of two recessive PD genes, Parkin and PTEN-induced putative kinase 1 (PINK1), in a novel mitochondrial quality control pathway. Upon disruption of the electrochemical potential ($\Delta\Psi_m$) across the inner mitochondrial membrane with the protonophore carbonyl cyanide m-chlorophenyl hydrazone (CCCP), the E3 ubiquitin ligase Parkin translocates from the cytosol to mitochondria. Parkin subsequently mediates the destruction of such defective mitochondria by autophagy (termed mitophagy) in a PINK1-dependent manner. Under basal conditions PINK1 is rapidly degraded in a process involving $\Delta\Psi_m$ -driven mitochondrial import and cleavage by mitochondrial proteases.

Disrupting $\Delta\Psi_m$ with CCCP blocks import and stabilizes PINK1 on the mitochondrial outer membrane, which in turn promotes Parkin recruitment. We previously identified a key step in PINK1 turnover, mediated by the mitochondrial processing peptidase (MPP). MPP knockdown stabilizes PINK1 at the mitochondrial surface, induces Parkin recruitment, and leads to mitochondrial clearance, much like CCCP. Here we show that MPP β silencing-induce PINK accumulation is dependent on the presence of MPP α . We suggest that free monomers of MPP α binds PINK1's MTS in a unique way, preventing it from being extruded to the inner membrane and promoting its accumulation at the outer membrane.

2-C-83 The role of the NOD-like receptor, NLRX1, in neuronal cell death

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Neuronal cell death is a phenomenon that occurs in normal conditions such as in brain development and in pathological conditions like in Multiple Sclerosis (MS). Although the exact cause of MS is unclear, neurons undergo cell death early in the disease. Apoptosis and autophagy are two processes that are implicated in neuronal cell death. A recently discovered protein, NLRX1, is a NOD-like receptor that is implicated in innate immunity by attenuating an inflammatory response. Also, NLRX1 plays a role in autophagy by interacting with Atg proteins. Using ShRNA against NLRX1 and True ORF cDNA clone in N2A cells, we evaluated the expression of apoptotic (caspase-3, caspase-8, caspase-9, and PARP) and autophagic (LC3 and Beclin-1) proteins after different stimuli. Using Western Blotting, NLRX1 knock-down (KD) cells showed a decrease in LC3 cleaved protein and Beclin-1, suggesting an overall reduction in autophagy. Additionally, these cells showed a higher expression of cleaved PARP and cleaved caspase-3 compared to wild-type cells after TNF- α treatment. Furthermore, NLRX1 KD cells are more prone to cell death in normal conditions and NLRX1 knock-in (KI) cells are more resistant to cell death compare to wild-type cells as observed by flow cytometry using Annexin V-PI staining. These results suggest that NLRX1 has a neuroprotective role and plays an important function in neuronal cell death. By understanding the mechanism of action of

NLRX1 in the process of autophagy and apoptosis, it could help develop novel treatments for MS.

2-C-84 Regulation of ischemic neuronal death by E2F4/p130 complexes.

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Inappropriate activation of cell cycle proteins, in particular cyclin D/Cdk4, is implicated in neuronal death induced by various pathologic stress including DNA damage and ischemia. Key targets of Cdk4 in proliferating cells include members of the E2F transcription factors which mediate the expression of cell cycle proteins as well as death inducing genes. However, the presence of multiple E2F family members complicates our understanding of their role in death. Presently, we focussed on whether E2F4, an E2F member believed to exhibit crucial control over the maintenance of a differentiated state of neurons, may be critical in ischemic neuronal death. We observed that in contrast to E2F1 and 3 which sensitizes to death, E2F4 plays a crucial protective role in neuronal death evoke by DNA damage, hypoxia and global ischemic insult both in vitro and in vivo. E2F4 occupies promoter regions of pro-apoptotic factors such as B-Myb under basal conditions. Following stress exposure, E2F4/p130 complexes are rapidly lost along with the presence of E2F4 at E2F containing B-Myb promoter sites. In contrast, E2F1 presence at B-Myb sites increases with stress. Furthermore, B-Myb and C-Myb expression increases with ischemic insult. Taken together, we propose a model by which E2F4 plays a protective role in neurons from ischemic insult by forming repressive complexes which prevent pro-death factors such as B-Myb from being expressed.

2-C-85 Delineating the role of the GIRK2 channel in the generation of Behavioral Spasms and Electrodecremental events in Infantile Spasms

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Infantile Spasms (IS) is a catastrophic childhood seizure disorder. It is characterized by extension and/or flexion jerking movements, cognitive deterioration and Electrodecremental events (EDR) which refers to the flattening of the EEG waveform. The mechanism/circuitry contributing to the disorder is unknown. We hypothesize that the overexpression of GIRK2 (G protein inward rectifying potassium channel) is necessary and sufficient for the GABAb-GIRK2 agonist-induced Infantile Spasms phenotype in the Ts65Dn mouse model of Down's Syndrome. We reduced the GIRK2 channel activity in the Ts65Dn mice pharmacologically using a GIRK antagonist tertiapin Q (TPQ) and observed a significant reduction in EDR activity. To further validate the role of GIRK2, we engineered the segmentally trisomic Ts65Dn mouse to be disomic for the GIRK2 gene. We then measured the difference in behavioural spasm number and EDR duration between the Ts65Dn mice, Ts65Dn disomic mice and Wild Type controls. We found a significant reduction in both behavioural spasms and EDR duration in the Ts65Dn disomic mice compared to the Ts65Dn mice. The data suggest that over expression of GIRK2 leading to excess hyperpolarization is the reason for this inhibitory epilepsy disorder. Our next aim is to create a transgenic GIRK2 over expressing mouse line to further strengthen our hypothesis. This study has important implications for future therapies for IS, Down's syndrome and other inhibitory seizure disorders.

2-C-86 FTY720 induces transcription of neuroprotective genes to regulate Ca2+ homeostasis in human astrocytes

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FTY720 is an S1P receptor (S1Pr) modulator approved for treatment of multiple sclerosis (MS). It prevents lymphocytes from leaving the lymph nodes and entering the CNS; however, FTY720 injections into the CNS reduce neuro-inflammation in mice in the absence of peripheral lymphopenia. Effects within the CNS were initially attributed to FTY720 inhibiting the actions of S1P. In contrast, we and others demonstrated that FTY720 continues to signal even while inhibiting S1Pr. When bound by S1P, S1Pr is recycled to the cell membrane. When FTY720 binds to S1Pr, the receptor-ligand complex is targeted to an endosomal compartment. FTY720 inhibits IL-1 beta induced calcium release from intracellular stores in human astrocytes. On the basis of microarray

analysis and studies of functional Ca²⁺ efflux, we propose that during MS progression or inflammatory challenge, ER stress in astrocytes leads to increased ER Ca²⁺ release, resulting in activation of store operated Ca²⁺ entry (SOCE) and further build-up of intracellular Ca²⁺ altering cell signaling. By studying the transcriptome signature of FTY720, we obtained evidence that FTY720 increases expression of Ca²⁺ regulating proteins, which reduce ER Ca²⁺ efflux and inhibit SOCE. We predict that this in turn will prevent the reversal of glutamate re-uptake and limit NO production, thereby reducing neuronal excitotoxicity and mitochondrial damage in axons. We propose that on this basis, the application of FTY720 protects astrocytes from Ca²⁺ induced excitotoxicity to facilitate neuroprotection.

2-C-87 Pharmacogenetic inhibition of eIF4E-dependent Mmp9 mRNA translation reverses Fragile-X syndrome-like phenotypes

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Fragile-X syndrome (FXS) is the leading genetic cause of intellectual disability and autism. Mutations in Fmr1 (Fragile-X mental retardation gene) engender exaggerated protein synthesis resulting in dendritic spine dysmorphogenesis, synaptic plasticity alterations and behavioral deficits in mice, reflecting a significant part of the FXS phenotype diagnosed in patients. In FXS postmortem brains and Fmr1 knockout mice (Fmr1^{-/-}), phosphorylation of the 5' mRNA cap binding protein, eukaryotic initiation factor 4E (eIF4E) at Ser209 is elevated concomitant with increased expression of Matrix Metalloproteinase 9 (MMP-9). Here we show that genetic or pharmacological reduction of eIF4E phosphorylation rescued core behavioral deficits, synaptic plasticity alterations and dendritic spine morphology defects via reducing exaggerated translation of Mmp9 mRNA in Fmr1^{-/-} mice. Furthermore, MMP-9 overexpression produced several FXS-like phenotypes. These results uncover a novel mechanism of regulation of synaptic function by translational control of MMP-9 in FXS, which opens new treatment avenues for the diverse neurological and psychiatric aspects of FXS.

2-C-88 Macrophage polarization after spinal cord injury is influenced by TNF- α and iron

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Macrophages/microglia appear to have both detrimental and beneficial effects in the injured CNS, which may be due to their polarization state. M1 polarization includes production of nitric oxide and pro-inflammatory cytokines, while M2 polarized macrophages are anti-inflammatory and pro-tissue repair. We have extended earlier work to show that myelin phagocytosis by bone marrow derived macrophages and microglia stimulated with M1 polarizing factors cause a shift in polarization to an M2 state. This is based on the expression of various cell surface and intracellular M1 and M2 makers assessed by flow cytometry. Interestingly, although myelin phagocytosis occurs in the first 2 weeks after spinal cord injury (SCI), macrophages/microglia are predominantly M1 polarized as assessed at the single cell level by FACS. We therefore assessed why these cells fail to switch to a M2 phenotype in the injured CNS. In vitro studies revealed that TNF- α can abrogate the effects of myelin phagocytosis on polarization. Furthermore, we show that TNF- α null mice have increased numbers of M2 macrophages after SCI. As hemorrhage and dying cells are a feature of SCI, we assessed the role of iron in polarization. We found that heme and non-heme iron induce M2 polarized cells in vitro to switch to proinflammatory M1 cells. In addition, iron loaded M2 macrophages transplanted into the injured cord transform into M1 polarized cells. This indicates that TNF- α and intracellular iron loading play a key role in modulating macrophage/microglia polarization to a detrimental proinflammatory M1 state.

2-C-89 Robust stress response does not alter adult hippocampal neurogenesis after acute predator stress

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Traumatic, stressful life events are thought to trigger a variety of neuropsychiatric disorders including post-traumatic stress disorder (PTSD), generalized anxiety disorder and depression.

Identifying the mechanisms underlying the stress response may aid in understanding the development or improving treatment options for these debilitating disorders. Neurogenesis, the production of new neurons, is known to occur in the subgranular zone (SGZ) of the adult mammalian hippocampus. While the reduction in adult neurogenesis following chronic stress is largely supported, acute stress models, particularly predator stress, have yielded inconsistent results. Thus, the goal of the current study is to help elucidate the effects of predator stress on adult hippocampal neurogenesis. Our study is unique as it implements a single, unprotected cat exposure (10 minutes) which produces anxiety-like behaviors and hyperarousal in rats for up to three weeks. We investigated the effect of predator stress on both proliferating and surviving cells in the adult hippocampus. Rats were injected with 5-bromine-2-deoxyuridine (BrdU) immediately following treatment (predator stress or handling) and brains were harvested 2 hours or 4 weeks later. Despite a robust stress response detected by corticosterone (CORT) in predator stressed rats, predator stress had no effect on the total number of proliferating or surviving cells in the SGZ at 2 hours or 4 weeks post-stressor. Further investigation of overall neuronal activity in the hippocampus following predator stress is currently underway.

2-C-90 Determining the oligomeric state of the beta-site APP-cleaving enzyme 1 (BACE1) in natural membranes and detergents

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The beta-site APP-cleaving enzyme 1 (BACE1) has a transmembrane sequence (TMS), which is necessary for effective BACE1 cleavage of the amyloid precursor protein (APP). An uncommon sulfur-rich motif, MxxxCxxxMxxxCxMxC, spans the entire TMS of BACE1. The sequence is highly conserved among homologues and is reminiscent of a high-affinity binding site for Cu(I) found in other copper-transporting proteins. We designed model peptides of the BACE1-TMS to investigate metal-ion binding and oligomerization uncoupled from the cytoplasmic and the ectodomain. We found that the sulfur-rich core motif MxxxCxxxM is involved in metal-ion coordination and oligomerization of BACE1. Addition of Cu(II) facilitated the formation of dimers and trimers of the BACE1

TMS. We find peptide trimerization to depend on (i) the presence of copper ions and (ii) the sulfhydryl group of Cys466. For the full-length protein, FLIM-FRET experiments revealed that BACE1 oligomers are naturally present in living cells. We determined a stable trimeric assembly of BACE1 in the plasma membrane by accurate single-molecule fluorescence counting. Although the oligomeric state of full-length BACE1 was not altered by the addition of copper in living cells, the addition of monovalent metal-ions was required to visualize di- and oligomers by Western blot analysis. Additionally, our results demonstrate a novel metal-ion controlled stabilization mediated by the TMS of the BACE1. We propose that BACE1 acts as a bona fide metalloprotein in an oligomeric form in vivo.

2-C-91 Changes in functional connectivity correlate with attentional deficits in Alzheimer's disease

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Default mode network (DMN) task-induced deactivation may contribute to selective attention (SA) errors. We assessed functional connectivity (FC) of DMN areas in Alzheimer's disease (AD) and healthy controls (HC). 11 AD (mean age 77.3) and age-matched HC participants underwent Stroop fMRI. Verbal responses identifying the ink colour in congruent, incongruent and neutral colour words were recorded. We contrasted neural activity preceding an incongruent error between HC and AD. Four regions of interest that showed group differences served as seeds for FC analyses. AD and HC connectivity maps were compared by t-test. Results were correlated with Stroop scores. The AD group had slower reaction times and more errors for incongruent trials than HC. There was decreased activation in numerous Stroop related areas in HC, including the left anterior cingulate cortex (ACC) and precuneus (PCu). AD participants had greater activity in parietal and posterior areas, including the right lingual gyrus and superior/inferior parietal lobules, as well as right lateralized ACC and PCu activity. FC increases in AD were between the left ACC and the right precentral gyrus and the left PCu and bilateral middle occipital gyrus. Decreases were found between the left PCu and parahippocampal gyrus. We found correlations between FC and Stroop scores. AD results in altered activity preceding an error on the Stroop

task. Precuneus task-related changes are related with FC changes and correlate with poor Stroop performance. These findings help identify neural correlates underlying SA deficits in AD.

2-C-92 Glial cells at the NMJ are maladapted for reinnervation in the SOD1G37R ALS mouse model.

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the loss of motoneurons. Glial cells are known to play a major role in the disease progression which can be either beneficial or detrimental to motoneurons. However, the contribution of Perisynaptic Schwann cells (PSCs), glial cells at the neuromuscular junction (NMJ), is still ill-defined. PSCs regulate both the synaptic and structural plasticity of the NMJ and should therefore contribute to the compensatory reinnervation observed in ALS. However, we previously reported that PSC properties are altered in the partially resistant Soleus muscle at a presymptomatic stage (P120) of the disease. We first tested whether PSCs' ability to detect synaptic activity would also be changed in vulnerable fast-twitch muscles. Using Ca2 imaging, we found that glial responses evoked by motor nerve stimulation were unaltered at P120 but greatly diminished at P180 in the Sternomastoid muscle. PSCs also displayed a reduced response to purines. Since decoding of synaptic activity by PSCs is essential for the maintenance of the NMJ, these results suggest that PSCs may not respond adequately to denervation and injury in ALS. Consistent with this possibility, PSCs at denervated NMJs of end-stage animals failed to upregulate Mac-2, a marker for axonal debris phagocytosis, while PSCs at innervated NMJs upregulated it. Together, these results suggest that synapse-glia communication is altered early in ALS leading to a maladaptive and disorganised glial response to denervation.

2-C-93 Lysosomal targeting of mitochondrial vesicles requires components of the endocytic system: implications for Parkinson's disease

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Proper mitochondrial function is essential for neuronal health. Whereas mitochondrial dysfunction has long been associated with Parkinson's disease (PD), we have only recently begun to understand the molecular mechanisms underlying this process. Notably, studies concerning the cellular functions of PARKIN and PINK1, two genes linked to familial PD, have indicated that a loss of mitochondrial quality control (MQC) mechanisms may underlie PD pathogenesis. In cells, parkin and PINK1 have been shown to target entire, dysfunctional mitochondria for degradation through the autophagy-lysosome system. Additionally, we have recently reported that, in response to oxidative stress, parkin and PINK1 are also required for the selective removal of damaged mitochondrial proteins from the organelle via mitochondria-derived vesicles (MDVs), which target to lysosomes independently of autophagy. Thus, PINK1/parkin MQC functions at two distinct, yet complimentary, levels. Our recent data indicate that, following induction of MDV formation, parkin accumulates on multivesicular bodies (MVBs), suggesting turnover of vesicles through the MVB-lysosome pathway - a targeting mechanism typically associated with cell surface receptor endocytosis and degradation. Moreover, an siRNA-based screen has identified components of the endocytic/MVB pathway required for turnover of parkin/PINK1 MDVs. We hypothesize that interactions between parkin and the endocytic system facilitate the targeting of MDVs to lysosomes, and that disruption of these delivery mechanisms contributes to the pathogenesis of PD.

2-C-94 GABA Neuron Inhibition in the Ventral Hippocampus Induces Behavioural Models of Schizophrenia

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Hyperactivity in the ventral hippocampus (vHPC) has been implicated in the pathophysiology of schizophrenia. A proposed mechanism underlying this hyperactivity is dysregulation in the GABA system. Studies on postmortem brains and animal models have provided indirect evidence of reduced activity in GABA neurons, specifically parvalbumin neurons. However, the role of vHPC GABA neurons in schizophrenia-associated behaviours has not been directly examined. Here, we tested the effect of

selective GABA or parvalbumin neuron inhibition in the vHPC on behavioural assays of schizophrenia. The DREADD (Designer Receptors Activated by Designer Drugs) receptor hM4D was expressed in vHPC GABA or parvalbumin neurons by infusing a Cre-dependent viral vector (AAV-FLEX-hM4D) into the vHPC of Gad65:cre or Parvalbumin:cre mice respectively. In hippocampal slice recordings, bath application of clozapine-N-oxide (CNO) reduced the firing rate of parvalbumin neurons expressing hM4D. GABA neuron inhibition with CNO increased both spontaneous and amphetamine-induced locomotor activity while parvalbumin neuron inhibition did not significantly change locomotor activity. The effects of GABA or parvalbumin neuron inhibition on prepulse inhibition and social interaction were also examined. These findings provide support for the role of reduced ventral hippocampal GABA neuron activity in symptoms of schizophrenia.

2-C-95 EARLY EFFECTS OF NEUTROPHILS AND IL-1 β IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS.

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Increasing evidence suggests that neutrophils are key players in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. In vitro and in vivo studies have revealed that neutrophils participate in dendritic cell recruitment, activation as well as immune activity in lymph nodes. Accordingly, our group and others found that neutrophil depletion delayed the onset and reduced the severity of EAE. Another key factor for EAE development is interleukin-1 β (IL-1 β). The effects of IL-1 β have been reported to be widespread and diverse, and include endothelial cell activation, leukocyte activation and recruitment and Th17 cell differentiation. Here, we show that neutrophils are important producers of IL-1 β in the blood and spinal cord of EAE mice. Flow cytometric analysis revealed that neutrophil counts are normal in the bone marrow of IL-1 β -knockout (KO) mice, but greatly reduced in the blood, both before and at disease onset (7 and 10 dpi, respectively). Furthermore, we found that neutrophils of IL-1 β -KO mice are significantly smaller than those of wild-type controls, a phenomenon that may reflect their

maturation or activation state. In contrast, lymphocytes were present in higher numbers in the bone marrow and blood of IL-1 β -KO mice after immunization, perhaps a sign that they were locked outside of the CNS. Together, these results add more weight to the implication of neutrophils in EAE. Further work is needed to study the impact of neutrophils and IL-1 β in antigen presentation and T cell activation and polarization.

2-C-96 Familial Prion protein mutants inhibit HRD1-mediated retrotranslocation of misfolded proteins

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Prion diseases are neurodegenerative disorders caused by a misfolded isomer of the ubiquitously expressed prion protein (PrP) (Prusiner, 1982). Mostly expressed at the cell surface, a small proportion of PrP is found in the cytosol (CyPrP) arising from the endoplasmic reticulum associated degradation (ERAD) pathway (Yedida, 2001, Roucou, 2003). This CyPrP has been shown to prevent Bax-mediated apoptosis in both human primary neurons (HPN) and MCF7 cells (Lin, 2008). Familial PrP mutants fail to undergo retrotranslocation, and this correlates with a loss of PrP's anti-Bax function (Jodoin, 2007, 2009). HPNs contain a considerable amount of endogenous PrP suggesting that mutant PrP may generally inhibit the retrotranslocation of proteins to the cytosol. To investigate this effect, we first identified that the E3 ubiquitin ligase, Hrd1, was involved in PrP's retrotranslocation in human CR7 cells submitted to Brefeldin A and proteasome inhibitor, epoxomicin. Overexpression of eYFP-Hrd1 increased levels of CyPrP, while siRNA against Hrd1 significantly decreased levels of CyPrP. The expression of PrP mutants V210I and M232R both decreased CyPrP levels and concomitantly prevented retrotranslocation of model Hrd1-mediated ERAD substrates, transthyretin mutant TTRD18G and α 1-anti-trypsin variant, A1AT-NHKKKK. These results indicate an inhibition of Hrd1-dependent retrotranslocation by familial PrP mutants. The accumulation of ERAD-substrate proteins, combined with a loss of anti-Bax protection, could explain the age-dependant neurodegeneration seen in familial prion disease.

2-C-97 Regional role of D1 receptor in responses to psychostimulants in new

conditional D1 dopamine receptor knockout mice**Quentin Rainer¹, Merouane Messekher¹, Bruno Giros¹**¹Douglas hospital research centre

There is considerable evidence that drugs of abuse converge on a common circuitry, the mesolimbic dopamine pathway, involving the ventral tegmental area (VTA) the nucleus accumbens (Nac). Nac is part of the striatum, involved in motor control and motivational processes, altered in drug addiction and schizophrenia. Its neuronal population is mostly composed of medium spiny neurons (MSNs). Dopaminergic signaling within the basal ganglia has been thought to occur in the striatonigral MSNs which contains the dopamine D1 receptor (D1R) and the striatopallidal MSNs which expresses dopamine D2 receptor (D2R). While there is still no clear consensus, increasing evidence indicate that D1R and D2R can be co-expressed in a subpopulation of striatal MSNs. A number of studies have shown that D1R and D2R are involved in cocaine-induced molecular and behavioral changes but required their co-activation. Therefore more selective approaches are needed, including cell-type-specific, region-specific manipulations of the dopamine receptors to better elucidate their functional roles in drug addiction. To target specific neuronal populations, we generated a novel strain of mice allowing a conditional deletion of the D1R gene, the D1loxP mice. This strain is under characterization using AAVcre virus injections in striatal regions. D1loxP mice will be bred with either D1Cre or D2Cre mice, which express Cre recombinase under the D1 or D2 promoters, respectively. This tool will allow a better understanding of the D1R involvement in drug of abuse mechanisms according to its expression in the brain.

2-C-98 Altered Dopamine Transporter and Caspase-3 Staining in the Prefrontal Cortex in Rats Following Neonatal Treatment With Domoic Acid**Mark Robbins¹, Catherine Ryan¹, Tracy Doucette¹**¹University of Prince Edward Island

Schizophrenia is a debilitating neurodevelopmental disorder which is characterized by positive, negative, and cognitive symptoms. Decreased glutamate and dopamine (DA) signaling in the medial prefrontal

cortex (mPFC) is postulated to underlie cognitive symptoms of schizophrenia. In order to improve our understanding of schizophrenia it is important to develop and further characterize neurodevelopmental animal models with relevance to the human condition. Previous research has demonstrated that rodents exposed to neonatal treatment with domoic acid (DOM) (20 µg/kg), a kainate receptor agonist, during a critical period of brain development (i.e. post-natal day 8-14) produces animals that exhibit several behavioural, neurochemical, and neuroanatomical abnormalities that are characteristic of schizophrenia. The current study assessed whether DOM treatment produces alterations in developmental apoptosis as evidenced by altered caspase-3 staining and DA system integrity as evidenced by altered DA transporter (DAT), D1R, and D2R in the mPFC. It was hypothesized that DOM administration would produce alterations in neurodevelopmental apoptosis with consequent alterations in the DA system. Results demonstrated increased DAT staining in the right prelimbic cortex (PRL) of DOM-treated females and decreased caspase-3 staining in the right PRL of both DOM-treated females and males. These results serve to strengthen the construct and etiological validity of the model as they demonstrate neurochemical alterations which are associated with behavioural symptomatology.

2-C-99 Transcriptional Deficits in an Isogenic Stem Cell Model of Parkinson's**Tammy Ryan¹, Scott Ryan¹**¹The University of Guelph

Neuronal loss in Parkinson's Disease (PD) is associated with both aberrant mitochondrial function as well as impaired proteostasis in dopaminergic neurons (DA) of the substantia nigra pars compacta. A strong association has been reported between PD and exposure to mitochondrial toxins such as the environmental pesticides paraquat, maneb, and rotenone. These toxins are associated with increased oxidative stress that may link mitochondrial dysfunction with aberrant proteostasis. Using a robust, patient-derived human induced pluripotent stem cell model (hiPSC) of PD that allows for comparison of A53T-SNCA mutant cells against isogenic mutation-corrected controls, in addition to a human embryonic stem cell (hESC) model with the A53T-SNCA mutation introduced, we generated A9-type DA neurons (hNs). An analysis of mitochondrial

function in these neurons identified perturbations in mitochondrial respiration specific to A53T mutant hNs. A decrease in maximal respiratory capacity was observed in A53T hNs following mitochondrial toxin exposure. Furthermore, we report a novel molecular pathway whereby basal as well as toxin-induced stress inhibited the MEF2C-PGC1 α -NRF2 transcription network in A53T hNs, leading to mitochondrial dysfunction and cell death. This occurred, in part, through redox-based modification of MEF2C that prevented the transcription factor from binding to the PGC1 α promoter. Our data provide mechanistic insight into gene by environment interactions in the pathogenesis of PD, and identify NRF2 as a promising target for therapeutic development.

2-C-100 Injury to distant neuronal fibers activates retinal glia, followed by neuronal cell cycle re-entry and DNA hyperploidy, and neuronal death.

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Retinal ganglion cells (RGCs) are neurons that relay visual signals from the retina to the brain. The RGC cell bodies reside in the retina and their fibers form the optic nerve. Full transection (axotomy) of the optic nerve is an extra-retinal injury model of RGC degeneration. Optic nerve transection permits time-kinetic studies of neurodegenerative mechanisms in neurons and resident glia of the retina, the early events of which are reported here. One day after injury, and before atrophy of RGC cell bodies was apparent, glia had increased levels of phospho-Akt, phospho-S6, and phospho-ERK1/2; however, these signals were not detected in injured RGCs. Three days after injury there were increased levels of phospho-Rb and cyclin A proteins detected in RGCs, whereas these signals were not detected in glia. DNA hyperploidy was also detected in RGCs, indicative of cell cycle re-entry by these post-mitotic neurons. These events culminated in RGC death, which is delayed by pharmacological inhibition of the MAPK/ERK pathway. Our data show that a remote injury to RGC axons rapidly conveys a signal that activates retinal glia, followed by RGC cell cycle re-entry, DNA hyperploidy, and neuronal death that is delayed by preventing glial MAPK/ERK activation. These results demonstrate that

complex and variable neuro-glia interactions regulate healthy and injured states in the adult mammalian retina.

2-C-101 Effects of Task-Irrelevant Emotional Face Processing on Bipolar Disorder and Attention-Deficit Hyperactivity Disorder Patients on an Antisaccade Task

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Differentiating symptoms between Attention-Deficit Hyperactivity Disorder (ADHD) and Bipolar Disorder (BD) is difficult because the core features of ADHD are quite similar to the symptoms of BD. Antisaccade tasks, which require inhibiting a prepotent visual response, probes differences between these groups which may differentiate diagnoses based on each group's distinct reaction profile. Performance deficits would represent the underlying neuropathology of these disorders, specifically for deficits of executive control. 20 ADHD, 20 BD, and 25 Control subjects performed an interleaved pro and antisaccade task (look toward vs away from visual target, respectively). In order to further differentiate between subject groups, standardized emotional faces (fear, happy, sad, neutral) were used as task-irrelevant stimuli, which are expected to influence emotion-processing deficits in BD, with control images (object, no picture). In all conditions, Controls had faster mean saccadic reaction times and reduced variability compared to ADHD and BD groups while the ADHD group was faster than the BD group. Controls also made less antisaccade direction errors (prosaccade on antisaccade trial). Emotion effects were group dependent: fear increased direction errors for ADHD subjects while neutral had the same effect on BD subjects. In conclusion, there is a clear pattern separating Controls from ADHD and BD groups and these patient groups from each other, demonstrating emotion-processing deficits that are disorder-specific as well as the usefulness of eye movements as a biomarker.

2-C-102 The Neuroprotective Role of Gap Junctions in Brain Glucose Deprivation

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Severe brain hypoglycemia, as the result of an insulin overdose in diabetic patients, can cause serious neurological complications such as seizures and coma, resulting in brain death. However, the mechanisms underlying hypoglycemic seizure generation and propagation remain unclear. Gap junctions not only play a role in neural synchrony, promoting seizure activity, but are also known to be involved in cell death mechanisms during metabolic inhibition. We found that mouse brain slices perfused with low-glucose (0.5 mM) artificial cerebral spinal fluid (aCSF) typically experienced several repetitive seizure-like events (SLEs), after which there is an irreversible loss of evoked potentials within 30 minutes unless immediately rescued by normal glucose aCSF. When gap junction blockers were added to the hypoglycemic perfusate, SLEs were blocked and neuronal death was prevented. Blockade of pannexins using a selective blocker, however, prevented neither the hypoglycemic SLEs nor the subsequent loss of field response. These data suggest that blockade of gap junctional communication plays a neuroprotective role during brain glucose deprivation. Supported by JDRF and CIHR

2-C-103 The overexpression of mutated tau in the entorhinal cortex: its effects on local neurons, cortical theta oscillations, and memory

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The accumulation of hyper-phosphorylated tau protein in the entorhinal cortex is one of the first abnormalities observed in Alzheimer's disease (AD). The entorhinal cortex is reciprocally connected with many cortical regions critical for memory formation and expression. Entorhinal tau pathology would therefore disrupt the transfer of information with the cortical memory network, resulting in memory impairments in AD. To test this hypothesis, we expressed, through transfection with a viral vector, an excess of human tau with the P301L mutation (Tau rats) or green fluorescent protein as a control (GFP rats), specifically in the entorhinal cortex of adult rats. We then tested the rats' ability to acquire an associative memory in trace eyeblink conditioning while monitoring local field potentials in the hippocampus, a major efferent target of the entorhinal cortex. One month after transfection, approximately 20% of neurons

were filled with hyper-phosphorylated tau and the area adjacent to the injection sites showed signs of astrogliosis and neurofibrillary tangles. When tested in trace eyeblink conditioning, the Tau rats formed the association between a tone and eyelid stimulation in a comparable manner to the GFP rats; however, the hippocampus of the Tau rats had relatively smaller amplitude of theta oscillations upon tone presentation in comparison to GFP rats. Thus, minor entorhinal tau pathology attenuates the transfer of stimulus information to the hippocampus, which is detectable at the level of neuronal activity even before memory impairments become apparent.

2-C-104 Deletion of C9ORF72 results in motor neuron degeneration and stress sensitivity in *C. elegans*

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An expansion of the hexanucleotide GGGGCC repeat in the first intron of C9ORF72 gene was recently linked to amyotrophic lateral sclerosis. It is not known if the mutation results in a gain of function, a loss of function or if, perhaps both mechanisms are linked to pathogenesis. We generated a genetic model of ALS to explore the biological consequences of a null mutation of the *Caenorhabditis elegans* C9ORF72 orthologue, F18A1.6, also called alfa-1. alfa-1 mutants displayed age-dependent motility defects leading to paralysis and the specific degeneration of GABAergic motor neurons. alfa-1 mutants showed differential susceptibility to environmental stress where osmotic stress provoked neurodegeneration. Finally, we observed that the motor defects caused by loss of alfa-1 were additive with the toxicity caused by mutant TDP-43 proteins, but not by the mutant FUS proteins. These data suggest that a loss of alfa-1/C9ORF72 expression may contribute to motor neuron degeneration in a pathway associated with other known ALS genes.

2-C-105 Differential changes in microglial ultrastructure in the APP^{sw}-PS1 mouse model of amyloid- β deposition

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Microglial activation is well-documented in Alzheimer's disease (AD), especially near the plaques of amyloid- β (A β), but their implication remains undetermined. In this study, we examined microglial ultrastructure in the APP^{swe}-PS1 mouse model of A β deposition, at 6 months of age where the impairment of synaptic plasticity, learning and memory is first observed, using immunocytochemical electron microscopy. To visualize microglial cell bodies and processes, immunohistochemistry staining for Iba1 (ionized calcium binding adaptor molecule 1) was performed with diaminobenzidine. Our analysis in the hippocampus CA1 reveals that diseased microglia present distinct morphologies, ultrastructural features and interacting partners, depending on their proximity to the plaques. For example, microglia displayed various signs of cellular stress including darkening of their cytoplasm and nucleoplasm, mitochondrial dysfunction, dilation of the endoplasmic reticulum, and overall shrinkage, especially in the vicinity of the plaques and blood vessels. In regions of cellular dystrophy, microglia with enlarged cell bodies and processes containing various types of inclusions were also encountered. Nevertheless, in regions distanced from the plaques, healthier-looking microglia frequently juxtaposed axon terminals, dendrites and dendritic spines as during normal physiological conditions, but their phagocytosis was significantly reduced. These results suggest distinct microglial phenotypes which could be involved in the complex mechanism of AD pathogenesis.

2-C-106 The role of the innate immune system in neuronal toxicity in *C. elegans* models of ALS

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Amotrophic lateral sclerosis (ALS) is the most common age-related neurodegenerative disease affecting motor neurons. Symptoms are characterized by progressive paralysis leading to respiratory deficiency. 90-95% of the cases are sporadic and 5-10% are inherited. Many of the genetic causes are now known but no curative treatments are available. Recent studies have shown there may be an abnormal contribution of the immune system (IS) in ALS. The Toll-Like Receptor (TLR) is one of the main components of the innate IS, and TLR mRNA expression is increased in the cerebrospinal

fluid of ALS patients. To better understand the innate IS role in this pathology, we have turned to the model organism *C. elegans*. We have created novel models of ALS in *C. elegans* based on the expression of ALS-associated human mutations in the worms motor neurons. We have focused on genes encoding TAR-DNA binding protein 43 (TDP-43) and fused in sarcoma (FUS). Mutations in these genes are found in 5-10% of familial ALS cases. Worms expressing mutant TDP-43 or FUS proteins show progressive motility defects accompanied by motor neuron degeneration. Our preliminary data suggests that genes encoding components of the innate IS, including TLR, promote neurodegeneration associated with mutant TDP-43 and FUS proteins. Genetically disabling the TLR signalling pathway improves movement and decreases neurodegeneration in our *C. elegans* ALS models. Our data suggest that TLR immune pathway genes may be new targets for therapeutic development. An update of our findings will be presented.

2-C-107 Overexpression of the insulin-like growth factor-II receptor increases β -amyloid production in fibroblast

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The insulin-like growth factor-II (IGF-II) receptor involves in the transport of newly synthesized lysosomal enzymes from the trans-Golgi network to endosomes. The endosomal-lysosomal system, the major site of IGF-II receptor expression, plays a critical role in the processing of amyloid precursor protein (APP) leading to the generation of β -amyloid (A β) peptide - a key player in the development of Alzheimer's disease pathology. However, the role of IGF-II receptor in APP processing remains unclear. To address this issue we used IGF-II receptor overexpressing and deficient fibroblasts to study the influence of the receptor on APP processing and A β metabolism. A variety of biochemical assays were used to measure mRNA or protein levels of APP, its processing enzymes and A β in these cells. Confocal microscopy and lipid raft isolation were used to detect the distribution of the IGF-II receptor, APP and its processing enzymes. Our data revealed higher mRNA levels of APP as well as β - and γ -secretases in IGF-II receptor overexpressing cells. Accordingly, we observed increased levels of APP, precursors of A β and activities of β - and γ -secretases in these cells. Secreted APP and A β 1-40/A β 1-42 were also

higher in their conditioned media. These changes were reversed by knocking down IGF-II receptor levels. Additionally, higher levels of APP and its processing enzymes were localized with IGF-II receptors on lipid raft, an active APP processing site. Our results suggest that overexpression of IGF-II receptors can increase APP level/processing leading to enhanced production of A β .

2-C-108 Inhibitor of Apoptosis Stimulating Protein of p53 (iASPP) is required for retinal ganglion cell survival after axonal injury

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Purpose: p53 apoptotic activity is regulated by the apoptosis-stimulating proteins of p53 (ASPP) family members ASPP1/2 and iASPP. We previously showed that pro-apoptotic members ASPP1/2 contribute to p53-dependent death of retinal ganglion cells (RGC), yet the role of the p53 inhibitor iASPP in the CNS is unknown. Here, we addressed the role of iASPP on RGC survival in a model of acute optic nerve injury. **Methods:** iASPP knockdown was carried out by intravitreal injection of small interference RNA (si-iASPP). Overexpression of iASPP in RGCs was achieved by intraocular delivery of adeno-associated virus (AAV.iASPP). Phosphoserine immunoprecipitation was performed on retinal lysates of intact, axotomized, and iASPP overexpressing retinas. iASPP, Fas/CD95, PUMA, Noxa and Bax protein levels were examined by retinal immunohistochemistry and western blot analysis. RGC densities were assessed by quantification of Brn3a-positive cells on retinal whole mounts. **Results:** Our data show that iASPP is expressed by intact and injured RGCs, and that iASPP phosphoserine levels are significantly reduced following axotomy. We show that iASPP downregulation by siRNA exacerbates RGC death, whereas selective AAV-mediated overexpression of iASPP promotes robust RGC survival. iASPP overexpression following axotomy results in an increase of iASPP phosphoserine levels and downregulation of PUMA and Fas/CD95. **Conclusions:** Our study demonstrates a novel role for iASPP in the death of RGCs, and provides further evidence of the importance of

ASPP family in CNS neuronal survival after axonal injury.

2-C-109 5HT receptor neurons differentially modulate locomotor recovery from anoxia in Drosophila

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Locomotor recovery from an anoxic coma is dependent on a rapid restoration of ion distribution in neural tissue followed by recovery of neuronal circuit function. The cellular and molecular mechanisms underlying the complete recovery of locomotor coordination are largely unknown. Preliminary data showed that knockdown of the Drosophila white gene (encoding an ABC transporter) in serotonergic neurons was sufficient to delay locomotor recovery. We aimed to identify the roles of different serotonin (5HT) receptor neurons in locomotor recovery from anoxia. 5HT1A, 5HT1B, 5HT2A and 5HT7 receptor neurons were targeted by (1) overexpressing the pro-apoptotic factor Grim and (2) white knockdown. We characterized locomotor recovery in a behavioural assay. We found that genetic ablation through Grim expression in 5HT receptor neurons was lethal when combined with some Gal4 lines, or caused severe delay of locomotor recovery with others. white knockdown in all 5HT receptor neurons caused significant delays of locomotor recovery. White transports small molecules, including cyclic guanosine monophosphate (cGMP) and biogenic amines, and may improve signaling efficacy. We expressed a cGMP-specific bovine phosphodiesterase (bPDE5) in 5HT receptor neurons and found that bPDE5 expression in 5HT1A neurons but not 5HT1B, 5HT2A or 5HT7 neurons caused a severe delay of locomotor recovery. Therefore, 5HT receptor neurons differentially modulate locomotor recovery, although normal White expression is also important during recovery from anoxia.

2-C-110 Role of ceruloplasmin on iron accumulation after permanent experimental brain ischemia.

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Several studies have shown the relation between iron and ferritin levels on clinical outcome in stroke patients. However, the

understanding of the mechanisms underlying iron accumulation after brain ischemia is still lacking. Ceruloplasmin (Cp) is a multicopper enzyme that oxidizes the ferrous form of iron (Fe²⁺) to its ferric form (Fe³⁺). We induced permanent cerebral ischemia in Cp knock-out (CpKO) and C57BL/6 wild type (WT) male mice of 8-10 weeks of age by permanently occluding the middle cerebral artery (pMCAO) with a 9-0 suture. Infarct volume and the adhesive removal test (ART) were done at different time points and tissue from pMCAO and sham control animals was collected to determine protein expression. CpKO mice had an increased infarct volume at 24h compared with WT animals. We also observed an asymmetry in the ART in the WT group 24h and 7 days post-MCAO with an spontaneous recovery 14 days after the occlusion. However, in CpKO animals the asymmetry kept progressing with time, being significantly different from the WT group 14 days after pMCAO. Western blot data on WT animals shows that ceruloplasmin is upregulated 24h post-pMCAO, peaks at 72h and starts decreasing 14 days after pMCAO. Ferritin expression is upregulated 24h after pMCAO and increases with time presenting the highest expression 14 days after pMCAO; the iron influx transporter, DMT1, reaches peak expression 72h post-pMCAO. These data suggests that Cp plays an important role in infarct evolution and is accompanied by changes in the expression of other iron homeostasis proteins after brain ischemia.

D - Sensory and Motor Systems

2-D-111 Influence of Visual Feedback on Gaze-Dependent and Location-Dependent Errors in Grasping Movements

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Previous studies have shown that visual feedback from the target and the hand is important to enhance the accuracy of reaching movement. A recent study found that online visual feedback from the hand suppresses gaze dependent errors (Dessing et al, 2012). In a previous experiment (AlOmawi et al, 2013) we investigated the influence of gaze and target positions on the transport, final grip size, and orientation components in a reach to grasp task during open loop condition. This paradigm

utilized rectangular 'virtual' targets presented at 3 orientations, 3 locations, and with 3 gaze fixation positions. Here we used the same paradigm to investigate the influence of online visual feedback (VF) on the grip components. 7 subjects reached to grasp a target during 4 VF conditions: brief target presentation with no online VF (NVF), vision of the target (TVF), hand (HVF), and both target and hand (BVF). We found that reach location errors related to gaze and target location were highly correlated between NVF and TVF, whereas, BVF errors correlated to HVF errors. However, the modulation of HVF and TVF was dependent on both of the stimulus and gaze directions. HVF increased the reach location errors for the central target and reduced these errors when targets were located to the left and right relative to the body midline. In addition, looking directly to viewed target led to accurate grip size, while HVF increased the grip size. The results suggest that processing hand visual information adds more complexity to the system and may rely on different mechanisms as compared to no VF task.

2-D-112 Involvement of foot afferents in corrective postural reactions

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Several studies suggest that corrective reactions to perturbations are mediated by plantar muscle afferents projecting to leg muscles, but their precise role is not fully understood. The goal of this study was to assess the contribution of foot afferents in corrective postural reactions. **METHODS:** Electrical stimulation to the right posterior tibial nerve (PTN) below the internal malleolus was applied at different delays during unexpected forward or backward tilts in 8 healthy subjects. EMG activity of right flexor digitorum brevis and soleus (SOL) were recorded. PTN stimulation (2-2.5 x motor threshold) was applied during quiet standing, prior to and during the perturbations. **RESULTS:** 1) Quiet standing: PTN stimulation induced a short-latency suppression (53±6 ms), a medium-latency facilitation (67±6 ms) and a long-latency facilitation (92±5 ms) in SOL. 2) Standing between perturbations: the short-latency suppression appeared more rapidly (difference = -9.1 ms, Student's t test, p<0.01) and amplitude of suppression was increased compared to quiet standing (35%, p<0.01). 3) Backward tilt: the short latency suppression observed during

standing reversed to a facilitation at 150 ms after tilt onset (185% vs 64% during standing; $p < 0.001$) and the facilitation observed at long-latency became an inhibition (57% vs 141% during standing; $p < 0.01$). 4) Forward tilt: the short-latency suppression increased but insignificantly. **DISCUSSION:** PTN stimulation induces responses in SOL that are modulated in a task-specific way and may contribute to induction of postural reactions. NSERC, REPAR

2-D-113 Expression of CB1, CB2, and GPR55 in the monkey retina

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The endocannabinoid system is present in the mammalian central nervous system including the retina, and is responsible for the regulation of many physiological processes. Recent anatomical and functional data collected in the rodent retina indicate that cannabinoid receptors are important mediators of retinal functions. While the expression pattern of cannabinoid receptors has been well established in the rodent retina, little data is available for the primate. Using confocal microscopy, we show here the differential expression of CB1R, CB2R, and GPR55 in the vervet monkey retina (*Chlorocebus sabaeus*). We found that CB1R is present mainly in cone photoreceptors and other retinal components. Indeed, higher CB1R expression was detected in the glutamatergic vertical signal pathway, namely bipolar and ganglion cells. Moderate to low expression of CB1R was also found in horizontal and amacrine cells. CB2R was exclusively located in the retinal glia, the Müller cells. Expression of GPR55, a recently de-orphanized receptor and a putative cannabinoid receptor, was restricted to rod photoreceptors. These results show that these receptors are differentially expressed in the retinal mosaic of monkeys and might have a functional role in visual perception.

2-D-114 The Toolish Hand Illusion: Motor experience facilitates incorporation of a tool.

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Almost 15 years ago, Botvinik and Cohen discovered that when subjects watched their own hand being brushed synchronously with a fake hand, they felt like the fake hand was their real hand. This illusion, called Rubber Hand Illusion (RHI), has since been studied by many researchers who found that a key aspect is the visual similarity between the fake hand and the subjects' hand. Indeed, the RHI arises only in presence of a fake hand and not other objects, as for example wooden blocks (even when they are shaped as a human hand). Here we tested whether functional similarity (instead of anatomical similarity) is sufficient for the illusion of ownership to extend to non-hand-shaped tools. In particular, we wanted to test whether it is possible to induce the illusion by stroking a grabber that shares the same functionality of a human hand (to grasp), despite its different visual appearance. We hypothesized that motor experience with the tool would be necessary to induce the illusion. We tested subjects in a modified version of the classical RHI paradigm. Subjects were asked to observe a grabber being stroked synchronously (test) or asynchronously (control) with their own (hidden) right hand, before and after a short period of tool-use consisting in grasping and lifting objects with the grabber. We used three different measures: proprioceptive drift, questionnaire and GSR (Galvanic Skin Response) to threat. Crucially, subjects had no previous experience with the tool prior to the experiment. Results from this study showed that it is possible to experience an illusory sen

2-D-115 The effect of training and cholinergic stimulation on visual capacities in healthy rats.

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Electrical stimulation of the cholinergic system paired with visual stimulation to a specific pattern induces long-term enhancement of visual acuity for this pattern in rats. We evaluated whether repeated visual exposure of a specific stimulus paired with pharmacologically induced build-up of acetylcholine (Donepezil administration) would change the cortical activity and visual acuity of the rats for the trained stimulus. Donepezil, a specific inhibitor of acetylcholine esterase, is used for the treatment of Alzheimer's patients. We recorded visual evoked potentials (VEPs) in rat's primary visual cortex before and after a 2 weeks visual

exposure of a sinusoidal grating pattern phase converting, shown in a pseudo-random manner for 30° and 120° orientations and spatial frequency. During the 2 weeks of visual exposure (10min/day, 0.12CPD training frequency), the cholinergic system was stimulated through an electrode implanted in the basal forebrain or by injecting Donepezil (i.p., 0.5mg/kg daily) 30min prior to visual exposure. Pharmacological and electrical stimulation of the cholinergic system during the visual training induced similar enhancement (70% increase) of the cortical response to visual stimulation. VEPs were enhanced in the electrically stimulated group ($p=0.046$) and in Donepezil-injected group at the trained frequency 0.12 cpd ($p=0.043$). Our study suggests that pharmacological stimulation of the cholinergic system via Donepezil administration is a potential method for improving visual capacities if coupled with visual training.

2-D-116 Local Adaptation of Feedback Responses and Voluntary Reaching Movements

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A hallmark of voluntary motor control is the ability to adapt our motor patterns to physical loads applied to the limb. This adaptation generalizes to movements that differ in amplitude, direction, and speed, with the amount of learning decaying rapidly with the distance from the training conditions. In parallel, recent studies have highlighted feedback responses that mirror the adaptation of voluntary behaviour, leading to the hypothesis that feedback responses should exhibit the same learning patterns expressed during voluntary actions. Here we investigate whether feedback responses compensate for novel interaction loads, and then measure how these adapted responses transfer across conditions requiring identical or different joint motion patterns. Participants reached to two targets while adapting to loads that altered the relationship between elbow and shoulder motion. On random trials, we applied elbow perturbations while subjects reached to a probe target that required only shoulder motion. We found that, as subjects adapted their reaching patterns, shoulder muscle responses compensated for the novel interaction loads. Importantly, these adapted feedback responses generalized when subjects reached from different workspace locations to targets requiring identical joint

motion patterns, but this transfer was non-existent when joint motion patterns differed from the training task. We propose that a common learning mechanism governs the adaptation of feedback control and voluntary action, and produces learning that is localized and sensitive to the training conditions.

2-D-117 Direct conversion of endogenous cells into functional neurons in the mammalian inner ear using defined transcription factors

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Hearing loss is the fastest growing and one of the most prevalent chronic conditions today affecting 600 million people worldwide. Primary auditory neurons (spiral ganglion neurons) are crucial in hearing as they transmit sound information from the inner ear to the brain. Auditory neurons are lost due to disease, excessive noise and aging; and like most neurons, once lost they do not regenerate. Hence, they are a primary target for regeneration since the induction of even a small number of neurons in a damaged ear could have significant impact on hearing restoration. One approach to hearing loss treatment is the use of gene therapy for the induction of endogenous cells. Two target cell populations in the mouse cochlea for induction are non-sensory epithelial cells and spiral ganglion glial cells. We have used neurogenic transcription factors known to directly reprogram cells and induce neurons in several systems as well as transcription factors required for the generation and survival of auditory neurons. Overexpression of these factors in vitro induced neurons at high efficiency at embryonic, postnatal and juvenile stages. The induced neurons expressed neuronal markers and were electrophysiologically functional producing action potentials. Thus, overexpression of transcription factors is sufficient to convert endogenous cochlear cells into functional neuron-like cells. We will investigate combinatorial factors that induce phenotypes that most closely resemble auditory neurons, and examine connectivity to the inner ear in the periphery and the cochlear nucleus in the CNS.

2-D-118 Optical inhibition of peripheral pain pathways in freely moving optogenetic mice

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The nerve endings of peripheral nociceptors are the initiation sites of nociceptive transduction, therefore their selective activation and/or silencing can control pain perception. Using a conditional genetic strategy, we sought to generate an analgesic model in which the activity of nociceptors is optically silenced using light-gated inhibitory opsins. This approach consisted of expressing the proton pump Arch or the chloride pump Halorhodopsin-3 (eNpHR3.0) in the Nav1.8-positive nociceptors, using the Nav1.8-Cre recombinase driver line. Cellular distribution of the Arch-EGFP and eNpHR3.0-EYFP constructs was assessed in fluorescence in dorsal root ganglia (DRG), trigeminal ganglia, sciatic nerve, glabrous skin and dorsal horn of the spinal cord, and showed a strong and selective expression of these opsins in nociceptive soma and fibers. Electrophysiological recordings on cultured DRG neurons revealed significant outward photocurrents and hyperpolarizations in response to yellow light (589 nm) stimulation. These light-evoked hyperpolarizations were sufficiently large to block electrically- as well as chemically ($\alpha\beta$ meATP)-induced action potentials in Arch-expressing neurons. Strong expression of Arch in the periphery translated into significant reduction in mechanical allodynia under inflammatory conditions, providing evidence for a novel promising analgesic model in which nociception is optically silenced in awake, behaving mice.

2-D-119 Pallidal neurons and their afferent projections are influenced by volume transmission of acetylcholine in primates

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The primate basal ganglia receive a highly heterogeneous cholinergic (ACh) innervation arising chiefly from the pedunculopontine tegmental nucleus (PPN) of the brainstem. The present light and electron microscopic study characterizes and compares the ACh innervation of the internal (GPi) and external (GPe) segments of the globus pallidus in squirrel monkeys (*Saimiri sciureus*) following labeling with an antibody against choline acetyltransferase (ChAT). Unbiased counts of

ChAT+ axon varicosities reveal that the total density of innervation is significantly lower in GPi (0.26 ± 0.03 million axon varicosities / mm³) than in GPe (0.47 ± 0.07 million), with an anteroposterior decreasing gradient and a dorsoventral increasing gradient in both pallidal segments. Unbiased neuronal counts on Nissl-stained adjacent sections indicate that the number of ChAT+ axon varicosities per pallidal neurons is not statistically different in GPi compared to GPe (74 ± 9 and 127 ± 29 varicosities / neuron, respectively). At the electron microscopic level, ChAT+ axon varicosities in GPi and GPe are comparable in size and shape. Only 20% of ChAT+ axon varicosities establish a synaptic contact, indicating that ACh neurotransmission occurs predominantly through volume transmission in both pallidal segments. The absence of axo-axonic contacts suggests that presynaptic ACh modulation of pallidal afferents is mainly asynaptic. Altogether, these results indicate that the PPN exerts a potent influence on the GPi and the GPe, mainly through volume transmission of ACh.

2-D-120 White Noise Visual Motion Reconstruction From MEG

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Decoding time-varying stimuli from human brain activity by means of functional neuroimaging has been difficult in part due to poor signal quality. Here we derived the relationship between randomly time-varying motion stimuli and MEG signals at both the sensor and source level. Subjects fixated on a cross hair between two circular random dot patches located in opposite visual fields for the full duration of the trial. Our stimuli consisted of pulses of coherent motion made from white random dot patterns on a grey background. The pulse sequence was random on every trial. Our approach to reconstructing the motion stimulus was derived from electrophysiology, where the latency at which neurons increase their firing rate to fast random stimuli ("white noise input") is assessed by means of an optimal reconstruction filter. Our model was a standard linear filter followed by a static nonlinearity (LN model), which was physiologically plausible and allowed comparison to single cell electrophysiological responses in visual cortex. Our analysis accounted for up to 3% of the variance in the stimuli. We observed filters in occipital and occipital-parietal areas with properties similar to

filters estimated with single cell electrophysiology.

2-D-121 TMS-induced plasticity causes changes in cerebral blood flow

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Plasticity in the human brain represents the intrinsic property of neurons to alter their activity in response to physiological changes and experiences, such as reorganization due to adaptation, training, or repair after injury. However, it is unclear if plasticity induced by repetitive transcranial magnetic stimulation (rTMS) and the resulting altered cortical excitability is coupled with changes in cerebral blood flow (CBF) delivery. In the present experiment, we sought to answer this question by monitoring CBF changes and cortical excitability using a combination transcranial doppler (TCD) ultrasound and TMS. In particular, we used single-pulse TMS to map out motor-evoked potentials (MEP) in the right primary motor cortex before and after 30 minutes of either real or sham rTMS at 1Hz. At the same time as the mapping process we monitored CBF. Results showed that cortical excitability and right hemispheric CBF increased following 30 minutes of real, but not sham, rTMS. Taken together, this suggests that cerebral blood flow can be modulated temporarily by altering the activity of neurons in the motor cortex using rTMS.

2-D-122 Functional connectivity of the subthalamic nucleus and substantia nigra pars reticulata depends on behavior

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The subthalamic nucleus (STN) is a common hub for the two major basal ganglia (BG) inhibitory pathways (hyperdirect and indirect). Consequently, current models of BG voluntary motor control predict that STN efferent signals inhibit movement or incorrect motor plans. However, STN output can also facilitate movement, via opposing pathways to the substantia nigra pars reticulata (SNr, BG output in the oculomotor loop) through the external segment of the globus pallidus. It is unclear how these conflicting signals from the STN contribute

to motor control. Here, we compare the influence of the STN on goal-directed, and non-goal directed eye movements in two monkeys, in order to resolve whether STN output can vary between inhibitory and facilitatory effects according to behavioral condition. In the same monkeys, we compared the STN to the downstream SNr. We found that: (1) electrical stimulation of the STN inhibited and facilitated saccades in goal directed and non-goal directed tasks respectively, while SNr stimulation inhibited saccades across tasks; and (2) STN and SNr local field potential was correlated at beta frequencies in goal directed tasks, but in the non-goal directed task was correlated at gamma frequencies only. We suggest that when a rewarding goal exists, the STN increases inhibition from BG output to decrease unnecessary movements in favor of goal-directed movements. Alternatively, when no explicit goal exists, the STN reduces inhibition from BG output to facilitate automatic movements toward unexpected stimuli.

2-D-123 The influence of remembered sensory information on sensory integration

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To plan a reach, one must identify the location of the target in space. When the target is one's hand, vision and proprioception can provide the brain with information regarding the target's spatial location. It has been proposed that these sensory signals are optimally integrated to estimate the object's location. In particular, according to the maximum-likelihood estimation (MLE) model, more reliable sensory inputs are assigned a greater weight (Ernst & Banks, 2002). In this study, we investigated whether the brain is able to adjust which sensory cue it weights the most. Specifically, we examined if the brain changes how it weights sensory information when the availability of sensory inputs was manipulated. Subjects reached to visual (V), proprioceptive (P), or visual proprioceptive (VP) targets under different delay conditions (i.e. subjects reached when the target was available or to a remembered target), using their right or left hand. Results indicated that subjects' reaches differed based on the hand used. Specifically, reaching movements performed with the left hand were less accurate and more biased to the right of the target compared reaches completed with the right hand regardless of target modality. However, subjects weighted sensory cues in accordance

with the MLE model across all delay conditions and these weights were similar regardless of the delay condition. Thus, manipulating the availability of the target did not cause sensory reweighting.

2-D-124 Changes in contrast responses of cells in the primary visual cortex after deactivation of the pulvinar

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The pulvinar establishes reciprocal connections with nearly all visual cortical areas and is thus in a strategic position to influence their stimulus decoding processes. Projections from the pulvinar to the primary visual cortex (V1) are considered to be modulatory, altering the response of neurons without changing their basic receptive field properties. Results from our laboratory, based on optical imaging, had lent support to this assumption (Soc. Neurosci. Abst. 2011. Vanni et al.). Here, we investigate further this issue by studying V1 single unit responses during the reversible deactivation of the lateral posterior (LP) - pulvinar complex in the cat through microinjections of gamma-aminobutyric acid. Recording and injection electrodes were positioned to obtain overlapping thalamic and cortical receptive fields. Results are as follows: no change in the preferred orientation or direction selectivity of V1 neurons was observed during pulvinar deactivation. However, for 67% of the cells tested (n=39/58), the response amplitude to the optimal stimulus was reduced by a mean of 65%. The contrast response function of neurons was modeled with the Naka-Rushton function and analysis of the effects of pulvinar deactivation revealed at least three types of modulation based on the function parameter predominantly affected: 24% of cells had a decrease in Rmax, 13% had an increase in the exponential factor and 11% had a C50 increase. Our results suggest that the pulvinar modulates activity of V1 neurons in a contrast-dependent manner. Supported by CIHR grant #MOP231122 to CC.

2-D-125 Rapid whole-body postural responses following mechanical perturbations to the upper limb.

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Postural adjustments precede reaching movements of the arm by ~100ms and can precede corrections to arm trajectory by 80-85ms after visuomotor perturbations. Such anticipatory postural adjustments appear implausible following mechanical perturbations to the arm given the rapid nature of corrective responses (muscle activity changes within 60ms). We hypothesized that in standing, the postural system is updated at the same latency as the arm, and postural responses are modulated by the behavioural goal, as observed for arm responses. Standing subjects held a robotic handle and reached to a target. Step torque perturbations were interleaved with unperturbed reaches. In order to probe the flexibility of the responses, targets were presented as a dot or rectangular bar. Following perturbation onset, arm muscle activity increased as early as 45-75ms. Leg muscle activity increased ~75-100ms post-perturbation, leading to deviations in center of pressure (COP) ~25-50ms later. Subjects corrected the hand back to the dot target but corrected to off-center locations on the bar. Task-related differences were observed as increased arm muscle activity (~60-75ms post-pert) and increased hand trajectory and velocity (~150-200ms post-pert) for the dot vs. the bar. As predicted, task-related differences were observed at similar latency in leg muscles (~75-120ms post-pert) and COP trajectory and velocity (~150-200ms post-pert). These findings suggest that the postural system is rapidly updated with feedback from the arm to elicit appropriate postural responses with changing task demands.

2-D-126 The Debate Is Over: Action and Perception Dissociate Using a 3D Variant of the Sanders Parallelogram Illusion While Controlling for Visual and Haptic Feedback

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According to the two visual systems hypothesis (TVSH), 'vision-for-action' and 'vision-for-perception' are mediated by two distinct cortical pathways. Supporting evidence for the TVSH has come from neuropsychological, neurophysiological, and neuroimaging studies of humans and non-human primates. One contentious line of evidence, however, comes from studies that find weaker effects of pictorial

illusions on action than on perception. Re-appraisals of these studies have rendered the perception-action dissociation interpretation problematic, noting confounding task-differences in attention, stimulus-response functions, obstacle avoidance, and visual and haptic feedback. Here, participants either reached out to pick up (length-wise) target bars embedded in the Sanders illusion or perceptually estimate their lengths. We removed visual feedback by suppressing the participants' vision throughout their grasps. We controlled for haptic feedback by allowing the participants the same opportunity to touch the targets in the perceptual estimation task as they had in the grasping task. In line with the TVSH, the illusory effect was significantly weaker on grasps than on perceptual estimates when the tasks were blocked separately and when the perceptual estimation and grasping tasks were alternated from trial to trial. In addition, an analysis of the illusory effects 'corrected' for any biases in response-functions supported our key findings. These results provide positive evidence for separate visual-perceptual and visuomotor systems in neurologically-intact individuals.

2-D-127 Moderate to Severe Degenerative Intervertebral Discs identified by T2-RARE MRI in alive SPARC-null mice.

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Introduction: Chronic low back pain (cLBP) affects 12-35% of the global population and intervertebral disc (IVD) degeneration is considered to be its primary source in 40-45% of the cases. SPARC-null mice are a clinically relevant model cLBP with signs of IVD degeneration. The objective of the present study is to use Magnetic Resonance Imaging (MRI) in SPARC-null mice to identify degenerative and possible pain generator discs. **Method:** T1 to S4 spines were dissected from fixed (PFA4%, intra-cardiac) or fresh (exsanguination) mice (2-20 mth old) and embedded in 15ml tubes with 10% agarose. Alive mice (3 or 18 mth old) were anesthetized with 2.5% Isoflurane. T2-RARE weighted MRI scan were collected with a 7T magnet with volumetric coil. After scanning, ex-vivo samples were processed for histological analysis using the FAST protocol. Alive animals were euthanized and spines were collected for further biochemical analysis. **Results:** Sagittal images of ex-vivo fixed and fresh samples as

well as alive mice presented similar results. In each condition, 5 different IVD patterns were identified (normal, lost of signal, black, bulged or herniated) that corresponded to different histopathologies (normal, desiccated, internal disc disruption, bulged and herniated, respectively). **Conclusion:** T2-MRI scan adds a new dimension to classical histology. It allows screening for abnormal and potentially pain generator discs in alive mice. Future studies will compare biochemical content of normal vs. degenerative discs and time-course of IVD degeneration intra-subject.

2-D-128 Investigating the neural encoding of linear self-motion

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Understanding how sensory neurons transmit information about relevant stimuli is a major challenge in neuroscience. Accordingly, we took advantage of the otolith system which is well-defined anatomically and physiologically and benefits from easily characterized sensory stimuli (i.e., head acceleration). Moreover, otolith afferents have a broad diversity in their spontaneous discharge regularity. Here, we employed multiple measures (i.e., gain, information theoretic, and spike timing precision) to probe the impact of background discharge regularity on the encoding of linear acceleration by otolith afferents. Specifically, we investigated how sensory information is processed in macaques' otolith afferents during translations with broad band (0-15 Hz) noise linear accelerations. We found an increase in gain for both regular and irregular afferents as a function of the stimulus frequency; however, the gain enhancement was more prominent for irregular units. Irregular units conveyed more information at higher frequencies (e.g., >7Hz), whereas regular afferents transmitted slightly greater information at low acceleration frequencies (≤ 2 Hz). Finally, our preliminary analysis shows that irregular units display more spike time precision in response to the same stimuli suggesting a role for spike timing in the encoding of linear motion. Taken together our results suggest that while highly sensitive irregular afferents are more advantageous for transient and dynamic stimuli, the regular units can provide accurate information when the stimulus is less dynamic (e.g. static tilt).

2-D-129 Neural Correlations in the Electrosensory Lateral Line Lobe of the Weakly Electric Fish, *Apteronotus leptorhynchus*: Analysis of Multi-Channel Recordings

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It is recognized that perception and behavior result from the activities of large neural ensembles. As such, it is key to understand the mechanisms that give rise to correlated activity in the brain. However, correlated activity is highly plastic as it is regulated during specific behavioral contexts. In this work, we aim to understand how activation of neural circuits can shape correlated activity by using the weakly electric fish, *Apteronotus leptorhynchus*. We performed multi-channel recordings in the electrosensory lateral line lobe, which benefits from well-characterized neural architecture. First, a spike-sorting algorithm was applied on the recorded signals to extract neural units. Then, correlated activity can be examined from pairwise population-averaged cross-correlograms calculated from all pairs of the extracted units. We found that the activities are positively correlated for neurons of the same type (ON-ON, OFF-OFF), but negatively correlated for neurons of opposite type (i.e. ON-OFF). Also, the effect of different stimulus characteristics on the correlation is observed. While the correlation is decreased by conspecific-like stimuli, it is increased by prey-like stimuli. Furthermore, some neurons tend to fire synchronously at particular portions of stimulus, e.g. at specific phases of sinusoidal stimuli. Thus, this work will give important insights in how correlated activity contributes to the processing of natural stimuli.

2-D-130 Dynamics of peri-saccadic receptive fields in monkey area V4

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Predictive remapping of receptive field (RF) has given some insight on the mechanisms that underlie stable vision during eye movements. Neurons transiently respond to stimuli flashed, prior to the eye movement, in their future RF parallel to the impending saccade vector (Sommer and Wurtz, 2006). Other findings suggest that RF dynamics of V4 neurons

change during a saccade, converging towards the saccade target (Tolias et. al., 2001). Our goal was to solve this confound by studying the peri-saccadic RFs of V4 neurons using 10x10 electrode arrays. The peri-saccadic spike RFs showed classical shift parallel to the saccade vector, but did not show convergence towards the saccade target. Such a shift in LFP RFs was accompanied by a subsequent shift towards the saccade target. When the flashed probe was replaced with the static probe, used by Tolias et al (2001), both the spike and LFP RFs shrunk and shifted towards the saccade target. The manifestation of predictive remapping is therefore paradigm-dependent. The dynamics of peri-saccadic LFP RFs implies sub-threshold activities competing for one of the two scenarios of remapping. Flashed probes, generating strong bottom-up attention, cause the neurons to shift their RFs parallel to the saccade vector. For static probe, the only salient feature on the screen during a saccade is the recently appeared saccade target. Hence, the RFs shift and compress towards the saccade target. By thus keeping track of momentary attentional loci on the visual field, our visual system is able to attain, what appears to us as stable vision.

2-D-131 Interhemispheric modulation of primary motor cortex outputs by the ventral premotor cortex in Capuchin monkeys (*Cebus apella*)

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In primates, the primary motor cortex (M1) receives numerous inputs from several premotor areas. These connections may allow premotor areas to modulate the corticospinal outputs of M1. To date, only the effects of the ipsilateral PMv (iPMv) on M1 outputs have been studied in detail. Here, we studied the interhemispheric influence of PMv on M1 outputs. We used paired-pulse stimulation protocols while recording electromyographic (EMG) activity from up to 8 forelimb muscles in each arm. Under ketamine anesthesia, we first used intracortical microstimulation (ICMS) techniques to identify the M1 hand area and iPMv in one hemisphere and PMv in the contralateral hemisphere (cPMv). Then, a stimulating electrode was placed in the hand representation of M1 to be used as our test electrode (T). We only used sites where a single pulse shock could elicit a clear motor evoked potential (MEP) in at least one of the EMG recorded signals. A second,

conditioning electrode (C) was placed in the hand representation of the cPMv. The intensity of the C stimulus was subthreshold. In total, we recorded 11 pairs of cPMv-M1 sites in 4 monkeys and compared the results to 11 pairs of iPMv-M1 in the same animals. Our results indicate that subthreshold stimulations of cPMv are more likely to produce facilitation of M1 outputs when C and T are separated by moderate latencies (5-10 ms). Longer latencies between C and T are more likely to have an inhibitory effect. Our results thus support that interhemispheric interactions between PMv and M1 are complex and strongly temporally modulated. --

2-D-132 Statistics of natural vestibular stimuli in primates: Implications for nonlinear coding.

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Understanding how neurons process sensory information requires not only a characterization of the neuronal responses, but also the natural stimuli encountered in an organisms sensory environment. The vestibular system has two kinds of afferents, canals afferents (sensitive to angular velocity) have been characterized by stimuli with standard deviations (std) around 40 deg/sec, and otolith afferents (sensitive to linear acceleration) with stimuli of std around 0.2G, and frequencies from 0-20Hz. We thus measured the head movements of monkeys during natural behaviours, using a micro-electromechanical systems module sensitive to linear acceleration and angular velocity in 3D. We compared the probability distributions of the head movement signals to Gaussians, and found that the former have significantly longer tails as quantified by the kurtosis, as seen for other modalities of natural stimuli. We found that head movement signals could reach values up to 500-1500 deg/sec and 2-6G, respectively, but had frequency content from 0-20 Hz, peaked around 2Hz. Based on current afferent models, we predict that natural stimuli will elicit cut-off and saturation in single afferent responses. We also predict that such nonlinear responses will synchronize the afferent population, in which case precise spike timing would carry essential stimulus information. These results challenge the traditional wisdom that early vestibular pathways use a linear rate code, thus motivating a comprehensive rethinking of sensory coding in the vestibular system.

2-D-133 TrkB receptor activity in the olfactory bulb is needed for long-term memory of odour-reward learning

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Understanding the molecular mechanisms underlying long-term memory (LTM) has been a long-standing goal in neuroscience. It is well established that LTM requires protein synthesis whereas short-term memory (STM) does not. While specific proteins have been identified as crucial for LTM consolidation, what remains unclear is the specific timecourse of their involvement, particularly in multi-trial appetitive learning. To further explore this idea, we examine the time course of brain-derived neurotrophic factor (BDNF) activity in an olfactory bulb (OB)-dependent incremental learning task. BDNF is a strong candidate due to its established involvement in promoting neuron survival, the activity-dependent nature of its secretion, and its established effect on OB-dependent learning mechanisms (Bath et al., 2008, J Neurosci). It has been shown to be necessary for LTM consolidation, but not for STM (Alonso et al., 2005, Learn Mem). In the present study, animals were trained over several trials to learn an odour-reward association and memory was probed 48 hours later. We found that, compared to controls, mice infused with the BDNF receptor antagonist K252a into the OB prior to training showed normal learning, but impaired memory at 48 hours. This finding suggests that early activation of the BDNF-TrkB pathway is necessary for the consolidation of OB-dependent LTM.

2-D-134 DF's Visual Brain in Action: The Role of Tactile and Visual Feedback

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Patient DF, who developed visual form agnosia following ventral stream damage, configures her hand in flight to match the geometric properties of novel objects when picking them up, despite her inability to use these same properties to explicitly differentiate amongst these objects. We have proposed that her spared grasping is mediated by a feedforward visuomotor system housed within the posterior parietal cortex. Alternatively, DF might use haptic feedback from

grasping the objects to calibrate egocentric visual cues to the object's surface, or she might use visual feedback during the grasp to appropriately scale her in-flight hand aperture to the target's size. To test these alternatives, we devised a grasping task that disrupted visual-haptic calibration by varying the visual size of the target from trial to trial while keeping its felt size constant. In a second condition, we removed visual feedback by suppressing her vision throughout her grasping movement. If the alternative accounts were true, DF's grasps should no longer reflect the visual size of the goal objects. Contrary to their predictions, however, DF continued to scale her grip aperture to the visual sizes of the targets. Furthermore, providing haptic feedback about perceptual judgments of visual size did not improve her chance performance. Together, these findings strengthen the proposal that DF's spared grasps are driven by visual feedforward processing. They also suggest that tactile contact with an object keeps DF's residual visuomotor system engaged, preventing the grasps from defaulting to pantomimes.

E - Homeostatic and Neuroendocrine Systems

2-E-135 The Involvement Of Tumor Necrosis Factor Alpha In The Neurophysiological Response Of CA1 Synapses To Acute Stress

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Stress is disruptive to immune function, but the reverse relationship - how the immune system can influence the animal's response to stress - is not clear. Here we investigate the neurophysiological response of adult mice to acute stress, and the potential involvement of Tumor Necrosis Factor Alpha (TNF α) - an immune factor that has been shown to modulate homeostasis of neuronal synaptic plasticity. To this end, we subjected adult wild-type (WT) male mice to a forced swim as a stressor, and found that the AMPA/NMDA ratio (a readout of synaptic strength) at hippocampal Schaffer collateral to CA1 synapses is altered one day following stress. However, when TNF α knockout (KO) mice were subjected to the same stressor, we found no alteration from baseline in the AMPA/NMDA ratio one day following stress, indicating a deficiency in the sustained neurophysiological response to stress in TNF α

KO. To test whether the deficiency in TNF α KO is limited to the prolonged phase following stress, we compared the AMPA/NMDA ratio of WT and TNF α KO shortly (1 -3 h) after stress, and found that TNF α KO mice lack the response to stress even at these early time points, suggesting an altogether absent neurophysiological stress response. Plasma corticosterone was similar between WT and TNF α KO at baseline, and following stress, suggesting an intact hormonal response in TNF α KO. Future experiments are aimed at delineating which component of the synaptic response is altered in response to stress at Schaffer collateral to CA1 synapses of adult mice.

2-E-136 Chronic intracerebroventricular administration of relaxin-3 induces sex-specific effects on food intake and body weight in rats

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Relaxin-3 (RLN3) is a neuropeptide that is thought to play a role in modulating physiological functions such as food intake and stress. Recent results suggest a sex-specific regulation of the central RLN3 system. While acute and chronic central administration of RLN3 has been shown to increase feeding and body weight in male rats, it has never been tested in female rats. Our goal was thus to examine the role of RLN3 on food intake regulation and body weight in both male and female rats by using chronic intracerebroventricular (icv) administration of RLN3. Two groups of male and female rats received vehicle or human RLN3 (400 pmol/d) during 14 days. During all the experiment, the RLN3 rats displayed persistently higher body weight than control rats and this increase was significantly greater in female than male rats. In both sex, the percentage of body fat of RLN3 rats was significantly increased. Accordingly, the RLN3 rats demonstrated higher intake of chow compared to the vehicle-treated rats. This hyperphagia persisted in female rats during all the infusion period whereas male rats showed an increase of food intake only during the first week of treatment. Furthermore, RLN3 female but not male rats had a greater mean feed efficiency compared to their respective vehicle-treated groups. In conclusion, female rats exhibited greater hyperphagia and overweight

than male rats when chronically icv infused with RLN3. These results confirm that RLN3 up-regulates food intake, body weight and adipose tissues and suggest, for the first time, that these effects are sex-specific.

2-E-137 Rimonabant peripheral injections attenuate the orexigenic effect of ghrelin infused into the VTA

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Ghrelin is an endogenous signal that targets the brain to increase food intake and energy balance. Recent evidence suggests that ghrelin increases appetite by acting on receptors in the ventral tegmental area (VTA), a brain region associated with reward seeking behaviors. The ability of ghrelin to induce appetite is reminiscent of the appetite inducing effects of endogenous cannabinoids (CBs). Interestingly, recent studies suggest that ghrelin's ability to stimulate feeding is dependent on a functional CB system in the hypothalamus. Here we hypothesize that a similar interaction between ghrelin and the CB system exists in the VTA to modulate feeding. To test this hypothesis, Long-Evans rats were subjected to VTA cannulation procedures and placed in one of the following 4 treatment groups (IP/intra-VTA): vehicle/saline, rimonabant (1.5 mg/kg)/saline, vehicle/ghrelin (1 µg /0.5 µl), rimonabant (1.5 mg/kg)/ghrelin (1 µg /0.5 µl) to determine if global pharmacological inhibition of the cannabinoid system would attenuate the ability of ghrelin within the VTA to acutely increase food intake (measured 1, 2, 4, 6 hours post-microinjection). Results show that ghrelin administered into the VTA significantly increased food intake ($p < 0.05$) and that this effect was attenuated to control levels when animals were pre-treated with rimonabant 30 minutes prior to ghrelin microinjections. This suggests that ghrelin targets the VTA to increase food intake through an interaction with the CB system.

2-E-138 Osmotic activation of phospholipase C in osmosensitive supraoptic neurons

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The magnocellular neurosecretory cells of the hypothalamus (MNCs) respond to increases in

plasma osmolality by increasing their firing rate and the release of vasopressin (VP). VP suppresses diuresis and thereby plays a critical role in maintaining the osmolality of body fluids near a "set point", which is 295 mosmol/kg in the rat. The mechanisms of osmosensitivity in the MNCs are incompletely understood, but could include osmotically-evoked changes in second messenger systems. We used immunocytochemistry to monitor phosphatidylinositol 4, 5-bisphosphate (PIP2) in the plasma membrane of acutely isolated rat MNCs. A 5 minute exposure to hypertonic solution (325 mosmol/kg) caused a marked decrease (~25%) in immunoreactivity to PIP2. This decrease was reversible and was prevented by the phospholipase C inhibitor U73122 (1 iM). The muscarinic agonist oxotremorine (10 µM) also decreased PIP2 immunoreactivity and this effect was also blocked by U73122. The osmotically-evoked decrease in PIP2 was prevented by minimizing the Ca²⁺ concentration in the external solution and by the L-type Ca²⁺ channel antagonist nifedipine (30 nM), suggesting that Ca²⁺ influx is necessary for this effect. PLC converts plasma membrane PIP2 to the second messengers diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) and all three of these signaling molecules regulate the activity of ion channels. The osmotically-induced activation of PLC may therefore make an important contribution to the osmosensitivity of the MNCs and therefore to the neural regulation of body fluid balance.

2-E-139 Feeding and Hormonal abnormalities in 5xFAD mice

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The 5xFAD mouse is a double transgenic model of Alzheimer's disease (AD), which carries an amyloid precursor protein transgene with three mutations (Sweden, London, Florida), and a presenilin-1 transgene with 2 mutations (M146L and L286V). These mutant transgenes act additively to produce massive increases in AB-peptides, and the development of AB-plaques by 2 months of age. Previously, we have observed age-related weight-loss in the 5xFAD mouse. Weight-loss is a common problem in human AD patients. Therefore, we investigated feeding behaviour and insulin levels in 5xFAD mice and their wildtype (B6SJLF2) controls. We measured food intake and body weight at 3, 6, 9

and 12 months of age and activity (grooming, climbing, stillness, and jumping), muscle mass, fat tissue, and insulin concentrations at 12 months of age. To investigate if weight loss was due to age-related motor impairments that made the retrieval of food difficult, we measured food intake at 12 months of age for 21 days when food was placed on the hopper (7 days), when food was placed on the floor of the cage (7 days), and when food was mashed with water (7 days). There were no significant differences between 5xFAD and WT mice in insulin levels, muscle mass, activity, or overall feeding, except for when food was placed on the hopper. However, fat mass and weight were significantly lower in the 5xFAD mice compared to WT controls. These results indicate that weight loss 5xFAD mice is not likely due to immobility or feeding impairments but rather to an impaired metabolic system.

2-E-140 Morphological and electrophysiological plasticity of tyrosine hydroxylase neurons in mouse arcuate nucleus

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The orexigenic peptide ghrelin has previously been demonstrated to induce changes in synaptic connectivity of NPY and POMC neurons within the arcuate nucleus of the hypothalamus (ARC). Yet the long-term effects of ghrelin on ARC tyrosine hydroxylase (TH) neurons, another ARC population which contributes to regulation of energy balance, have not been investigated. We carried out experiments using dissociated neuronal cultures from mice expressing EGFP under the control of the TH promoter to investigate whether ghrelin treatment could cause alterations in morphological and electrical properties of putative ARC dopaminergic neurons. Ghrelin administration for 5 days to dissociated ARC neurons significantly increased the number and length of neurites on TH-EGFP neurons. Using electrophysiology, we next examines whether ghrelin caused changes in intrinsic electrical properties of ARC TH-EGFP neurons. While resting membrane potential and spontaneous action potential frequency remained unchanged after ghrelin treatment, ghrelin did cause a significant 4 mV hyperpolarization of the action potential threshold. This increased excitability is correlated with a significantly increased input resistance and 6 mV hyperpolarizing shift in the activation of voltage-gated Na channels. The

effects of ghrelin on morphological and electrical properties were abolished by pretreatment with 100 uM of the ghrelin receptor antagonist, D-Lys-GHRP-6. Together these data indicate that ARC dopaminergic neurons are subject to plasticity induced by ghrelin.

2-E-141 Methyl-CpG-binding domain protein 2 (MBD2) is associated with levels of maternal care in C57/BL6 mice

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The methyl-CpG-binding domain (MBD) family of proteins is known to interact with regions of methylated DNA in the genome. Recent work has begun to elucidate the specific molecular function of each of the MBDs. However, little is known regarding the role of MBDs on phenotypic outcomes. Therefore, the aim of this project was to characterize the role of MBD2 on maternal care. To address this question, we developed a novel protocol for characterizing and quantifying maternal behaviours in a line of MBD2 null, heterozygous and wild type C57/BL6 mice during the early postnatal period. Our results from maternal observations across multiple cohorts show that MBD2 null dams displayed lower levels of licking and grooming towards their offspring over the first six days of life when compared to heterozygous and wild type litter mates. MBD2 null dams also showed deficiencies in the level of active maternal behaviours when assayed in retrieval and nest building performance tests on postnatal day 7. Moreover, we found estrogen receptor alpha (Esr1) mRNA expression in the medial preoptic area (mPOA) of MBD2 null dams is significantly decreased when compared to heterozygous and wildtype litter mates, and that Esr1 expression is positively correlated with licking and grooming levels. These studies demonstrate the association of MBD2 with levels of Esr1 and the role of MBD2 in maternal behaviour. This work was funded by the Canadian Institutes of Health Research

2-E-142 Chronic social stress influences maternal behavior in rats

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Environmental epigenetics has attracted considerable attention but little is known about the mechanisms implicated in environmentally

induced phenotypic variation in adulthood. In mammals, parent-offspring interactions are influenced by the quality of the environment. There are highly stable individual differences in maternal behavior among lactating rats, especially in the frequency of pup licking and grooming (LG). However, adult female offspring of High LG mothers reared under conditions of social isolation early in development show a decreased frequency of pup LG. The objective of this study is to investigate the cause-effect relationship between impoverished housing conditions and variations in maternal care. Female offspring of High and Low LG mothers were socially isolated or standard housed at post-natal day (PND) 70 for 35 days. At PND 105, tissues and blood were collected or the females were bred and the maternal behavior was observed. Results show that socially-isolated virgin females are more fearful, have elevated levels of plasma corticosterone and significant alterations in gene expression in the paraventricular nucleus of the hypothalamus and medial preoptic area. Importantly, the frequency of LG is significantly reduced in socially-isolated dams. Altogether, these experiments indicate that chronic stress in adulthood leads to significant alterations in gene expression, which have a profound influence on the maternal behavior. Ongoing experiments are focusing on the epigenetic regulation of the genes associated with stress and maternal care.

2-E-143 α -MSH exerts direct postsynaptic excitatory effects on NTS neurons and enhances GABAergic signaling in the NTS

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The melanocortin system plays a critical role in the control of feeding. While anorexigenic effects of α -melanocyte stimulating hormone (α MSH) acting in the medullary nucleus of the solitary tract (NTS) have been shown, the cellular events underlying these effects are less well known. We thus used whole cell patch clamp electrophysiology to examine the effects of α MSH on rat NTS neurons in slice preparation. In normal aCSF, α MSH (500 nM) depolarized 39% of cells (n=16, mean: 6.14 ± 0.54 mV) and hyperpolarized 22% of cells (n=9, mean: -6.79 ± 1.02 mV). The use of tetrodotoxin revealed α MSH exerts direct depolarizing effects on some NTS neurons and indirect inhibitory effects on others. A third

subset of neurons is simultaneously directly depolarized and indirectly hyperpolarized by α MSH, resulting in a net lack of effect on membrane potential. The inhibitory inputs influenced by α MSH were identified as GABAergic, as α MSH increased the frequency, but not amplitude, of inhibitory post synaptic currents (IPSCs) in 50% of cells. Pharmacological blockade of GABAA and GABAB receptors, and physical removal of synaptic inputs via cellular dissociation, abolished α MSH induced hyperpolarizations. We conclude α MSH exerts direct, postsynaptic excitatory effects on some NTS neurons. By presynaptically enhancing GABAergic signaling, α MSH indirectly inhibits other NTS cells. These findings provide critical insight into the cellular mediators of medullary melanocortin anorexigenic effects, and expand knowledge of the circuitries involved in melanocortin signaling. Funding: NSERC, HSFO, FQRNT

2-E-144 Modulation of corticotropin-releasing factor (CRF)-mediated stress and anxiety-related behaviours by teneurin C-terminal associated peptide (TCAP)-1

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The neuropeptide, teneurin C-terminal associated peptide (TCAP), exists in four isoforms, each of which is encoded by the terminal exon of the teneurin transmembrane protein. However, TCAP-1 is expressed as a separate mRNA distinct from teneurin-1 and its mature peptide acts as a ligand to β -dystroglycan (β -DG), thereby inducing a MEK-ERK1/2 signal transduction cascade in neurons to stimulate cytoskeletal reorganization and glucose transport. At the onset of stress, corticotropin-releasing factor (CRF) is released, stimulating the HPA axis and stress-associated behaviours. Previous studies show that TCAP-1 inhibits the CRF-mediated stress response in mice and rats. Intracerebroventricular (ICV), intravenous (IV) or subcutaneous administration of synthetic TCAP-1 can attenuate anxiety-related behaviours, and inhibit CRF-induced cocaine reinstatement at picomole and nanomole concentrations. Also, rats with ICV-administered TCAP-1 and acute CRF injections show altered behavioural responses in the elevated plus maze, open field test, and acoustic startle test. However, TCAP-1 has no effect on the HPA axis or on CRF receptor-

binding activation. Interestingly, neuronal cells treated with TCAP-1 show enhanced neurite formation, axon growth, and dendritic spines, suggesting that TCAP-1's effects are associated with neuronal circuitry and synaptic plasticity. Thus, TCAP-1 is a novel regulator of CRF-dependant behaviours, and may function by targeting downstream components of the extrahypothalamic CRF pathway. We suggest a plausible model on the regulatory actions of TCAP-1 on CRF.

F - Cognition and Behavior

2-F-145 Effects of housing conditions on neurogenesis in black-capped chickadees (*Poecile atricapillus*)

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In captive black-capped chickadees, neurogenesis in the hippocampal complex is suppressed compared to free-flying birds. However, it is not known whether varying housing condition influences the degree to which neurogenesis is suppressed. We captured groups of chickadees during winter, spring, and fall, and housed them in four different conditions: Outdoor aviary, indoor aviary, and outdoor cage, and indoor cage. Birds were injected with BrdU within two days of capture and behaviour was observed for six weeks, after which time their brains were assessed for neuronal proliferation and survival. Preliminary results from behavioural data seem to show that enclosure size did not affect behaviour as much as being housed indoors. Results from this study will allow us to determine whether being housed outdoors and/or in a larger enclosure relieves effects of captivity on both behaviour and neurogenesis. These results will provide insight to what factors underly differences in neurogenesis between captive and free-flying birds. Further, results will aid in understanding the influence of environmental context in field and laboratory studies using songbirds.

2-F-146 Central GPR120 activation inhibits food intake, food reward and anxiety-like behavior

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GPR120 is a g-protein coupled receptor that is activated by polyunsaturated fatty acids (FA) and reported to mediate the anti-inflammatory and insulin-sensitizing effects of omega-3 FA. Omega-3 FA have been linked to central anorectic and anxiolytic actions. The objective of our study was to test the effects of acute pharmacological GPR120 stimulation in CNS on feeding, the rewarding effects of high-fat and -sugar food, energy expenditure and anxiety-like behavior. Methods: Intraventricular (ICV) cannulas were stereotactically implanted in adult male C57Bl6 mice. Mice (N=11) received vehicle injection (saline+1%DMSO/1 μ l), a 0.1 μ M and a 1 μ M dose of GPR120 agonist. Mice (n=7) were also placed in metabolic chambers for assessment of locomotor activity and indirect calorimetry. Operant responding on a progressive ratio schedule was used to evaluate food reward. Elevated plus maze and open field tests were employed to assess anxiety-like behavior. Results and conclusion: GPR120 agonist produced a significant reduction in chow intake at 1, 2 and 4 hours post-injection (up to 80% reduction), an effect not observed with GPR40 agonist. GPR120 (1 μ M) also produced a brief increase in locomotor activity, a decrease in respiratory exchange (suggesting increased utilization of fat), reduced breakpoint responding for high-fat/sugar food and produced a modest decrease in anxiety-like behavior. Together, these findings suggest that GPR120 stimulation in the brain has catabolic and anxiolytic actions and that it may mediate the central effects of omega-3 FA. Funded by NSERC

2-F-147 Pre-ischemia CRH receptor 1 blockade attenuates hippocampal cell death and prevents spatial memory impairments in rats.

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Recently, findings from our laboratory demonstrated that Corticotropin releasing hormone receptor 1 (CRHR1) blockade, prior to ischemia, significantly attenuated elevations of basal and stress-induced corticosterone secretion post ischemia. CRHR1 is known to mediate the rapid effects of stress on learning and memory through changes in neuroplasticity, thus providing a potential molecular basis for impaired spatial memory by cerebral ischemia. Thus, the current study investigated whether ischemic hippocampal cell death, and impaired spatial memory ability may be improved by

pretreatment of Antalarmin, a selective CRHR1 antagonist. Forty-eight male Wistar rats (n=12 per group) were subjected to sham surgery or global cerebral ischemia using the four vessel occlusion (4VO) model. ICV injection of Antalarmin (2µg/2µl) or saline was administered 30 min prior to ischemia. Spatial learning and memory ability was measured using the Barnes Maze task. Results show prevention of ischemia-induced spatial impairment in Antalarmin-pretreated rats, achieving comparable memory performance to that of sham groups. Hippocampal cell death, assessed thirty days following ischemia, indicated increased neuronal survival in Antalarmin-pretreated rats, as compared to the saline-treated ischemic group. These results suggest neuroprotective effects of CRHR1 blockade associated with improved spatial learning and memory after global cerebral ischemia.

2-F-148 Early memory processes are altered by Lithium administration

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Recently, lithium has been proposed as a treatment for neurodegenerative conditions, but clinical trials have been hampered by its prominent side effects in the elderly. The mechanisms underlying both the positive and negative effects of lithium are not fully known. We have investigated the effect of lithium treatment on the memorization processes associated with motor learning. Lithium was administered in drinking water for two weeks prior testing mice motor behaviors. Lithium did not alter the general motor capacity of mice on three motor execution tests, the wire suspension, the pole and the stepping tests. On the other hand, lithium-treated mice displayed a delay in the early phase of rotarod learning compared to vehicle-treated mice. As lithium inhibits glycogen synthase kinase-3 (GSK-3) in vivo, we have investigated the role of GSK-3 in motor learning. We performed intracranial injections of the selective GSK-3 inhibitor SB216763 directly into the dorsal striatum prior each rotarod training sessions. This inhibitor did not alter any of the motor behavior tested. At the biochemical level, we have investigated the temporal changes of GSK-3 α and GSK-3 β activities after the rotarod learning in different brain regions. Learning the rotarod task did not affect levels of total GSK-3 α and β , but induced a selective modulation of p-GSK-3 α and p-GSK-

3 β in the striatum, anterior cortex and hippocampus. Altogether, our results demonstrate that lithium affects early encoding processes of motor learning, but whether this effect is due to GSK-3 activity remains uncertain.

2-F-149 Optogenetic investigation of septal GABAergic modulation of hippocampal theta rhythm.

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Hippocampal neurons oscillate in synchrony at theta (4-10 Hz) frequencies during periods of wakefulness and rapid-eye-movement (REM) sleep, and evidence suggests that these theta rhythms are required for cognitive processing. The hippocampus receives cholinergic, glutamatergic and inhibitory GABAergic inputs from the medial septum (MS), a brain region required for normal theta rhythm generation in vivo. Previous work using lesional, pharmacological or electrical modulation of MS cell activity suggested that septal GABAergic neurons may be important for theta rhythm generation. However, due to the difficulty in achieving both temporal precision in combination with cell-type specificity using these methods, the causality of this neural pathway on hippocampal theta rhythms remains to be clarified. Here, we genetically targeted archaerhodopsin (ArchT), a silencing opsin to GABAergic neurons of the MS. We found that yellow light pulses reliably hyperpolarized ArchT-expressing cells in the MS in brain slices in vitro. Using a combination of optogenetic and electrophysiological (field potential and unit recording) techniques in freely-moving mice, we further found that theta power was significantly and reversibly attenuated when septal GABAergic neurons were optically inhibited during periods of active wakefulness or REM sleep. These results demonstrate that septal GABAergic neurons are critical for normal hippocampal theta rhythm in vivo and may implicate this neuronal population as an important component of cognitive processing mechanisms during wakefulness or REM sleep.

2-F-150 Utilization Behaviour after Lesions Restricted to the Prefrontal Cortex

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Utilization behaviour refers to the tendency of patients to pick up and use objects presented to them, in the absence of instructions to do so (Lhermitte, 1983). It has been ascribed to damage to the frontal lobe, with the assumption of critical involvement of the frontal cortex. However, careful examination of studies of patients presenting with utilization behaviour shows that these patients had sustained widespread cerebral lesions extending beyond the frontal cortex and involving subcortical neural structures. The present study examined utilization behaviour in patients with lesions restricted to the prefrontal cortex, and no more than the immediately subjacent white matter. There was no difference in the presence of utilization behaviour between patients with lesions restricted to the prefrontal cortex, patients with temporal lobe lesions and carefully matched neurologically intact participants. The results suggest that the exhibition of utilization behaviour in patients with large damage to the anterior part of the hemisphere reported in previous studies may have been due to the extensive damage to subcortical structures. The present results emphasize the need to base claims about frontal cortex functions on damage limited to the frontal cortex. Lhermitte F.

'Utilization Behavior' and its relation to lesions of the frontal lobes. *Brain* 1983; 106: 237-255.

2-F-151 Aberrant dopamine in the salience network and parahippocampal gyrus contributes to memory impairment in Parkinson's disease

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Patients with Parkinson's disease (PD) and mild cognitive impairment (PD-MCI) represent a vulnerable group for the development of dementia. Many patients experience memory impairment, however its neurochemical basis in PD-MCI is unknown. Large-scale brain networks interact to facilitate memory, and are known to be dysfunctional in PD. The objective was to investigate dopamine (DA) changes in the salience, central executive and default mode networks in patients with amnesic PD-MCI (PD aMCI) using PET. PD aMCI (n=8), PD non-

amnesic MCI (PD naMCI)(n=10), PD noMCI (n=11) and age-matched healthy controls (HC) (n=15) Patients were classified according to the Level 2 MDS task force criteria, and were considered PD aMCI if 2 memory tests were impaired (≥ 1.5 std below normative mean) on a neuropsychological test battery. They were scanned with [¹¹C] DTBZ to examine striatal DA depletion, and [¹¹C] FLB 457 to measure D2 receptor availability in the cortex. The PD aMCI group had significantly more striatal DA depletion compared to HC, PD noMCI and PD naMCI in the associative striatum. D2 receptor levels were also significantly reduced compared to HC, PD noMCI and PD naMCI in the bilateral insula, anterior cingulate cortex (salience network) and the parahippocampal gyrus (PHG). This PET imaging study showed that dopaminergic changes in the salience network and PHG underlie PD aMCI. These findings also demonstrated the first evidence of the contribution of dopamine to memory impairment in PD.

2-F-152 Interacting with bots online: users reactions to actions of automated programs in the virtual community of Wikipedia

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With the drastic rise of social media, large-scale collaborative online projects such as Wikipedia are now dealing with incredible large amount of data. This growth forces the community to provide tremendous efforts in order to maintain the accuracy and structure of the database. To deal with such amounts of data, Wikipedia users have developed automated programs - bots - to help them to do some of the maintenance tasks. However, it is unclear how human users react to the actions of these bots. Based on a corpus of 6,528 interventions (2,353 different discussions) of users on talk pages of 50 bots active on English-language Wikipedia pages between January 4, 2012 and January 2, 2013, we analysed the reactions of users depending of the characteristics of the bots' actions. Bot activity was strongly associated with the functioning Wikipedia internal community. Bots whose activity was mostly related to the work of other users (e.g. high degree of constraint or visibility) elicited more reactions. By combining the different characteristics of the bots, we were able to define two opposite "ideal types" of bots with distinct behavior: "servant bots" which mainly do repetitive and laborious work instead

of human users, and policing proactively enforcing Wikipedia's guidelines and norms, which elicited more polarized reactions from users (either negative or positive rather than neutral). Our results demonstrated differential reactions of humans in fonction of the behavior of bots, and unveiled a surprising dichotomy in the level of acceptance of control by automated programs.

2-F-153 Slow oscillations augmentation during sleep increases object recognition performance in mice

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The slow oscillation of slow-wave sleep induces (long-term potentiation) LTP and it is also associated with cortical memory consolidation. We hypothesized that an experimental increase of the slow wave activities during the natural sleep would improve memory. The memory was tested using the novel object recognition test (NOR) in mice as a well-accepted memory test in rodents. This test does not use fear (amygdala-dependent) or space (hippocampal-dependent) task and appears to depend on the frontal cortex. We used Thy1-COP4/EYFP mice, which express channelrhodopsin 2 channels in a large number of cortical neurons. A fiber-optic microcannula and an LFP electrode were implanted in the frontal cortex. After a recovery period, mice performed the NOR and LFPs were recorded continuously. Blue light stimulations at 0.8 Hz induced slow waves (Stim group) during the first 4 hours of the light cycle and the sham group had the same surgery but did not receive stimulation. We performed intracellular recordings in vivo and in vitro to characterize the pattern of neuronal activity using the same stimuli. The stimulation induced neuronal excitatory responses in vitro and in vivo. In implanted mice it induced a slow-wave type of responses and an overall increased delta power in the LFP. The NOR test showed that mice from the Stim group spent much less time exploring the old object, suggesting that they remembered more the old object than the sham group of mice. These experiments showed an improvement in memory after an optogenetic increase in slow-wave activity in the frontal cortex.

2-F-154 Correlations between brain and behavior: insights into the processing of statistical information.

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The processing of a complex auditory signal relies, in part, on the ability to decipher the statistical structure of the incoming sounds. Here we studied inter-individual differences in statistical processing to identify regions in which cortical thickness (CT), and/or surface area (SA) correlate with sensitivity to statistical structure. Participants heard short auditory sequences consisting of either syllables or bird songs and completed two behavioral tasks: estimation of the number of discrete elements (numerosity ratings targeting grouping of sounds) and estimation of the degree of statistical order (regularity ratings targeting sensitivity to structure). We then correlated measures of CT and SA taken from supratemporal cortices against (1) sensitivity to regularity and (2) the impact of regularity on perceptual grouping. Results from the supratemporal plane analysis demonstrate that CT and SA correlate significantly with both regularity-induced perceptual grouping and sensitivity to structure, but in markedly different regions, and that the areas implicated differ partially depending on whether the input consists of speech or non-speech sounds. We conclude that inter-individual differences in the influence of regularity on the perception of statistical structure and numerosity in the auditory domain can be detected using thickness and surface based morphometry. In addition, examining brain/behavior correlations to uncover the neural underpinning of statistical information processing can provide novel insights into the biological basis of human cognition and behavior.

2-F-155 Memory or attention? The effect of early auditory experience on neural immediate-early gene expression in female zebra finch (*Taeniopygia guttata*) auditory forebrain areas.

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The auditory forebrain regions caudo-medial nidopallium (NCM) and caudo-medial mesopallium (CMM) of songbirds are associated with auditory perception and complex auditory processing. Neural activation measure through the expression of the immediate-early gene ZENK in these areas varies in response to

different sounds. Two hypotheses are proposed for this variation. First, ZENK may reflect access to a representation of song memories created early in life. Second, ZENK may reflect attentional processes. We tested these hypotheses by measuring ZENK in response to tutored heterospecific or isolate songs compared to non-tutored wild-type song. Young zebra finch females were exposed during development to one of three different tutoring conditions; 1. Conspecifics that sang an isolate song, 2. Heterospecific, Bengalese finches (*Lonchura striata domestica*), 3. Conspecifics that sang a wild-type song. After maturity females were exposed to one of five different playback types; wild type song, isolate song, their own tutor song, heterospecific song (Bengalese finch song), or white noise. Subsequently, the expression of ZENK in CMM and NCM was measured. We found that ZENK responses varied across playback stimuli in CMM and NCM, and this variation seemed to interact with early auditory tutoring conditions. Females tutored by wild type conspecifics or heterospecific showed more activation in response to conspecific or isolated song, but isolate females did not. In conclusion, these results do not support the hypothesis that ZENK activation reflects early auditory memories.

2-F-156 Quinine adulteration allows for an access-induced consumption difference to emerge with higher sucrose concentrations

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Rats with ad lib access to food and water will consume significantly more 4% sucrose solution when it is available for 24h every 3rd day (E3DA) as opposed to every day access (EDA). This difference is maintained when all rats are switched to every 2nd day access (E2DA). The E3DA/EDA difference becomes smaller and, at times, unnoticeable with more concentrated (8% and 16%) sucrose solutions. When rats with a history of E3DA or EDA to 16% sucrose are given E2DA to 4% sucrose a significant consumption difference emerges immediately. The lack of an initial consumption difference may be attributed to the higher caloric consumption with 16% sucrose. In the current study quinine (Q) was added to an 8% sucrose solution to reduce intake and allow an E3DA/EDA difference to emerge. Sprague-Dawley rats (n=64) were given 4% or 8% sucrose with or without added Q and then

assigned to E3DA or EDA based on initial 24h consumption. Q solutions were consumed in lower quantities than their non-Q counterparts, and an E3DA/EDA difference emerged in the 8%+Q rats but not the 8% sucrose rats. This suggests that access-induced consumption differences are acquired across sucrose concentrations but are masked as rats approach an intake-limit with higher sucrose concentrations.

2-F-157 The effect of JDTC on stress-induced reinstatement of sugar seeking

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It is known that food restriction acts as a stressor in rats, and stressors tend to increase consumption of palatable food in rats and humans. The kappa opioid (KOR) receptor modulate stress responses, and controls consumption of palatable foods in both rats and rhesus monkeys. Interestingly, the KOR antagonist JDTC attenuated stress-induced reinstatement of cocaine and alcohol seeking, suggesting that JDTC may also be effective in reducing seeking of palatable food produced by food restriction stress. We recently developed a novel method to test self-administration of sugar, and reinstatement of sugar seeking, in an automated radial arm maze. Twenty-four free-fed Sprague Dawley rats were trained for 14 days to nose poke for sugar pellets. Rats then received 3 extinction sessions, and they were subsequently either free fed (FF) or food restricted (FR) to 85% of their ad-lib body weight. When rats were tested for reinstatement of nose poking behaviour 4 days later, it was found that only FR significantly elevated responding over extinction. We are currently evaluating the effect of JDTC (0, 3 and 10 mg/kg) on reinstatement produced by FR. These studies are supported by NSERC.

2-F-158 The relationship between schizotypy and willingness to play social roles

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Studies investigating the varying degrees of schizotypy in healthy volunteers provide a valuable method for assessing symptoms observed in schizophrenia, such as delusions,

jumping to conclusion bias and disorganization. In addition they allow for studying how these symptoms could influence social functioning. Emerging research has explored the performance of individuals with different degrees of schizotypy in tasks evaluating social related skills such as empathy, facial expression and emotion recognition and Theory of Mind. Thus far, their results suggest that healthy non-clinical individuals with higher schizotypy perform significantly worse than individuals with lower schizotypy. The present study aims to further contribute to this area of research by investigating the performance of low and high schizotypy but healthy volunteers in a novel decision task with large ecological validity, i.e., decide whether or not social roles were suitable for them. Four categories of names of social roles were randomly presented and participants had to decide whether they could consider themselves playing each role. This task was done to test if participant's (n=120) selection of unsuitable or inappropriate roles correlated with their degree of schizotypy (measured with the Schizotypal Personality Questionnaire (SPQ) and the Peter's Delusions Inventory (PDI)). The total SPQ and PDI scores significantly correlated with the percentage of acceptance of unsuitable and inappropriate roles, further contributing to the relationship between schizotypy and social functioning.

2-F-159 Effect of sleep deprivation on EphA4 and response to sleep deprivation in EphA4 knockout mice

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Introduction: Sleep is required in mammals and its recovery aspect was hypothesized to depend on mechanisms controlling synaptic strength. EphA4 is an adhesion molecule implicated in the regulation of synaptic function. The aim of the study is to understand the impact of sleep deprivation [SD] on the expression of EphA4 and on gene expression in EphA4 knockout [KO] mice. Methodology: 1) The expression of EphA4 and its partners was measured by quantitative PCR [qPCR] after a 6h SD in 3 different mouse brain regions (cortex [CTX], hippocampus [HP], and a thalamic/hypothalamic [TH/H] region). 2) EPHA4 synaptic and total protein level was measured by Western blot after a 6h SD in the same 3 brain regions. 3) Mice from 3 genotypes (wild-type, heterozygous and homozygous EphA4 KO mice) were

submitted to SD followed by qPCR measurements of markers of sleep need in the forebrain. Results: 1) The expression of EphA4 and its partners was not changed by SD in the CTX or HP. However, SD significantly increased EphA4 mRNA in the TH/H region. 2) SD did not significantly affect synaptic and total protein level of EPHA4 in the 3 brain regions studied. 3) The absence of EphA4 did not significantly impact on SD-dependent increase in Bdnf, Per2, Homer1A, Fos and Arc. Discussion: These preliminary results suggest that sleep loss may increase EphA4 expression in the thalamus or hypothalamus. Current experiments are assessing the genome-wide response to SD in EphA4 KO mice.

2-F-160 Effects of chronic prenatal MK-801 treatment on cognitive flexibility in the adult rat offspring

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Patients with schizophrenia exhibit impairments in executive functions. The neurodevelopmental hypothesis of schizophrenia posits that disruption of the developing brain predisposes neural networks to lasting structural and functional abnormalities resulting in the emergence of such cognitive symptoms in adulthood. Given the critical role of the glutamatergic system in cognitive performance, we investigated whether chronic prenatal exposure to the glutamate NMDA receptor antagonist, MK-801, would induce impairments in cognitive flexibility in adult male offspring. Pregnant Long-Evans rats were administered saline or MK-801 (0.1 mg/kg; s.c.) at gestation day 7 through 19. Cognitive flexibility was assessed using a maze-based set-shifting procedure. We tested the effects of prenatal MK-801 on (1) acquisition of response or visual-cue discrimination and (2) acquisition of the shift from a response to visual-cue discrimination task and vice versa. The shift procedure required rats to suppress the use of the previously relevant strategy. Prenatal MK-801-treated rats showed impaired acquisition when shifting to a new strategy compared to saline-treated rats, though this difference was not statistically significant. Analysis of the errors revealed that the deficit was due to regression to the previously learned behaviour. These findings suggest that glutamate dysfunction during early development may mediate cognitive deficits in

adulthood and therefore may shed light into the cognitive symptoms observed in schizophrenia.

2-F-161 Decision-related eye movement patterns during virtual navigation in non-human primates (*Macaca mulatta*)

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Virtual reality can be used as a tool to study the neurobiology of cognition and behaviour, including processes such as decision-making and memory. To this end, we have used an open-source video game engine (Unreal Engine 3) to create immersive virtual reality environments that can be modified in real-time through a custom-built Matlab interface. We have trained rhesus monkeys (*Macaca mulatta*) to freely navigate through these environments using a two-axis joystick. In one paradigm, monkeys were presented with a two-alternative forced choice paradigm (similar to a Y-maze) and learned an arbitrary object/reward value hierarchy through trial and error. Analysis of navigational and eye-movement patterns at the point of decision-making yielded patterns of visual fixation that correlate with object choice. That is, when faced with differentially-rewarded and choice-exclusive objects, non-human primates will rapidly saccade between them; the proportion of time fixating on each object is predictive of the object choice. Further, the proportion of time fixating on the object of higher reward value increases as the monkeys learn the object/reward value association. These findings are congruent with human and animal models of decision-making. Importantly, they support the utility of dynamically controlled virtual environments in understanding the neurobiology of complex cognitive behaviours in non-human primates.

2-F-162 Investigating the role of orbitofrontal cortex in crossmodal object recognition in rats

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We have previously shown that rats rely on the interaction between posterior parietal (PPC), perirhinal (PRh) and orbitofrontal (OFC) cortices to recognize objects across the tactile and visual sensory domains. Whereas past studies suggest that PPC and PRh process tactile and visual

object properties, respectively, the current study was conducted to better establish the precise contributions of OFC to crossmodal object recognition (CMOR). We began by examining the temporal involvement of OFC during the tactile sample and visual choice phases of CMOR. Transient lesions induced by intra-OFC infusions of lidocaine produced a delay-dependent impairment when given prior to the sample but not the choice phase, suggesting a role in information encoding. We next tested the hypothesis that this putative function may help to prevent interference effects during extended delay periods. When rats were exposed to a sensory restriction procedure in order to reduce potential interfering stimulation during the retention delay, the impairment produced by pre-sample intra-OFC lidocaine was reversed. Finally, we further explored the circuitry underlying CMOR by transiently disconnecting communication between the OFC and other regions previously implicated in the task. Thus far, we have established that CMOR performance relies on communication between OFC and PPC but not between OFC and PRh. We are currently examining the possibility that OFC interacts with areas located caudal to PRh along the ventral visual stream to facilitate crossmodal encoding of object representations.

2-F-163 Oscillatory responses to sentence embedded semantic and syntactic violations: Effect of bilingualism

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EEG studies employing time-frequency analysis have revealed modulations of theta and alpha power in a variety of language and memory tasks. Semantic and syntactic violations embedded in sentences evoke well-known event-related potentials, but little is known about the oscillatory responses to these violations. We investigated oscillatory responses to both kinds of violations, while monolingual and bilingual participants performed an acceptability judgement task. Both violations elicited power decreases (event-related desynchronization, ERD) in the 8-30 Hz frequency range, but with different scalp topographies. In addition, semantic anomalies elicited power increases (event-related synchronization, ERS) in the 1-5 Hz frequency band. The 1-5 Hz ERS was strongly phase-locked to stimulus onset and highly correlated with time-domain averages, whereas the 8-30 Hz ERD response varied

independently of these. In addition, the results showed that language expertise modulated 8-30 Hz ERD for syntactic violations as a function of the executive demands of the task. When the executive function demands were increased using a grammaticality judgment task, bilinguals but not monolinguals demonstrated reduced 8-30 Hz ERD for syntactic violations. These findings suggest a putative role of the 8-30 Hz ERD response as a marker of linguistic processing that likely represents a separate neural process from those underlying event-related potentials.

2-F-164 Intraoral self-administration of sweeteners in laboratory rats

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The food addiction hypothesis predicts that some foods may share with drugs of abuse the ability to reinforce behaviors leading to their consumption. The current study in male Sprague-Dawley rats was designed to compare the reinforcing effect of sucrose (S) and high fructose corn syrup (HFCS) using procedures commonly used to study the reinforcing properties of drugs. To assess the palatability of these sweeteners, rats implanted with intraoral cannulas received taste reactivity (TR) tests with isocaloric solutions of S (20%) or HFCS (25%). Then, rats self-administered the same solutions (one 3 hour session/day) for 40 days. To this end, rats pressed a lever to receive an intraoral infusion (90µl/inf) of either S or HFCS on continuous and progressive ratio (PR) schedules of reinforcement. Preliminary data indicate that HFCS engendered greater hedonic reactions in tests of TR; however, palatability was not related to intake when lever pressing on a continuous schedule in self-administration (SA) as rats maintained higher intake of S while binge-like behavior only emerged in rats that self-administered HFCS. Finally, group differences did not emerge when lever pressing on a PR schedule. Taken together, these data suggest that isocaloric solutions of S and HFCS do not have the same effect on mechanisms of reward and reinforcement as these sweeteners engendered differences in palatability, overall intake, and binge-like behavior. Future studies will focus on replicating these results as well as identifying features of S and HFCS that may contribute to these notable effects.

2-F-165 Differences of fructose/glucose ratios on operant self-administration and c-fos expression in the hypothalamus and nucleus accumbens

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One potential contributor to obesity is over-consumption of sugars. More specifically, it is believed that foods containing high levels of fructose (instead of glucose) are more likely to be "abused" and hence promote overeating. The current study explored the hypothesis that ratio of fructose/glucose influences reward-related behaviors and neuronal activity in areas of the brain involved in reward and energy balance. Using a radial arm maze, male Sprague-Dawley rats self-administered high ratio (HR; 55%F-45%G, typical ratio used in the food industry) or low ratio (LR ; 30%F-70%G, typical commercial sucrose pellets ratio, Bio-Serv, Frenchtown, NJ) fructose-glucose pellets for 10 minutes daily, over 14 days. Control rats received the same treatment but did not receive pellets after nose pokes. On day 14, rats were sacrificed 90 minutes after the session, and brains were extracted and processed for Fos-like immunoreactivity in the hypothalamus and nucleus accumbens. Initial results indicated no significant differences between HR and LR in consumption of pellets, or c-fos density (count/um²) within the nucleus accumbens. However, within the perifornical area of the lateral hypothalamus, there was a significant trend for the HR group to display lower c-fos density. In light of these preliminary findings, it is concluded that 5%-30% differences in ratio of fructose/glucose may not be large enough to induce significant changes in rewarded behavior and underlying neural substrates.

2-F-166 Lack of sex differences but menstrual cycle phase dependent modulation of craving for cigarettes in female smokers : An fMRI study

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While overall more men than women smoke cigarettes, women and girls take less time to

become dependent after initial use and have more difficulties quitting the habit, than men and boys. One of the factors contributing to these differences may be that women crave cigarettes more than men and that their desire to smoke is influenced by hormonal fluctuations across the menstrual cycle. Therefore, the purpose of the present study was two-fold: a) examine potential sex differences in functional neuroanatomy of craving; b) delineate neural correlates of cigarette cravings in women across their menstrual cycle. Fifteen tobacco-smoking men and 19 women underwent a functional MRI during presentation of neutral and smoking-related images, known to elicit craving. Women were tested twice; once during early follicular (low levels of estradiol and progesterone) and once during mid-luteal (high levels of estradiol and progesterone) phase of their menstrual cycle. The analysis did not revealed any significant sex differences in the cerebral activations associated with craving. Nevertheless, the pattern of activations in women varied across their menstrual cycle; significant activations in the precuneus, anterior and posterior cingulate, medial frontal, inferior temporal and angular gyrus during follicular phase, and only limited activations in the right hippocampus and precuneus during the luteal phase. Present findings may provide some preliminary clues to design better programs to quit smoking for women.

2-F-167 The effect of atypical antipsychotics on an index of semantic processing

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Semantic processing relates to the access of meanings about incoming information from semantic memory. Deficits in this process have been proposed to account for the symptomology of schizophrenia as well as the schizotypal tendencies seen in the general population. For instance, an abnormal spread of activations within semantic memory networks could underlie disorganization while inefficient use of context to inhibit incongruent activations could underlie delusional ideation. Antipsychotic medications could thus alleviate these symptoms by acting on the neural networks underlying semantic processing. To test this hypothesis, we examined a well-known index of semantic processing: the event-related potential (ERP) peaking negatively 400 ms post stimulus onset

(i.e., the N400). We investigated the immediate effect of a single dose of risperidone (n=45) and a placebo (n=25) on this N400 in healthy, drug-naïve individuals, as they performed a semantic categorization task. An increase in the frontal N400 amplitudes was observed in both the medication and placebo groups in the 300-500 ms time window, while an increase of this ERP in the medication group only was seen in the 200-300 ms time window. We conclude that risperidone has an immediate impact on the early part of the N400, while practice to task augments the later part of this ERP.

2-F-168 Profile differences in 50 kHz vocalizations induced by systemic or intraaccumbens application of amphetamine

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Ultrasonic vocalizations in rats serve as a behavioural index of their affective states. It has been established that production of 50 kHz calls, associated with appetitive states, involves activity of the ascending mesolimbic dopamine (DA) pathway from the VTA to the nucleus accumbens. The 50 kHz call category can be further subdivided into flat and frequency modulated subtypes based on sonographic characteristics. Little is known about the role of these subtypes and how they are generated. The purpose of the current study was to investigate whether the route of DA agonist application can have effect on parameters and subtypes of elicited 50 kHz calls. Injections of saline or the indirect DA agonist, amphetamine (AMPH) were made both systemically (1.5-2.0 mg/kg, subcutaneous) and directly to the brain (7 µg, intraaccumbens) with the resultant call profiles analyzed (N = 24). Systemically-induced AMPH calls were found to differ significantly in total number of generated calls, acoustic parameters, and subtype proportions when compared with those after saline (n = 12), as well as compared to intraaccumbens AMPH microinjections (n = 12). These results support the hypothesis that different routes of drug application differentially elicit 50 kHz call subtypes and modulate their acoustic parameters. Systemic application appears to more effectively induce affective signaling presumably by more complete activation of the mesolimbic DA system than local intraaccumbens injection.

2-F-169 Haloperidol-Environment Interaction Mediates Expression of c-Fos Proteins in the Ventral Pallidum of Rats

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Enhanced ventral pallidum (VP) activity has been shown to mediate dopamine (DA) neuronal activity and facilitate the strength of motivationally salient environmental stimuli. Repeated treatment with antipsychotic drugs reduces the salience of biologically significant stimuli and gradually produces motivational impairments observed as a loss of motor engagement with the environment. We examined the neural mechanisms of context-dependent catalepsy sensitization in rats with an immunohistochemical assay for c-Fos, a marker of recent neuronal activity. During the training phase (15 daily sessions), the paired group received haloperidol (0.25 mg/kg; n=9), a DAD2 receptor-preferring antagonist, 1 h prior to a catalepsy test carried out in a specific environment by placing the rats with their forepaws resting on a horizontal bar. The unpaired group (n=9) was treated with saline before catalepsy test and then injected with haloperidol 1 h later; a third group (n=9) was treated with saline before and after catalepsy test. On a test day when all groups were treated with haloperidol prior to the catalepsy test, the paired group showed significantly longer descent latency from the bar compared to the unpaired and saline control groups. Immunohistochemical results found that haloperidol paired animals (n = 5) had significantly attenuated c-Fos expression in the VP compared to both the unpaired (n = 6) and saline controls (n = 3). No differences were found in the parietal cortex. Results implicate the VP in the integration of contextual and motivational information. (Funded by NSERC)

2-F-170 Sex Differences In Myelination Of The Song Control System

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The song control system (SCS) is an intensively studied network in the songbird brain. Nuclei within this network are responsible for learning, production, and maintenance of song, and furthermore adult singing behavior. There are

extreme sex differences in these nuclei and in singing behaviour, making songbirds an excellent model to study sex differences in the brain. Myelination of the SCS, while potentially vital to its function, has not been extensively examined. The current study used male and female adult zebra finches to examine sex differences in myelination of the SCS. Male zebra finches sing but females do not. Immunohistochemical labeling of myelin basic protein (MBP) was used to measure myelination within the SCS. Regions of interest included HVC, RA, and LMAN. Fibre tracts analyzed included the HVC to RA tract and the HVC to Area X tract contained within lamina mesopallium ventralis (LMV). Results show a significant male-biased sex difference in MBP immunoreactivity within HVC and the HVC to RA tract, but not within RA, LMAN, or LMV. These results suggest that the myelination of HVC and the HVC to RA tract is important to adult singing behaviour, as the males sing and females do not. Furthermore, the non-significant findings in other areas of the SCS suggest that these regions are still important for females, potentially for the perception of song, and/or the production of other vocalizations. Determining how sex differences in myelination of the SCS are regulated will provide an important advance in basic neurobiology.

2-F-171 Set, reversal, and long-term olfactory learning in the 3xTG-AD mouse model of Alzheimer's disease.

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The 3xTG-AD transgenic mouse model of AD develops both amyloid- β plaques and tau tangles between 3 and 6 months of age. We tested female 3xTG-AD mice (n=14), and their wildtype controls (B6129S/F2) (n=13), at 6 to 18 months old on a series of 18 olfactory discrimination and reversal tasks in an operant olfactometer. Mice were trained to discriminate between two odour stimulus, an S and an S-, on a go, no-go task. The mice were then presented with a reversal task in which the S and S- odours were switched. Following the reversal task, the mice were given a new pair of odours to discriminate, followed by a reversal, and this was repeated until the mice had completed a series of 18 discriminations and reversals. Mice were retested on the final odour pair 1, 2, or 3 months later. Mice made more errors learning the reversal tasks than the discrimination tasks.

This difference was most pronounced on the earliest odour pairs and decreased as the mice advanced through the series. Many of the mice showed near errorless learning, making only 1 or 2, in the final stages of the series. During the retest phase performance returned to levels comparable to the start of the series. Transgenic mice reached their maximum performance earlier in the series than the wildtype mice, but with the wildtype mice reached a higher level of performance in the later odour pairs. This high level of performance suggests that the mice were able to develop a strategy during the earlier stages of the experiment and apply this to the later stages.

2-F-172 Synaptic impairment of cortical and hippocampal fast-spiking basket cells induces cognitive deficits in *Cacna1a* mutants.

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Background. CACNA1A encodes the $\alpha 1$ sub-unit of Cav2.1 channels. CACNA1A deletions result in episodic ataxia (EA2) and epilepsy in humans. We recently demonstrated that a targeted deletion of *Cacna1a* in forebrain GABAergic interneurons (IN) leads to selective synaptic impairment of parvalbumin-positive (PV) fast-spiking basket cells and is sufficient to induce epilepsy in mice. Altered function or maturation of cortical PV INs have been reported to impair cognition in mice. We propose that impaired perisomatic inhibition resulting from PV INs synaptic dysfunction in neocortical and hippocampal circuits leads to cognitive deficits in *Cacna1a* mutants. **Method.** We assessed the cognitive abilities of 12 patients from 3 different families carrying CACNA1A deletions. Furthermore, we generated mutant mice carrying a targeted *Cacna1a* deletion restricted to telencephalic PV populations (PVcre;*Cacna1a*^{ac}/). We assessed their cognitive abilities and behaviour in the Open Field, T-maze and Morris Water Maze. We also investigated cortical and hippocampal perisomatic inhibition in vitro. **Results.** Patients carrying CACNA1A deletions displayed a spectrum of neurocognitive deficits including inattention, impulsivity and/or intellectual disability. We show that *Cacna1a* haploinsufficiency in PV populations alters perisomatic inhibition and is sufficient to cause

hyperactivity, cognitive rigidity and reduced spatial memory in mice. **Conclusions.** Our results demonstrate the critical role of Cav2.1 in regulating perisomatic inhibition during cognitive processes.

2-F-173 Dorso lateral corticoid area and its neuronal classes: possible role in vocal learning and cognition in Indian Ring Neck parrot (*Psittacula krameri*)

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Detailed study of avian pallium provides apprehension, how birds are able to carry out higher order cognitive functions without a laminated cerebral cortex. However, birds are able to do so as major part of avian telencephalon contributes to pallium and is comparable to mammalian cortex, Parrots possess quite sophisticated cognitive abilities and vocal abilities carried out by nuclear pallial areas. Most of the telencephalic vocal control nuclei are in the pallium with one vocal nucleus in the striatum. Dorsolateral corticoid area (CDL) in aves is a part of corticoid complex and is located as a thin narrow part at dorsolateral surface of telencephalic pallium. The present study was designed to explore the neuroarchitecture of parrot telencephalon with CDL in particular. Using Nissl stain and Golgi impregnation techniques, the types of neuronal sub-classes in parrot CDL were identified and studied in detail. Neurons of this area are distinguished in two prominent cell types, spinous projection neurons and local circuit neurons. Detailed morphology and the possible roles of these cell types in regulating cognitive and emotional processing including vocal abilities are also discussed. We also conclude about how the CDL interact with other pallial regions as a part of circuits involved in the regulation of cognitive functions. The CDL of birds is most recently proposed as homologous to mammalian cingulate cortex, suggests that complex cognitive function of birds are due to similar neuronal components of homologous regions of brain.

2-F-174 Muscarinic cholinergic receptor activation destabilizes object memories, possibly via proteasome-mediated protein degradation

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Consolidated memories can become destabilized and open to modification upon retrieval. Destabilization is most reliably prompted when novel information is present during memory reactivation. We hypothesized that acetylcholine (ACh) plays an important role in novelty-induced memory destabilization due to its established involvement in new learning. Accordingly, we investigated the effects of cholinergic manipulations in rats using an object recognition paradigm that requires reactivation novelty to destabilize object memories. The muscarinic receptor antagonist scopolamine, systemically or infused directly into the perirhinal cortex (PRh), a brain region strongly implicated in object memory, blocked this novelty-induced memory destabilization. Conversely, systemic injection or PRh infusion of a muscarinic receptor agonist (oxotremorine or carbachol) mimicked the destabilizing effect of novel information during reactivation. Furthermore, preliminary data suggest that carbachol-induced destabilization requires proteasome-regulated protein degradation in the PRh. The bidirectional cholinergic effects suggest a crucial influence of ACh on memory destabilization and the updating functions of reconsolidation. This is a hitherto unappreciated mnemonic role for ACh with implications for its potential involvement in cognitive flexibility and the dynamic process of long-term memory storage. A connection between muscarinic receptor activation and proteasome activity provides a basis for investigation into the intracellular pathways involved in object memory destabilization.

2-F-175 Decoding the focus of visual attention from prefrontal ensemble activity

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The lateral prefrontal cortex (LPFC) is thought to play an important role in visual selective attention. Traditional single cell studies in non-human primates have revealed that the activity of individual LPFC neurons pooled over many trials can filter behaviourally relevant from irrelevant visual information. However, in real situations the brain must filter information in a

single trial based on the activity of many neurons. Thus, whether and how the activity of simultaneously active LPFC neurons can effectively filter relevant targets in the presence of distractors remains unclear. Here we chronically implanted multielectrode arrays in area 8r of the LPFC of two non-human primates and showed that the activity of small assemblies of ~50 simultaneously recorded neurons can be reliably decoded to determine the location of an attended target among distractors. The decoding was robust to unexpected transient changes in the distractors' features, predictive of behavioral errors, and stable across a timespan of multiple weeks, suggesting that it can be of potential use in the implementation of cognitive brain machine interfaces.

2-F-176 Muscarinic Control of Rostromedial Tegmental Nucleus GABA Neurons and Morphine-induced Locomotion

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Opioids induce rewarding and locomotor effects mainly via rostromedial tegmental (RMTg) GABA neurons that express μ -opioid and nociceptin receptors. These GABA neurons then strongly inhibit midbrain dopamine neurons. Opioid rewards and locomotion also depend on dorsal tegmental cholinergic and glutamate neurons that project to and activate VTA dopamine neurons. Here we show that many pedunclopontine and lateral tegmental cholinergic neurons project to both RMTg and VTA, and that M4 muscarinic receptors are co-localized with μ -opioid receptors on RMTg GABA neurons. To inhibit or excite RMTg GABA neurons, we bilaterally transfected designed muscarinic receptors (M4D or M3D) in GAD2-Cre mice with AAV. In M4D-expressing mice, clozapine-N-oxide (CNO) increased morphine-induced, but not saline-induced locomotion. In M3D-expressing mice, CNO blocked morphine-induced locomotion, but not saline-induced locomotion. We propose a disinhibitory model of opioid-induced locomotion in which cholinergic inhibition of RMTg GABA neurons via M4 muscarinic receptors facilitates opioid inhibition of the same neurons. Collateral cholinergic activation of VTA dopamine neurons via M5 muscarinic receptors activates dopamine neurons and facilitates dopamine-dependent locomotion.

2-F-177 Sensitization of the Activity- Decreasing Effects of Haloperidol in Rats: Preliminary Results

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Increases in brain dopamine (DA) produced by repeated administration of cocaine or amphetamine in a particular environment result in sensitization of locomotor activity in rats, activity increasing over sessions. This effect is specific to the drug-paired environment. We investigated whether decreased activity could be sensitized using a similar sensitization paradigm. In previous work in rats, low doses of the D2 receptor-preferring antagonist haloperidol (HAL; 0.25 mg/kg, i.p.) led to context-dependent increases in catalepsy, a phenomenon marked by immobility on a suspended horizontal bar. We hypothesized that decreases in brain DA neurotransmission produced by repeated administration of HAL would lead to context-dependent sensitization of decreased activity. The paired group (n = 10) received HAL (0.1 mg/kg, i.p.) 1 h before placement in the test chamber, where activity was measured for 3 min daily during 13 sessions. The unpaired group (n = 10) received haloperidol 1 h after the daily activity test, serving as a drug-history control, but without the drug-environment pairing. A third group (n = 10) received saline instead of HAL. The groups did not differ during the early test sessions but the paired group showed less activity than the unpaired and saline groups on days 12 and 13, revealing sensitization to the activity-decreasing effects of HAL. Results suggest that DA D2 neurotransmission plays an important role in producing context-dependent sensitization of decreases in activity. (Funded by NSERC)

2-F-178 The Nature of Forgetting: Storage or Retrieval Impairment in Experimental Amnesia

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Experimental amnesia can reflect impaired storage (memory erasure), or impaired retrieval (inaccessible memory). We have previously proposed a novel paradigm to test this unresolved issue. We proposed that exploiting the differences in brain mechanisms which are selective to first versus second learning is a constructive approach to dissociate these two theories. Specifically, by infusing AP5 into the

rodent dorsal hippocampus (dHC) prior to training, we show that first object location learning acquisition requires NMDA receptors, while subsequent learning of the same task does not. We used this difference in a second learning protocol to determine whether amnesia for first learning induced by ZIP infusions in the dHC would affect second learning. Rats were taught first learning of a novel object location task in context A and then infused with ZIP or scrambled ZIP (SCR) 24 hours after the end of training. The following day both groups underwent AP5- or vehicle-infusions immediately prior to second learning training in Context B. We show that only ZIP-infused amnesic rats required NMDA receptors to acquire second learning memory of object location, as if there was no previous memory of first training. These data are consistent with the interpretation that the amnesia induced by ZIP-infusions does not impair retrieval, but erases memory.

G - Novel Methods and Technology Development

2-G-179 Examining astrocyte morphology using DiOlistic labeling with the PDS-1000/He Particle Delivery System

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Morphological changes in astrocytes have profound effects on nervous system function, yet detailed characterization of their 3-D morphology is not complete. Astrocytic morphology is highly complex; numerous spines, lamellae and filopodia radiate along the lengths of astrocyte processes. To visualize these morphological structures it is necessary to use a membrane associated dye or membrane targeted fluorescent protein. Typically, transgenic mouse lines have been used to express fluorescent proteins within individual cells; however, these are costly and difficult to maintain. DiOlistics, a less costly method, has been used to label cells with membrane-targeted dyes. DiOlistics involves coating 1.0 µm tungsten particles with lipophilic dyes and delivering them into tissues using a blast of helium. When these particles come in contact with a cell, the entire membrane is labelled with the fluorescent dye, enabling an examination of its structure in its native environment. The labeling also occurs quickly and persists for long periods of time (at least 9 months). Finally,

DiOlistic labeling can also be combined with immunocytochemistry to examine the subcellular distribution of proteins within individual cells. Where DiOlistic labeling normally requires a BioRad Helios gene gun, we have adapted this methodology to the PDS-1000; this permits a larger number of brain sections to be bombarded at one time, increasing the number of labelled cells. We are currently combining confocal microscopy and 3-D reconstruction using Imaris to localize proteins within labelled astrocytes.

2-G-180 Focused ultrasound-mediated blood-brain barrier opening in a mouse model of Alzheimer's disease

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Focused ultrasound (FUS) can temporarily open the blood-brain barrier (BBB) to permit access of therapeutic agents into localized brain regions. FUS-mediated BBB opening has reduced amyloid plaque load in the cortex of a mouse model of Alzheimer's disease (AD). Here, we correlate reduced plaque pathology with changes in cognition. Further, we use two-photon microscopy to evaluate the changes in BBB permeability in the presence of plaque pathology. 8 month old transgenic (Tg) mice exhibiting advanced pathology and compromised vasculature, were treated with weekly MRI-guided FUS treatments in the hippocampus. In the Y maze, untreated Tg mice spent significantly less time in the novel arm compared to their non-Tg littermates but following FUS, Tg mice performance was restored to levels of non-Tg mice. Increases in the number and complexity of doublecortin-expressing cells in the dentate gyrus suggest that FUS promotes neuronal plasticity and improves cognitive behaviour even in Tg mice with advanced pathology. Further, using two-photon microscopy and fluorescent intravascular dye, we demonstrated that dye leakage following FUS-induced BBB permeability was significantly less in Tg compared to non-Tg mice. Changes in vessel diameter after FUS treatment were diminished in plaque-coated vessels suggesting that the mechanisms of BBB opening may be different in Tg mice. These studies further the understanding of FUS-induced BBB permeability in presence of amyloid pathology, and they contribute to evaluating the potential of FUS in the development of treatments for AD.

2-G-181 A novel method of electrical stimulation to reduce tibialis anterior contraction fatigue

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Neuromuscular electrical stimulation (NMES) can be used to produce contractions for people who have had a stroke or a spinal cord injury. Traditionally, NMES is applied either over a muscle belly (mNMES) or nerve trunk (nNMES). Unfortunately, NMES is limited by rapid contraction fatigue, due in part to abnormally high discharge rates of recruited motor units. To overcome this problem, we developed interleaved NMES (iNMES), in which stimulus pulses are alternated between the muscle and nerve sites. Since mNMES and nNMES recruit different motor unit populations, we propose that iNMES will halve motor unit discharge rates compared to mNMES or nNMES delivered alone. Presently, we compare contraction fatigue during mNMES, nNMES and iNMES. We hypothesized that iNMES will generate the most fatigue-resistant contractions, followed by mNMES and nNMES respectively. Able-bodied subjects participated in 3 experimental sessions on separate days. The fatigue protocol consisted of 250 trains of NMES (50 μ s pulse duration, 40 Hz, 2 s "on", 1 s "off"). At the start of the protocol, contraction amplitudes were 10-15% of a maximum voluntary contraction. Contraction fatigue was quantified as the fatigue index: the average torque for the last 5 contractions divided by the average torque for the first 5 contractions, multiplied by 100. Data collected from 6 subjects thus far show that iNMES generated the most fatigue-resistant contractions (75 \pm 61), followed by nNMES (67 \pm 75) and mNMES (51 \pm 25) respectively. iNMES demonstrates potential to reduce contraction fatigue for rehabilitation.

2-G-182 A new standalone software for interactive filtering of movement artifacts generated during multiphoton intravital imaging of neuroinflammation

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Two-photon intravital microscopy is a powerful and versatile tool to investigate with high spatial

and temporal resolution biological samples in their unaltered environments. Notably, it allows the live visualization of neuro-immunological events taking place in the central and peripheral nervous systems both in physiological and pathological contexts, which is of the utmost interest for studying animal models of neurological disorders. The acquisition of high quality intravital time-lapse data is however hampered by movement artifacts generated by cardiac and respiratory cycles as well as peristalsis, which even deep anesthesia and animal stabilization cannot eliminate. This is a major obstacle to studying dynamic morphological changes in living tissue at the cellular level. To overcome these caveats, we recently developed a post-acquisition software that allows to minimize the impact of movement artifacts through image treatment, filtering and alignment techniques (Soulet et al, PLoS ONE, 2013). In this communication, we present a new, standalone multiplatform version of our algorithm that was completely rewritten with MathWorks® MATLAB. Among the new functions available, we have developed a user-friendly graphical interface that greatly facilitates the navigation through the different features of the software. Of importance, execution time has been optimized to allow more efficient multidimensional image processing in 4 channels in the Z-axis over time. This results in a dramatic increase in image quality and better interpretation of dynamic biological events.

2-G-183 Development of BRET-based biosensors for nuclear receptors associated with dopamine neurotransmission

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The Nur (Nr4a) subgroup includes 3 members (Nur77, Nurr1 and Nor-1). They are transcription factors of the orphan nuclear receptor (NR) family. Nurr1 is expressed in dopamine (DA) neurons, whereas Nur77 and Nor-1 are normally expressed in brain structures innervated by DA neurons. Previous works from our laboratory indicated that Nur77, along with its cognate partner retinoid X receptor (RXR), is involved in antipsychotic and anti-parkinsonian drug activities. However, Nur-dependent transcriptional activity in the brain remains elusive. Nurs can interact with distinct gene promoter response elements through monomer, homodimer, or heterodimer forms with RXR. Very few drugs targeting Nurs have been

identified so far and drugs acting at RXR remain poorly characterized. Furthermore, presently available assays cannot recapitulate the complexity of NR activities and generate indirect measures of drug activities. For this purpose, we have developed and optimized luciferase protein complementation-based Bioluminescence Resonance Energy Transfer (BRET) assays for the recruitment of a YFP-tagged co-activator by specific NR species. We validated the assays with available compounds using dose-response curves. EC50 obtained were very similar to previously reported values. Our results indicate that these BRET assays can be used as biosensors to monitor selective drug activities at RXR/RXR and Nur77/RXR complexes. Thus, this technology can be used to identify new compounds showing specificity profiles to individual dimeric species formed by one NR. Supported by the GRUM.

2-G-184 Kinematic Assessment Effectively Guide Botulinum Neurotoxin Type A Injections for Essential Tremor Treatment

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Efficacy of botulinum neurotoxin type A (BoNT-A) injection in treating essential tremor (ET) has been poor due to the difficulty of visual assessment in arm tremor. 24 ET patients were assessed over 48 weeks using kinematic sensors placed on the wrist, elbow and shoulder joints. Recordings were taken with the patient's arm in rest, posture, load and kinetic states. Measurements were processed and kinematic results were provided to the physician. Based on the physician's own clinical experience, BoNT-A dose and injection sites were determined. Kinematic assessment and clinical tremor rating scales were completed at each visit. Injection cycles were 4 months apart. Following the first injection, kinematic measurements showed a significant tremor reduction at the shoulder, elbow and wrist. The Quality of Life scale showed a significant improvement, dropping from an average of 40 to 31 by week 22. Similarly, the Fahn, Tolosa, Marin Tremor Rating Scale showed significant improvement by week 6 with a reduction in total score of 47 to 36. The Unified Parkinson Disease Rating Scale action tremor assessment declined from 2.6 to 1.6 by week 16. Maximal grip strength was reduced by 25% following the first injection; patients perceived mild weakness with limited to no loss of function. Using this kinematic

assessment technique, overall arm tremor post BoNT-A injection showed remarkable reduction. The kinematic device and software were able to deconstruct the complex movements of tremors and thus increase BoNT-A injection accuracy, an outcome not possible by visual assessment alone.

2-G-185 BrainDir: A Public Online Repository for Healthy Control Neuroimaging Data

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A matched control group is often a requirement of well-designed imaging studies; however, the elevated costs associated with scanning can prohibit the recruitment of adequately large samples of healthy controls. A sufficiently large pool of publicly available healthy control imaging data would allow researchers to reduce their imaging costs by searching for and reusing healthy control data shared by other researchers. Previous attempts at creating online repositories of neuroimaging data have been undertaken, but there is a lack of options for cross-laboratory sharing of healthy control imaging data. Here we propose a freely available web application and companion cross-platform desktop application to facilitate the exchange of neuroimaging data between research groups. Our application consists of an online repository of anonymised imaging datasets stored in the DICOM image format searchable by criteria such as participant age, sex, handedness, and years of education. The web application also provides facilities for easily importing author information into popular reference managers. The companion desktop application provides a simple interface allowing for the anonymisation of DICOM metadata. As well, it provides functionality for reliably uploading and downloading large neuroimaging datasets to and from BrainDir's centralised online repository. Finally, in accord with ethical principles and Canadian regulation, we provide a set of standardised consent forms for researchers to include in their studies to obtain participant consent for secondary use of their imaging data.

2-G-186 Optimization of SYBR Green real time quantitative PCR for single neurons

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To understand the heterogeneous properties of single neurons, real time quantitative polymerase chain reaction (qPCR) is a sensitive technique to measure gene expression. Using SYBR Green in qPCR experiments can be cost-effective compared to the taqman approach. However, the sensitivity of this technique for quantification of genes expressed at low number of copies is typically lower than with taqman. In the present study, we tested the hypothesis that the use of nested qPCR, in which two different pairs of primers are designed for each gene of interest, could allow to overcome the lower sensitivity of SYBR Green qPCR. Using single mouse dopamine neurons, we optimized nested qPCR for 2 genes: the vesicular glutamate transporter 2 (VGLUT2) and tyrosine hydroxylase (TH). Specific primers for VGLUT2, TH and a house keeping gene (GAPDH) were designed. The technique was performed in three steps: the reverse transcriptase reaction, an outer round of PCR and finally qPCR. For a low copy gene such as VGLUT2, a higher number of cycles were needed in the outer round. The specificity of the primers used was confirmed by analyzing the melt curves and by performing regular nested RT-PCR. We also performed for each gene a linear standard curve from a dilution of a pool of cells in order to confirm an efficiency between 90-110 % of the qPCR reaction. Our results show that such a three steps single-cell reverse transcriptase nested qPCR using SYBR Green is a sensitive and reproducible technique to quantify low abundance mRNA in single neurons.

2-G-187 Whole-brain mapping of neural activation in mice

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The ability to visualize behaviourally-induced neural activity patterns across the rodent brain is critical for understanding the distributed brain networks mediating particular behaviours. However, current imaging methods are limited in their spatial resolution and/or ability to efficiently image the entire brain. Here we describe a new automated method for mapping behaviourally-evoked neural activity over the whole mouse brain at cellular resolution. This method

combines the use of transgenic immediate-early gene reporter mice to visualize recent neural activity; serial two-photon tomography for high-resolution whole-brain imaging; image processing algorithms to count the activated neurons and align the datasets to the Allen Mouse Brain Atlas; and statistical analysis to identify the network of activated brain regions evoked by behaviour. To validate our approach, we used this method to determine the whole-brain networks activated during the retrieval of fear memories. Consistent with previous studies, we identified a large network of amygdalar, hippocampal, and neocortical brain regions implicated in fear memory retrieval. This imaging pipeline can thus be used to map the networks mediating the expression of normal behaviours, as well as to investigate circuit dysfunction in mouse models of neurobiological disease.

I - Neuroengineering

2-I-188 A spinal analogue of memory reconsolidation enables the reversal of hyperalgesia

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The development of persistent pain through the sensitization of pain relays in the spinal cord dorsal horn shares many mechanistic and phenotypic parallels with memory formation. Memory reconsolidation, in which the reactivation of memories renders them labile and susceptible to erasure by inhibition of protein synthesis, may thus be of particular relevance to the treatment of persistent pain. Yet, it is unknown if the reactivation of sensitized pain pathways initiates a process similar to memory recall and reconsolidation and renders hyperalgesia labile. We discover that both acute and long-lasting mechanical hyperalgesia can be reversed after reactivation of the sensitized pain pathway and the concomitant inhibition of spinal protein synthesis. This process was dependent on the activation of spinal AMPA, NMDA, and NK1 receptors, and on the activation of CaMKII and ERK. Additionally, the activation of spinal AMPA or NMDA receptors was sufficient to render hyperalgesia labile, suggesting this process is mediated by post-synaptic activation of dorsal horn neurons. Synaptic long-term potentiation (LTP) in the superficial dorsal horn, a cellular model of hyperalgesia, was similarly reversed by reapplying the LTP induction stimulus in the presence of anisomycin. These findings provide

the first demonstration of a reconsolidation-like phenomenon in a sensory system, suggesting that reconsolidation may exist more broadly throughout the CNS than previously known. These findings may further provide a novel therapeutic strategy for the treatment and erasure of persistent pain.

2-I-189 In Vivo 2-Photon calcium imaging of the brain : active neurons revealed by spatio-temporal correlation analysis and region-growing segmentation.

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Two-photon calcium imaging in the nervous system has proven to be a key tool to characterize single neuron activity in intact brains. However, the low signal-to-noise ratio in an in vivo system and the high concentration of neurons makes the distinction of single neurons challenging. So far, no image-processing algorithm has been reported for calcium imaging that provides effective and tangible identification of neuronal cells. We present a new image segmentation technique using the calcium activity as the base for cell identification. The spatio-temporal correlation of the pixel calcium signaling and the method of region growing are employed to identify single calcium-active cells. On simulated calcium imaging data of neurons with irregular morphologies, the algorithm correctly assigns the ROIs within the attributed cell boundaries of all active neurons. The algorithm was tested on images of Oregon Green BAPTA-1 taken in vivo in tectum *Xenopus laevis* tadpole, revealing ROIs within the boundaries of neuronal cells. These results demonstrate that the combination of correlation analysis with region growing method is a promising image segmentation tool with the potential to identify and isolate active neurons consistently, opening the possibility of the automatic analysis of neuronal activity in vivo via calcium imaging.

2-I-190 Creating Artificial Neuronal Connections

G Monserratt Lopez Ayon¹, Margaret Magdesian¹, Megumi Mori¹, Xue Ying Chua¹, Alexis Goulet Hanssens¹, David Oliver¹, William Paul¹, Dominic Boudreau², Delphine Gobert¹, Ricardo Sanz¹, Yoichi Miyahara¹,

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Many forms of brain and spinal cord (CNS) injuries cut axons. When axons can regenerate, as in peripheral nerves, they can bring back function. However in the CNS axon regeneration fails. This is the main reason why paralysis and loss of sensation is permanent in conditions such as spinal cord injury. First because the environment surrounding CNS lesions is inhibitory to axonal growth, and second because most CNS axons only mount a feeble regeneration response after they are cut. Here we show that we can manipulate the growth of CNS axons using Atomic Force Microscopy (AFM) and optical microscopy. Axons adhere to functionalized beads precisely positioned at the tip of an AFM cantilever. Once the adhesion is created we can pull the beads, thereby extending a new neurite for several hundreds of microns. When in contact with another neuron the newly formed neurite forms a stable connection. To test the functionality and the type of connection, we put together an electrophysiology setup which enables paired recordings. We faced several instrumental and biological challenges to keep neurons healthy and responsive to perform paired recordings after artificially connecting the cells. From connection to recording, the pieces of the puzzle are finally together. Now we have instrumentation reliability allowing experiments to be carried out routinely. We will discuss potential biological factors that may lead to a strong, mature and stable artificial connection.

2-I-191 Gephyrin clusters are absent from small diameter primary afferent terminals despite the presence of GABA(A) receptors

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While both GABAA receptors and glycine receptors play a role in control of dorsal horn neuron excitability, their relative contribution to inhibition of small primary afferent terminals remains controversial. To address this, we designed an approach for quantitative analyses of the distribution of GABAAR subunits (SU),

GlyR α 1 SU and their anchoring protein, gephyrin on terminals of primary afferents identified by CGRP/IB4 labeling. An algorithm was designed to recognize structures with dimensions similar to those of the microscope resolution. To avoid detecting false co-localization, the latter was considered significant only if the degree of pixel overlap exceeded that expected from randomly overlapping pixels given a hypergeometric distribution. We found that both CGRP(+) /IB4(+) terminals were devoid of GlyR α 1 SU and gephyrin. The α 1 GABAAR was also absent from these terminals. In contrast, the GABAAR α 2/ α 3/ α 5/ β 3 SU were significantly expressed in both terminal types, as well as other GABAAR-associated-proteins (α -Dystroglycan/Neurologin-2/Collybistin-2). Ultrastructural immunocytochemistry confirmed the presence of GABAAR β 3 SU in small afferent terminals. RT qPCR confirmed the results of light microscopy immunochemical analysis. These results indicate that dorsal horn inhibitory synapses follow different rules of organization at pre vs. postsynaptic sites (i.e. in nociceptive afferent terminals vs. inhibitory synapses on dorsal horn neurons). As a consequence, presynaptic GABAAR-mediated inhibition may be more diffuse than at dorsal horn neuron synapses.

2-I-192 Interfacing synthetic and native membranes: model lipid membrane domains for evaluating specific cellular responses

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Lipid membrane domains (also known as lipid rafts) are dynamic assemblies of membrane components (phospholipids, cholesterol, proteins, etc.) that provide a platform for many cellular functions including signaling, protein transport, lipid sorting and membrane fusion. In this study, co-existing lipid microdomains, supported on spherical substrates (SS-BLMs), are utilized to interact with living cells (primary hippocampal neurons) in order to evaluate their responses with different cellular entities and processes. Spherical solid substrates (silica beads) combine the convenience of a mechanically stable platform for performing experiments while providing an environment that closely resembles the biological system being modeled. In addition, they provide a new way to observe relationships between curvature, lipid organization, and phase behaviour in lipid mixtures. Confocal fluorescence microscopy and

cryo-EM are used to characterize the SS-BLM formation and the co-existence of microdomains. The interactions of these model membrane domains with living cells as well as the cellular membrane organization in relation to the co-existing microdomains are examined using immunofluorescence confocal microscopy. The SS-BLM approach is now extended to geometries such as silica fibers in order to better mimic the axonal surface shape in a neural cell culture.

2-I-193 Digital Nanodot Gradients with Adjustable Reference Surfaces to Explore Growth Cone Navigation on Gradients of Nanopatterned Protein Cues

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¹McGill University

Cell navigation operates in response to an inhomogeneous distribution of extracellular cues. There is therefore an incentive to create deterministic protein patterns in vitro to address how the density and distribution of these cues directs cell migration. Although many experimental results were reported, (i) the gradients are often limited in range, produced as continuous patterns, and difficult to quantify; moreover (ii) the reference surface (RS) - the area surrounding the patterns - is often not well controlled nor characterized, and in fact methods to adjust it had not been developed. Here, we address these two points by introducing digital nanodot gradients (DNGs) and a RS with tunable affinity. We present new DNG algorithms that fit monotonic and non-monotonic functions, composed of 200 nm dots that follow ordered or random arrangements. An array of 100 distinct DNGs was then designed and printed using lift-off nanocontact printing to pattern the 57 million protein dots. Cell-surface affinity was then identified to critically influence cell response to a patterned cue. We therefore developed means to easily adjust the RS to the experiment to maximize specific cell response. Substrate-bound protein gradients with adjustable RSs will facilitate the study of axonal migration by enabling the attribution of movement to patterned protein. Gradient geometries that most readily promote axonal migration will enhance our understanding of the mechanisms that underlie development and may facilitate the design of efficient therapies for regeneration.

2-I-194 Quantification of Cellular Mechanotransduction Force with Micropillar Array Detectors

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Cells sense molecular cues in the ECM and mediate force through focal adhesions in a process termed mechanotransduction, relaying information from the cell's surroundings to the cytoskeleton. Even though the emergence of micro and nanotechnologies has allowed the role of mechanotransduction in defining cellular motility and differentiation to be addressed, the role of mechanotransduction in neurons is still poorly understood owing to the lack of adequate tools to measure these fragile cells. We measure discrete traction forces in neuronal growth cones by adopting micropillar array detectors (mPADs). Cells are seeded on top of the mPAD pillars and the force matrix exerted by a cell is visualized and quantified as the magnitude of pillar tip displacement. A novel printing technique was used to pattern the pillar with a desired protein, as well as specific protein patterns to investigate growth cone traction force. C2C12 myoblast traction force was assayed on mPADs with 6 μm high pillars and a variety of surfaces with low and high affinity for the cells. Average force measurements ranged from 4.89 nN on low affinity surfaces (high % PEG) to 7.11 nN on high affinity surfaces (RGD or high % PLL). We fabricated 12 μm long (and 2 μm wide) pillars that are 8 times softer than any previously reported and can respond to the weak forces of neurons down to pN resolution. Studies focusing on neuronal growth cone traction forces are in progress. Mechanotransduction information will be particularly valuable in the areas of tissue engineering and nerve regeneration.

2-I-195 A novel Fluorescence Lifetime Nanoscopy approach to resolve protein interactions inside dendritic spines.

Christian Tardif¹, Daniel Côté¹, Paul De Koninck¹

¹CRIUSMQ

Having the ability to localize protein interactions inside the synaptic area is important for deciphering the signaling cascades implicated in learning and memory. Fluorescence Lifetime Imaging (FLIM) to quantify Foster Resonant Energy Transfer (FRET) is useful to study

protein interactions. However, FRET-FLIM approaches provide limited spatial resolution due to the diffraction of light (~250nm), particularly for studying interactions in synaptic domains. Super resolution methods have been developed to beat this resolution limit. We combined STimulated Emission Depletion (STED) with FRET-FLIM technique to study molecular interactions within spines at nanoscale. We built a STED microscope that currently achieves an x/y resolution of 60 nm. To measure protein interactions, we used an immuno-FRET technique, which we validated by measuring a known, activity-regulated, interaction between the Calcium/Calmodulin-dependent protein kinase β (β CaMKII) and actin filaments (F-actin) inside the spines of cultured hippocampal neurons. We are now investigating the interaction of α CaMKII with the N-methyl-D-aspartate receptor (NMDAR), which is believed to be critical for long term potentiation. Our preliminary results indicate that we can resolve which specific clusters of NMDARs interact with CaMKII inside a dendritic spine. Since the specific location of NMDARs with respect to the post-synaptic density is thought to critically impact on their functions, our Fluorescence Lifetime Nanoscopy (FLIN) approach should help us understanding NMDAR-dependent signaling and remodeling.

2-IBRO-196 Purinergic receptor activation induces Ca²⁺ waves in a stem cell niche of the rat spinal cord.

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¹Instituto de Investigaciones Biológicas Clemente Estable

The ependyma of the spinal cord (SC) harbors stem cells which are activated and recruited by traumatic SC injury. Progenitor-like cells are organized in spatial domains around the central canal (CC). On the lateral aspects, cells combine characteristics of ependymocytes and radial glia (RG). On the poles of the CC, RG cells display complex electrophysiological phenotypes. The mechanisms regulating the behavior of these progenitors -in particular their reaction to injury- remain unknown. During development, purinergic signaling regulates progenitor cell proliferation, migration and differentiation. Thus, we explored the effects of adenosine triphosphate (ATP) on ependymal progenitor-like cells in the neonatal (P1-P6) rat SC. Cells in midline and lateral domains express

the ionotropic P2X7 purinergic receptor. Patch clamp whole-cell recordings in SC slices revealed that BzATP, a P2X7 receptors agonist, generated inward currents in most RG and ependymocytes. Inward currents were larger than currents induced by equimolar concentrations of ATP, had a reversal near 0mV and were reduced by bath application of the P2X7 receptor antagonist brilliant blue G. Ca²⁺ imaging (Fluo-4) revealed that BzATP generated Ca²⁺ waves in RG that propagate along the entire cell. We conclude that progenitor-like cells in the ependyma of the rat SC have functional ionotropic P2X7 receptors. The intracellular Ca²⁺ signaling triggered by P2X7 receptor activation may be an epigenetic mechanism that regulates their biology in response to ATP released after tissue damage.

POSTER SESSION 3**A - Development****3-A-1 Role of the precursor form of the brain-derived neurotrophic factor, proBDNF, and its receptor p75NTR on GABAergic synapse maturation in neocortex****Elie Baho¹, Bidisha Chattopadhyaya¹, Marisol Lavertu Jolin¹, Graziella Di Cristo¹**¹Université de Montréal/CHU Ste-Justine

Basket cells innervate hundreds of postsynaptic targets with synapses clustered around the soma and proximal dendrites. They are important for gamma oscillation generation and for the regulation of developmental cortical plasticity. Although the function of basket cells within cortical networks is being explored, the mechanisms that control the development of their extensive arborisation and synaptic contacts have not been entirely resolved. BDNF has been shown to be a strong modulator of activity-dependent maturation of GABAergic synapses. BDNF is initially synthesized as a precursor, proBDNF, which is cleaved to produce mature BDNF. Whether proBDNF per se plays a role in the development of basket cell synaptic territory is unknown. Our results show that treating organotypic cultures prepared from mouse cortex during the synaptic proliferation phase with exogenous cleavage-resistant proBDNF (proBDNFmut) strongly reduces the synaptic territory of basket cells. To increase endogenous levels of proBDNF, we treated cultures with a tPA-inactivating peptide, PPACK, which also reduced basket cell synaptic innervation. We further showed that proBDNF acts through p75NTR, by knocking down p75NTR specifically in basket cells and treating them with proBDNFmut. p75NTR^{-/-} basket cells form exuberant innervations compared to control cells, an effect that is not rescued by proBDNFmut. All together, these results suggest that proBDNF negatively regulates the synaptic territory of basket cells through direct activation of p75NTR. In vivo studies are underway.

3-A-2 Expression of Kirrels in vomeronasal sensory neuron axons controls their coalescence into glomeruli of the AOB**Alexandra Brignall¹, Janet Prince¹, Jean-Francois Cloutier¹**¹Montreal Neurological Institute, McGill University

The accessory olfactory system controls social and sexual interactions in mice that are crucial for survival. Vomeronasal sensory neuron (VSN) axons form synapses with dendrites of second order neurons in glomeruli of the accessory olfactory bulb (AOB). Axon guidance molecules control the anterior-posterior segregation of VSN axons in the AOB, however less is known about the mechanisms regulating the coalescence of axons into specific glomeruli. We have previously shown that Kirrel-2 and Kirrel-3 of the Kirrel family of cell adhesion molecules are differentially expressed in subpopulations of VSNs, thereby defining a molecular code of axonal recognition. Also, germline ablation of Kirrel-3 expression led to a loss of male-male aggression in mice. Here, we show that germline ablation of either Kirrel-2 or Kirrel-3 expression results in the formation of fewer yet larger glomeruli in the posterior AOB while removal of both prevents the formation of distinct glomeruli altogether. In addition, the coalescence of specific tau-lacZ-labeled populations of VSN axons is severely disrupted in kirrel-2^{-/-}; kirrel-3^{-/-} mice. More precisely, Kirrel function is required in VSN axons as its specific ablation in VSNs (kirrel-2lox/lox; OMP-Cre) leads to defects in glomeruli formation that phenocopy kirrel-2^{-/-} mice. We are currently assessing whether the wiring defects observed in kirrel-2lox/lox; OMP-Cre mice cause changes in VNO-mediated behavior. Altogether, our results show that differential expression of Kirrels on VSN axons dictates their proper coalescence into glomeruli in the AOB.

3-A-3 Early changes in the offspring mesolimbic dopaminergic system induced by perinatal maternal high-fat diet**MinGi Cho¹, Greg Dal-Bo², Hong Long², Claire-Dominique Walker²**¹McGill university, ²Douglas Mental Health University Institute

Recent evidence demonstrates that maternal high-fat consumption during pregnancy and lactation increases the risk of metabolic disorders and obesity in the offspring, although the mechanisms remain unknown. We previously showed that adult offspring of mothers exposed to a high-fat (HF) diet during the last week of gestation and lactation have altered mesolimbic dopamine (DA) activity, a system that regulates the hedonic value of food. In particular, adult HF male rats display increased tyrosine hydroxylase (TH) activity and

reduced D2 receptor expression in the VTA, but the time of onset of these changes is unknown. Here we examined when these changes first appear and whether they are dependent of the post weaning diet. In contrast to the adult offspring, pre-weaning HF pups on PND 10, 16 or 25 did not show significant differences in the concentration of TH, but showed a trend towards increased D2 receptor expression in the VTA. These data suggest that changes induced by the maternal diet in the mesolimbic DA system observed in adult HF offspring do not appear before weaning and might be dependent upon the post-weaning diet. We are currently testing offspring during puberty (PND 45) after weaning on either a HF or CD diet. Together with the onset of molecular changes in the DA system, we are also examining whether the expression of Nurr-1, a transcription factor required for the development of DA cells and upregulation of DA synthesis, is increased in HF rats concomitantly to TH activity in the VTA. Supported by CIHR to CDW.

3-A-4 Inflammation elevates the rate of axonal structural remodeling in a developing neural circuit

Nasr Farooqi¹, Edward Ruthazer¹

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Exposure to infection and inflammation in early development is associated with an increased incidence of subsequent neuropsychiatric disease. To investigate the effects of inflammation on a developing neural circuit in vivo, we exposed larval zebrafish to bacterial lipopolysaccharide (LPS) to induce an inflammatory response. We demonstrate that exposure to this stimulus results in morphological activation of microglia, confirming an active immune response. We expressed EGFP under a retinal ganglion cell (RGC) promoter to sparsely label axons arborising in the tectum and imaged them at high spatio-temporal resolution with in vivo two-photon microscopy. Morphometric analyses of RGC axons arborising in the tectum before and after acute immersive exposure to LPS or a control solution demonstrate that exposure to an inflammatory stimulus increases arbor motility as measured by rates of axonal branch addition and retraction. Increased structural plasticity in early development may reflect a failure to stabilize synaptic contacts, leading to aberrant circuit formation. Microglia have been implicated as regulators of normal neural development and of inflammatory responses in the CNS. We are

currently investigating whether the presence of microglia is necessary to mediate the effect of an inflammatory stimulus on developing axons. This research was funded by a CIHR grant to ESR and a CIHR Vanier Canada Graduate Scholarship to NF.

3-A-5 Effects of prenatal and neonatal nicotine exposure on the 3 β -hydroxysteroid dehydrogenases (3 β -HSD) enzymes of steroidogenesis in the rat hippocampus

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Early life exposure to nicotine is known to have deleterious effects on development. While smoking during pregnancy has decreased, nicotine replacement therapy is abundant. NRT is commonly used in order to aid individuals in quitting smoking. Our recent characterization of de novo steroidogenesis in the developing brain has shed new light on the role of these sex hormones in brain development. 3 β -HSD is one key enzyme in the steroidogenic pathway, of particular interest to us is the synthesis of androgens (And) and estrogens (E). This steroidogenic enzyme has been found to be expressed in brain regions not necessarily involved in reproduction, specifically the hipp, cortex, cerebel, among others. Given the diversity of developmental effects associated with maternal smoking, the aim of the current project is to assess the effects of early life nicotine exposure on expression of 3 β -HSD in rat hipp tissue via quantitative PCR. Dams were injected subcutaneously with 1mg/kg of nicotine bitartrate or a saline vehicle once daily for two weeks prior to mating until the time of weaning. Male pups were selected for this experiment and were housed in sibling pairs until sacrifice at post natal weeks 15 or 26. Brains were carefully extracted and flash frozen, dissected and kept at -80°C until processed for qPCR assays. While the exact role of And and E synthesized in the telencephalon during development is still not well understood, results from this experiment will provide a glimpse as to the influence of the environment on this steroidogenic pathway in the developing brain.

3-A-6 Pre-pubertal rats behaving badly: Assessing behaviour in the Morris water maze

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When sex specific behaviours are being assessed in animal models, the tests are either conducted in pre-pubertal animals or in gonadectomized adults, in order to control for circulating gonadal hormones. Given that rats are not seasonal breeders we would expect similar latencies in finding the submerged platform in the Morris water maze, regardless of the time of year when these animals are tested. Early confusing results prompted us to assess the performance of separate cohorts of pre-pubertal animals in this task. Shockingly, we found that grouping the behavioural results of multiple cohorts of animals in a seasonal fashion, or in a long versus short day one, revealed that learning to use the spatial cues to locate the hidden platform did not occur at the same rate across the "seasons" and sexes. Is this effect truly a seasonal one? One explanation for this finding is related to season, but not the season when the rats were born or when behavioural tests were conducted. Rather, it may be related to seasonal variations in the phytoestrogen content of the rat feed. Other potential explanations will also be discussed. These findings have obvious consequences and implications for those conducting behavioural studies using this and potentially other paradigms. Confounding factors appear to be playing a significant role in altering behaviour, thus, any comparison to a treatment group may be seriously impacted.

3-A-7 Integration and functional role of different sub-populations of newborn granule cells in the adult olfactory bulb.

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In the adult brain, the olfactory bulb is continuously supplied with new neurons differentiating into granule and periglomerular cells. Newborn granule cells (GCs) have usually been considered as a homogenous population of neurons, but growing evidence suggests that they can be differentiated into several sub-populations. It is still not clear what is the pattern of maturation and integration of distinct sub-populations of newborn GCs, as well as their role in the bulbar network functioning and odor behavior. It is also unknown if structural and functional differences exist in the same sub-population of GCs born during early and late

postnatal life. Using viral injections, transgenic mice, confocal imaging and whole-cell patch-clamp recordings we analyzed the structural and functional properties of different sub-populations of GCs at different maturational stages with particular emphasis on calretinin-expressing newborn cells (CR). Our morphological analysis performed at 1, 3 and 5 weeks after generation of newborn GCs revealed that CR GCs display similar maturational pattern as compared to CR-cells. Patch-clamp recordings from early postnatal and adult-born CR GCs showed similar frequency and amplitude of spontaneous inhibitory currents. In contrast, our preliminary results suggest that adult-born CR GCs may receive less frequent excitatory inputs as compared to early-born CR neurons. These data are essential to better understand the integration and the specific role in the neuronal network functioning and odor behavior of diverse sub-populations of newborn cells.

3-A-8 Controlling postsynaptic receptors expression as a model to investigate synaptic refinement

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As neural circuits become established, synaptic connections undergo considerable rearrangement. It is commonly believed that only functional synapses are maintained, whereas non-functional synapses are eliminated; this suggests that postsynaptic activity plays an important role in synaptic refinement. Yet, the underlying mechanisms are poorly understood. To learn more, we are developing a new model to control postsynaptic receptor expression. Our experiments focus on cholinergic-nicotinic synapses in mouse sympathetic ganglia. The postsynaptic receptors at these synapses are $\alpha 3$ -containing nicotinic receptors. In our model, the $\alpha 3$ gene is deleted from its normal location on chromosome 9 and its cDNA is expressed elsewhere in the genome under the control of the human ubiquitin promoter. To ensure functional $\alpha 3$ expression in this model, we are using intracellular recordings to measure synaptic transmission in intact sympathetic ganglia, as well as whole-cell recordings from isolated sympathetic neurons in culture. We find that nerve-evoked EPSPs are present as early as P7 and persist throughout postnatal development; however, interestingly, synaptic refinement seems to be impaired. In addition, we find that whole-cell acetylcholine-

evoked inward currents from mutant neurons are considerably smaller than those from wildtype neurons. Our results suggest that controlling postsynaptic receptors expression is a useful approach to investigate how synaptic activity refines connections. Funding: CIHR (EC)

3-A-9 Prenatal paternal stress and postnatal enhanced home cage affect maternal care and anxiety like behaviour in juvenile rats

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Paternal effects have recently drawn interest from neuroscientists, within the context of epigenetic transmission, largely following evidence from behavioural ecology and epidemiology. Recent studies have demonstrated offspring effects of paternal age, obesity, enrichment, and psychological stress. These outcomes may be partially manifested through differences in maternal care. For the current study, we used predator odour exposure to stress male Long-Evans rats for 7 days, immediately preceding a partner preference test, followed by breeding. On the day of parturition, dams were transferred to enhanced home cages (EHC), which are approximately twice the size of a standard cage (SC) and contain a burrow, or SC. Maternal care was observed for 10 days in both the EHC and SC conditions. Offspring were weighed at birth and at weaning, postnatal day 22 (P22). Following weaning, two male and two female pups were pair-housed while the rest of the litter was sacrificed. At P25, offspring were observed for social play behaviour for 5 days. Anxiety-like behaviour was examined using the elevated plus maze and open-field test on P32-35. Then, between P35-40 offspring were exposed to a predator odour for 30-minutes and post-fixed in PFA for immunohistochemical detection of GH and/or NPY. Females spent less time with stressed males, compared to control males in the partner preference test. Further, maternal care was affected by both paternal stress and EHC rearing. Anxiety behaviour and immunohistochemistry results have yet to be analyzed. Supported by an NSERC Discovery Grant to TP.

3-A-10 The Rho guanine nucleotide exchange factor beta-Pix is required for netrin-1 mediated chemoattraction

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Netrin-1 is a secreted chemotropic guidance molecule that attracts extending spinal commissural neurons towards the ventral midline of the developing spinal cord. Through its receptor Deleted in colorectal cancer (DCC), netrin-1 promotes cytoskeletal rearrangement and changes in growth cone morphology necessary for commissural axon extension. The exact mechanisms by which this occurs are however not fully understood. We have previously demonstrated that netrin-1 chemoattraction in commissural neurons requires the activation and recruitment of the rho GTPases rac1 and cdc42, as well as the serine-threonine kinase Pak1 to the intracellular domain of DCC (Shekarabi et al., 2005). To further our understanding of the mechanisms that regulate rac1 and cdc42 downstream of DCC, we have now demonstrated that the rho guanine nucleotide exchange factor (rhoGEF) beta-Pix, along with the adaptor protein Git2, associate with DCC in commissural neurons following netrin-1 stimulation. We also report that the dimerization of beta-Pix and the association with its downstream effector Pak1 are essential for filopodial formation and growth cone expansion induced by netrin-1. Additionally, we provide evidence that beta-Pix function is required for proper guidance of commissural axons to the ventral midline in the embryonic spinal cord. Together, these findings provide evidence that beta-Pix plays a key role in netrin-1 dependent commissural axon chemoattraction.

3-A-11 mTOR signaling in oligodendrocytes

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Mammalian target of rapamycin (mTOR) is a central regulator of multiple cellular functions including cell growth, protein synthesis and metabolism. The mTOR pathway exists as two distinct signaling complexes: mTORC1 (mTOR-raptor) and mTORC2 (mTORC-ricor). Both complexes are intimately linked with the PI3K-Akt signaling network, which is a major player in CNS myelination. Previous studies showed that Akt/mTOR signaling is essential for oligodendrocytes precursors differentiation and for the expression of myelin proteins during active myelination (Narayanan et al, 2009; Tyler et al, 2009). However, very little is known about the specific role of mTORC1 or mTORC2 in regulating oligodendrocytes myelination during

development and for the myelin maintenance in the adult. Our research aims is to determine the specific role of the two mTOR complexes during myelination of the CNS.

3-A-12 Effects of exogenous neuregulin-1 on maturing adultborn neurons in the hippocampus

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Introduction Neuregulin-1 (NRG1) is a neurotrophic factor implicated in schizophrenia and depressive disorders. We have shown that peripheral NRG1 administration increases cell proliferation and neurogenesis in the dentate gyrus (DG) of the hippocampus, and these effects are associated with antidepressant-like behaviour. However, effects of NRG1 on maturing adultborn DG neuronal growth and survival have not been characterized. **Methods** Adult male C57BL/6 mice were injected with BrdU (3x50mg/kg) and implanted 15 days later with s.c. minipumps delivering either saline (n=9) or NRG1 (n=10; 10µg/day) for 3 days before perfusion. Sections underwent BrdU immunohistochemistry (IHC) and BrdU DG cells were counted. To analyze dendritic extension BrdU/DCX IHC was performed and neurons were reconstructed. **Results** NRG1 administration did not affect adultborn cell survival overall (p=0.26) or in the dorsal (p=0.19) or ventral (p=0.41) DG. Cytogenesis was higher in the ventral DG than in the dorsal DG (p=0.0061). Dorsal and ventral cytogenesis were correlated (r=0.5, p=0.03). Preliminary analyses suggest that NRG1 did not affect overall cell body perimeter, area, feret min or max, aspect ratio, form factor, or roundness (ps>0.22), and did not affect overall dendritic nodes, length, surface area, or volume (ps>0.51). **Conclusions** NRG1 does not affect survival or morphology of immature cells in the adult DG, suggesting that neurogenic effects of NRG1 administration are restricted to increased proliferation. We are currently assessing synapse formation to further test this hypothesis.

3-A-13 Characterization of a cleavage-resistant EphA4 receptor in spinal motor neurons

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The correct wiring of many neural circuits in the nervous system depends on the spatiotemporally-controlled binding of contact-mediated axon guidance molecules, but also on the adequate detachment of axons following contact in order to continue growing. Ephrins and their Eph receptors, cleaved by metalloproteases after binding to each other, mediate the guidance of motor neuron axons in the developing limb mesenchyme by being expressed in both tissues and signaling to the Eph-expressing cell (forward) and to the ephrin-expressing cell (reverse). To investigate the role that Eph receptor cleavage plays in its signaling, we created a mutant version of EphA4 that is unable to be cleaved, but whose signaling properties are otherwise unaffected. A knock-in mouse mutant in which cleavage was abolished in both neurons and limb exhibited axon guidance errors, raising the question of whether cleavage is important in forward or reverse signaling, or both. To specifically ask whether forward ephrin:Eph signaling requires receptor cleavage, we unilaterally overexpressed the mutant, its wild type form, or GFP alone in the spinal cord of chick embryos. We found that the ability of ectopic EphA4 expression to reroute axons in this choice point was similar in the cleavage-resistant mutant compared to the wild type receptor, suggesting that cleavage is not critical for forward signaling and arguing that the misrouting seen in the mutant mouse is due to impaired reverse signaling alone.

3-A-14 Hypocretin/Orexin receptors in the chick embryo brain

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Hypocretin/Orexin (H/O) neurons in the posterior hypothalamus of adult mammals and birds regulate the sleep-waking cycle, among other physiological functions. H/O receptors (2 types) are widespread in the adult rodent brain. H/O neurons first become detectable in rats at day 18 out of a 21-day gestation period. However, H/O neurons are detectable in chick embryos as early as embryonic day (E) 3 of a 21-day fetal period, and their number progressively increases and reaches a plateau in the second half of embryonic development (Godden et al, submitted). In order to better understand the possible roles of H/O neurons in the developing chick, expression of the single H/O receptor type expressed in birds was surveyed in embryo

brains of different ages using standard immunohistochemistry. At E12, labelling was very low in the forebrain while high levels of expression were seen in some brainstem areas (cranial nerve sensory and motor nuclei, cerebellum). At E16 and E20, H/O receptors were seen at high levels in analogous brain areas to those that contain H/O receptors in adult rodents (pallium, medial septum, hippocampus, amygdala, paraventricular hypothalamic nucleus, cranial nerve sensory and motor nuclei, locus coeruleus). Strong signals were also seen in the cerebellar Purkinje cells and nuclei. These observations suggest that H/O could exert an early effect on the development of sensory and motor brainstem circuits. At later ages, the widespread expression of H/O receptors including forebrain areas suggests that H/O could be involved in regulating brain state changes.

3-A-15 AMIGO-1 regulates the targeting of olfactory sensory neuron axons

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Proper sensory system functioning relies on the development of precise connections between sensory and second order neurons in a topographic manner. Sensory axons are guided toward their correct synaptic partners by various axon guidance and cell adhesion molecules (CAMs). In the olfactory system, olfactory sensory neurons (OSNs) expressing the same olfactory receptor (OR) innervate specific stereotypically located glomeruli in the olfactory bulb (OB). While several guidance molecules help segregate OSN axons into broad zones of the OB, less is known about molecules regulating their coalescence into individual glomeruli. The few families of CAMs implicated in local axon sorting are unlikely to be sufficient to coordinate the sorting of axons expressing over 1000 different OR types into their appropriate glomeruli. Aiming to identify other CAMs in this process, we have found one member of the 'amphoterin-induced gene and ORF' (AMIGO) family of transmembrane proteins as a regulator of axonal coalescence in the OB. AMIGO-1 expression is confined to the ventrolateral regions of the olfactory epithelium, and AMIGO-1-expressing OSN axons project to the ventral region of the OB. AMIGO-1 null mutant mice show improper targeting of MOR28-positive OSN axons within this ventral

region but do not display defects in MOR174-9-positive axon targeting to the dorsal region of the OB. Furthermore, MOR28 glomeruli, formed in the ventral region of the OB, are significantly smaller in size in these mice. These findings identify Amigo-1 as a key regulator of OSN axonal targeting in the mouse OB.

3-A-16 Proteolytic cleavage of IgLON adhesion proteins by MMPs promotes neurite outgrowth

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Matrix metalloproteinases (MMPs) are a family of zinc-peptidases capable of cleaving extracellular matrix and cell surface proteins resulting in degradation or release of biologically active fragments. MMPs process ligands and receptors that regulate neuronal plasticity and neurite growth following injury in the Central Nervous System. In the present study, we evaluated the role of MMPs in regulating neurite growth. We find that pan-MMP inhibitors inhibit outgrowth of cortical neurons and dorsal root ganglion neurons (DRGs) and that this effect is dependent on the stage of neuronal maturity. Through tandem mass spectrometry we identified the IgLON family of glycosyl-phosphatidyl inositol (GPI)-anchored cell adhesion molecules as proteins that are shed in an MMP-dependent manner. IgLONs are the earliest and most abundant GPI-anchored proteins expressed in the nervous system and are implicated in the process of neuronal outgrowth and cell adhesion. Using reverse-transcription PCR and cell surface biotinylations, we observed a correlation between the expression of IgLON family members in vitro and our outgrowth phenotype with MMP inhibitors. Our findings suggest that a near full length fragment of the IgLON ectodomain is cleaved from the surface of cortical neurons in an MMP-dependent manner. Outgrowth experiments on immobilized full-length IgLON proteins identified NTM and LSAMP as IgLON family members that promote neurite extension in cortical neurons. Together our findings support a role for MMP-dependent shedding of IgLON family members in regulating neurite extension.

3-A-17 Investigating the role of Disrupted in Schizophrenia 1 (DISC1) in cortical inhibitory interneuron development

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Aberrant development of cortical inhibitory interneurons is hypothesized to play a role in Schizophrenia etiology. This hypothesis has been supported by post-mortem studies, which show reduced inhibitory markers, as well as patient studies which show disruptions in GABA-ergic interneuron-driven gamma oscillations. However, the molecular mechanisms underlying interneuron development remain largely unknown. The schizophrenia risk gene Disrupted in Schizophrenia 1 (DISC1) works through its many interacting partners to regulate various aspects of neuronal development. However, studies on DISC1 have been largely confined to excitatory neurons, and therefore, the role of DISC1 in interneuron development has not been clearly defined. Using a mouse in vitro model, we are examining the effects of shRNA-induced knockdown of DISC1 on dendrite growth and synapse formation in cortical inhibitory neurons. Preliminary results show that DISC1 knockdown causes a decrease in dendritic complexity in inhibitory neurons. We are also investigating the possibility that the Schizophrenia risk genes Neuregulin 1 (NRG1) and its receptor ErbB4 function through DISC1 to regulate inhibitory interneuron development. Preliminary results show that application of NRG1 to cortical cultures increases DISC1 levels in the primary dendrites of inhibitory neurons. These results suggest that DISC1 is an important regulator of dendritic growth in cortical interneurons and that its expression may be regulated by NRG1-ErbB4 signaling.

3-A-18 Repeated predator odour stress across the adolescent period alters γ 2 GABAA receptor subunit levels in hippocampus of male and female rats

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Prior work has demonstrated that juvenile stress in rats can alter adult levels of gamma amino butyric acid (GABA)A receptor subunit levels in the hippocampus and amygdala, and this has functional consequences with respect to the role of the receptor in controlling physiological responses to stress and anxiety. In particular, changes in adult levels of the α 2 GABAA receptor subunit have been observed following stressor exposure during the juvenile period; however, it is unknown at what point between the juvenile period and adulthood these changes arise. In the present study, we exposed male

and female rats to a cat odour cue or a control cue on five half-hour occasions during the adolescent period (between postnatal days 38-48) and sacrificed the animals after the final exposure. We then measured levels of α 2, γ 2, and δ GABAA receptor subunits in hippocampus using western immunoblotting. We found significantly decreased levels of the γ 2 GABAA receptor subunit ($p=0.028$) in stress-exposed animals relative to control but no changes in the α 2 and δ GABAA receptor subunits. There were no sex differences observed. These findings suggest that more work should be conducted in determining the timeline of juvenile stress-induced changes in GABAA receptor subunits in brain regions important for regulating the stress response, as well as in determining the functional consequences of these changes.

B - Neural Excitability, Synapses, and Glia: Cellular Mechanisms

3-B-19 Emotional and cognitive behaviour changes are associated with increased glutamatergic transmission in the early stages of experimental allergic encephalomyelitis (EAE)

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Multiple sclerosis (MS) is often associated with co-morbid behavioural and cognitive impairments, affecting around 50% of MS patients. We have identified similar behaviour co-morbidities, many of which are reflective of amygdalar abnormalities, in a mouse model of MS, Experimental Autoimmune Encephalomyelitis (EAE). These include anxiety- and depression-like behaviour even prior to the onset of motor deficits. The aim of the current study was to investigate mechanisms underlying these behaviour changes. We hypothesized that inflammation results in alteration of neuronal excitability and neurotransmission in the amygdala. EAE was induced in C57/BL/6 mice with MOG35-55 /CFA and pertussis toxin (PTX). Control mice received CFA and PTX only. Whole-cell recording was carried out in the principal neurons of the basolateral amygdala (BLA), a key region involved in emotional behaviour regulation. Hyperpolarization activated current (I_h) was increased in the EAE mice. Investigation into glutamatergic synaptic transmission revealed increased frequency, but not amplitude of mini-excitatory postsynaptic currents; this is indicative of increased glutamate release.

AMPA:NMDA ratio was also increased in the EAE animals. No changes in the GABAergic transmission were observed at this stage. In conclusion, emotional and cognitive deficits observed in EAE (and possibly MS) were associated with alteration in the hyperpolarization activated current (I_h) and glutamatergic transmission in the BLA. Future studies will explore the possible involvement of inflammatory mediators in these changes.

3-B-20 The distribution of cytoplasmic and membrane-associated TrkB in the dendrites of adult spinal motoneurons

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The majority of synapses in motoneurons are positioned on the dendrites. The strength of these synapses is regulated by several neuromodulators. The neurotrophin BDNF is conventionally associated with the growth, guidance, and survival of developing motoneurons. There is however emerging evidence that activation of TrkB receptor by BDNF is vital in the maintenance and strengthening of different classes of synapses in adult motoneurons. While previous studies have shown that TrkB is present in the soma and proximal dendrites of motoneurons, it remains unclear whether its regulatory role is restricted to these regions or if it also involves synapses in other dendritic regions. We used immunohistochemical, microscopy, and 3D image analysis techniques to examine the proximal to distal dendritic distribution of TrkB on the membranes and in the cytoplasm of adult spinal motoneurons. We found that in intracellularly stained motoneurons, TrkB immunoreactivity is arranged in punctae and is widely distributed in all regions of the dendrite tree. The density of TrkB immunoreactivity on the membranes and in the cytoplasm of dendrites was the same regardless of distance from the soma and the diameter of the dendrite. Additionally, via Western blotting, we detected several immature TrkB isoforms; a number of which are reported to be involved in the strengthening of synapses. Together, these results suggest that TrkB may be involved in the maintenance and regulation of synapses that are located throughout the dendritic tree of motoneurons.

3-B-21 Microglia respond to brain anoxia with rapid morphological changes

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Microglia are highly motile cells that play a pivotal role in monitoring brain homeostasis by constantly probing the environment and responding to extracellular cues. They are involved in stroke-related pathologies, mediating a nonspecific neuroinflammatory reaction that could lead to long-term deleterious effects following transient anoxia. However, the acute functional response of microglia during anoxic periods remains unclear. Here, we used real-time two-photon imaging in acute brain slices to monitor the initial effect of anoxic insults on the morphological phenotype and dynamic properties of microglia. Microglia in resting conditions display a highly branched morphology with motile processes, however oxygen depletion induces a rapid, actin-dependent retraction of processes that is fully reversible upon reoxygenation. This finding indicates that microglia sense decreased oxygen levels in brain tissue within minutes. The rapid loss of major processes, normally needed by microglia to probe the environment, translates into a significant change in microglia function. Under normal conditions, microglia quickly extend their major processes towards focal injury, however this mechanism is inhibited during anoxia. We show that this phenotype is mimicked by multiple signaling molecules hypothesized to be released during brain anoxia, indicating a common intracellular pathway is activated to induce microglial process retraction. Characterizing the molecular cues responsible for this functional switch of microglial behaviour will likely provide valid targets for stroke treatment.

3-B-22 Dual Role of Acyl-CoA Binding Protein (ACBP) in the Hypothalamus: Regulator of Astrocytes Fatty Acid (FA) Metabolism and Gliotransmitter Targeting Pro-opiomelanocortin (POMC) Neurons.

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The arcuate nucleus (ARC) of the hypothalamus detects changes in circulating nutrients levels (glucose, FA) via two antagonist neuronal populations, orexigenic neuropeptide Y and anorexigenic POMC neurons to in turn regulate energy balance. However, little is known regarding the cellular mechanisms involved in FA regulation of feeding and glucose homeostasis. In the periphery, ACBP binds and creates an intracellular pool of Acyl-CoA's that is important for their metabolism. In the brain, ACBP is secreted by astrocytes and inhibits the GABAA receptor. We show here that ACBP is mainly localized in hypothalamic astrocytes and its central administration inhibits food intake (FI). Thus, we propose that ACBP plays a dual role in the hypothalamus as a regulator of FA metabolism in astrocytes and a gliotransmitter targeting ARC neurons to inhibit FI. Using in vitro and ex vivo models generated from ACBP KO and WT mice, we show that ACBP deficiency reduces FA oxidation without affecting esterification into complex lipids in hypothalamic astrocytes. Our electrophysiological data show that ACBP reduces the frequency of inhibitory postsynaptic currents in anorectic POMC neurons leading to increased action potential frequency. These findings suggest that the anorectic effect of ACBP in the brain may be dependent on POMC neurons activation and that ACBP may be part of FA-sensing mechanisms by regulating their oxidation in hypothalamic astrocytes. Targeted ablation of ACBP in brain astrocytes is ongoing to determine its role in the hypothalamic control of energy balance.

3-B-23 Increasing extracellular potassium excites and then depresses cortical seizure activity in vitro and in vivo

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Elevated extracellular potassium ([K⁺]) causes seizures and also is increased by seizure activity. Whole cell recordings were made in vitro from layer II/III pyramidal cells with local field potentials recorded from within 150µm. Membrane properties, measured in control [K⁺] of 3.5mM, were subsequently compared with higher [K⁺] of 6mM, 9mM, 12mM and 15mM. As the [K⁺] increased from 3.5mM to 12mM, cells depolarized and changed their firing from silent to bursting and/or irregular firing, and in 15mM [K⁺] there was further depolarization (20-25mV from the resting membrane potential in control

[K⁺] of ~-70mV) and cessation of spontaneous firing, without evidence of spreading depression in the extracellular recordings. Concomitantly the input resistance decreased. Addition of 100µM 4-aminopyridine (4AP) generated seizure like events (SLEs) in slices in control [K⁺] of 3.5mM. Moderately raised [K⁺] up to 9mM increased the frequency of SLEs. In contrast, 12mM [K⁺] blocked or diminished the SLEs and left only inter-ictal bursts. When [K⁺] was pushed to 15mM, no local field activities could be recorded. SLEs and single cell action potentials reappeared within seconds after changing perfusate back to regular aCSF from any raised [K⁺]. Focal cortical seizures, generated in vivo by application of 4AP onto mouse somatosensory cortex, were also blocked by local application of raised [K⁺]. These results show a concentration dependent bidirectional regulation of cortical seizure activity by raised [K⁺]. Supported by CIHR

3-B-24 Omega-3 fatty acid prevents pro-inflammatory induced dendritic spine loss and synaptic deficits in the mature hippocampus

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It is widely accepted that the soluble oligomeric β -amyloid (A β) peptide accumulation exacerbates brain microcircuitry defects in Alzheimer's disease (AD). This may occur through an inflammatory-mediated response. Here, we investigated the initial effects of A β exposure prior to plaque and tangle formations. We compared synaptic structure and function of CA1 pyramidal neurons in the mature hippocampus using lipopolysaccharide (LPS), an inflammatory agent, to gain insight to AD pathophysiology. Our study focuses on emerging evidence that lipid abnormalities are crucial in AD. We applied oligomeric A β peptide to mature organotypic hippocampal slices to model the initial AD insults and compared that to LPS. We report an abnormality in neuronal and microglial lipid components by finding observable changes in lipid droplets (LDs) formation following A β treatment. The size distribution of LDs was altered after A β treatment, where an increase in large (> 1.0 µm) LDs was found. Similar alteration in lipid compartmentalization was observed with LPS-elicited inflammatory response. Furthermore, dendritic spine densities were decreased while AMPA-mediated mEPSC frequency was

increased after both A β and LPS treatments. When the omega-3 fatty acid, docosahexanoic acid (DHA), was supplemented during A β or LPS treatments, abnormal LD distribution, morphological, and electrophysiological phenotypes were restored to that of control slices. Our findings suggest that DHA could reduce functional impairments in neuroinflammation and may provide an innovative approach to AD therapies.

3-B-25 Importance of astrocytic coupling for rhythmogenesis in neurons of the masticatory central pattern generator.

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In the trigeminal main sensory nucleus (NVsnpr), a key component of the trigeminal circuit thought to be involved in mastication, rhythmic neuronal bursting can be induced by repetitive stimulation of glutamatergic afferent fibers or local applications of NMDA. This bursting is mediated by a sodium persistent current (INaP) and is promoted when the extracellular concentration of calcium ([Ca²⁺]_e) decreases. Our previous work has shown that a Ca²⁺-binding protein released from astrocytes can lower [Ca²⁺]_e and that integrity of the astrocytic syncytium is required for bursting to occur in NVsnpr. Here we examine the potential role of astrocytic coupling through gap junctions in determining the firing pattern of NVsnpr neurons. We postulate that burst inducing stimuli activate astrocytes, increase their coupling and by doing this induce bursting. To assess this hypothesis, we measured the size of astrocytic syncytia revealed by injections of biocytine in a single astrocyte in whole-cell patch clamp recordings in different conditions. Local NMDA applications and [Ca²⁺]_e decreases increased coupling between astrocytes, while NMDA-induced neuronal bursting was abolished by Carbenoxolone (20 μ M), a blocker of gap junctions. Once blocked, this bursting could be rescued by local application of the astrocytic Ca²⁺-binding protein. This work suggests that in addition to regulating the extracellular ions concentrations which determine the neuronal firing pattern of NVsnpr neurons, astrocytes may also play an important role in synchronizing entire assemblies of neurons in this nucleus.

3-B-26 Functional consequences of cysteine mutations at the kainate receptor dimer interface

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Glutamate is the principle excitatory neurotransmitter in the central nervous system. It acts at three ionotropic receptor-types (iGluRs): AMPA, NMDA and kainate receptors, which have similar gating processes. Glutamate binds to the ligand binding domain (LBD) causing conformational changes leading to channel activation. These structural changes are highlighted by the relative positions of adjacent pairs of LBDs to one another in an area called the LBD dimer interface. Previously we reported that restricting movement of the LBDs by crosslinking the dimer interface with the Y521C/L783C GluK2 mutation severely hampered single channel activity. For this study we investigated the equivalent mutation in AMPA receptors (L504C/L772C GluA2) in order to determine if its activation process was similarly disrupted. Macroscopic current responses of the double cysteine and each single cysteine mutations were similar for AMPA and kainate receptors. The double cysteine receptors produced currents that did not decay in the continued presence of agonist, the L504 GluA2 and Y521 GluK2 single cysteine mutations had long decay kinetics (decay ~50 ms) compared to wild-type receptors (~5 ms) and the L772 GluA2 and L783 GluK2 single cysteine mutations were both non-functional. Discrete channel openings were recorded from cross-linked AMPA receptors and, like kainate receptors, displayed many brief channel openings and closures, with apparently low conductance. Together these data suggest that this location in the dimer interface is a conserved functional domain of AMPA and kainate receptors.

3-B-27 Glutamate receptor activation involves stabilization at the apex of the ligand-binding domain

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Fast excitatory neurotransmission in the central nervous system involves the transient activation of AMPA and kainate-type (AMPA and KAR)

glutamate receptors (iGluRs), which desensitize within milliseconds of glutamate binding. Crystal structures of AMPAR ligand-binding domain (LBD) dimers have been used to propose a mechanism whereby separation between subunits at the LBD underlies desensitization. We identify the importance of an electronegative pocket at the apex of the LBD dimer interface in facilitating KAR activation. Using a combination of outside-out patch clamp electrophysiology and molecular dynamics simulations, we show that occupancy of this pocket by a positive charge correlates with receptor activation and prevents subunit separation (i.e. desensitization). For the KAR subunit GluK2, sodium fulfills this charge requirement, and is therefore necessary to achieve activation once glutamate is bound. Interestingly, the occupancy of the pocket by a mutant lysine residue tethered between subunits permits continuous receptor activation. In contrast, disulfide crosslinking of both KARs and AMPARs at a lower position in the LBD prevents stable activation, despite yielding nondecaying population responses. An intermediate phenotype occurs following binding of the allosteric modulator cyclothiazide (CTZ) to the LBD of the AMPAR subunit GluA2, as channels alternate between periods of high and low activity. Our data indicate that the arrangement of subunits at the apex of the iGluR LBD regulates the transduction of ligand binding into channel opening.

3-B-28 Weakly coupled oscillators interactions between the medial septum and the hippocampus mediated by the septum long-range GABAergic projections.

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The rodent hippocampus displays oscillations at various frequencies including those in the theta range (3 to 10 Hz). During theta, the activity of neurons is tightly coordinated by the ongoing rhythm such that synchronous interactions between neurons are governed by the local rhythm, thus promoting synaptic plasticity and memory formation. Lesion studies have suggested that the medial septum (MS) is responsible for hippocampal theta rhythm generation. However, work in our laboratory has shown that the isolated hippocampus can generate theta oscillations intrinsically, which prompted us to revise the role of the MS in

theta. Thus, we investigated the influence of the septo-hippocampal GABAergic projections on theta rhythm. To do so, we used the isolated septo-hippocampal preparation developed in our laboratory together with optogenetics to specifically control GABAergic projections from the MS. We found that rhythmic stimulation of the MS GABAergic fibers increased both theta power and oscillation strength. In addition, we found that such stimulation synchronized separate theta generators within the hippocampus. However, these stimulations only influenced theta when the stimulation frequency was close to the natural frequency of the ongoing oscillation. Importantly, when the stimulation was given at specific phases of the oscillation (to mimic the normal activity pattern observed in vivo) we found that stimulation of the septo-hippocampal GABAergic fiber increased or decreased theta power in a phase-dependent manner.

3-B-29 Sleep deprivation impacts on Neuroligin-2 and -3 protein levels in the mouse brain

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Sleep loss affects brain functioning and the molecular mechanisms underlying these changes remain mostly unclear. We previously showed that sleep deprivation (SD) changes the forebrain expression of Neuroligin 1 (NLGN1), a synaptic adhesion molecule involved in synaptic plasticity. In this study, we assess the impact of SD on other members of the NLGN family, NLGN2 and NLGN3, in different brain regions. C57BL/6J mice were subjected to a 6-h SD starting at light onset and achieved by gentle handling. Mice were then sacrificed along with a non-SD control group and the cortex, striatum, hippocampus and a region covering the thalamus/hypothalamus were extracted. Synaptoneurosomal and total protein extractions were performed, and NLGN2 and NLGN3 protein levels were quantified by Western blots. SD altered the level of NLGN2 in the cortex, increasing its synaptic protein level and decreasing its total protein level. SD also decreased NLGN2 synaptic level in the striatum. These changes altered the synaptic/total protein ratio, with a SD-dependent increase in ratio in the cortex and a decreased ratio in the striatum. Total NLGN3 levels tended to decrease after SD in the hippocampus and the thalamus/hypothalamus region, which was accompanied by a significant increase of

synaptic/total protein ratio at the hippocampus. These results suggest that the levels and trafficking of NLGN2 and NLGN3 are affected by SD in a brain-dependent manner, therefore providing a mechanism by which excitatory and inhibitory synaptic transmissions in the brain are modulated by elevated sleep pressure.

3-B-30 Network models provide insight into how oriens-lacunosum-moleculare (OLM) and bistratified cell (BSC) interactions influence local CA1 theta oscillations

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Although hippocampal theta, a 4-12 Hz rhythm associated with episodic memory, has been studied extensively, the cellular mechanisms underlying its generation are unclear. OLM cells have been considered pacemakers of local CA1 theta, but recent experimental work has disputed this role (Kispersky et al. 2012). The complex interactions that OLM cells have with other cell types, such as BSCs (Leão et al. 2012), make their contribution to network rhythms difficult to determine experimentally. Thus, we created a network model that is tied to experimental work on multiple levels, and explored how cell interactions affect the power of local oscillations. We derived cellular properties from patch clamp recordings of fast-spiking parvalbumin-positive interneurons (comprising basket cells, BSCs, and axo-axonic cells) and of somatostatin-positive putative OLM cells in the CA1 region of an intact hippocampus in vitro, and used them to constrain Izhikevich-type models. Our network model size, connectivity, and synaptic properties were also experimentally constrained. We examined various synaptic strengths and connectivities between OLM and BSCs to determine their influence on network power. Spike characteristics and firing behaviors in our network models approximated those determined experimentally. Our models distinguish between regimes in which OLM cells minimally or strongly affect the power of network oscillations, and predict that the dis-inhibitory effect of OLM cells on BSC to pyramidal cell interactions plays a critical role in the power of network theta oscillations.

3-B-31 Functional Dissection of Descending Medial Prefrontal Cortex Inputs to the Dorsal Raphe Nucleus

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The serotonin (5-HT) system has been widely studied in part because of its involvement in mood regulation and major depressive disorders. Anatomical and functional studies have described a number of descending cortical inputs that regulate 5-HT neuron activity, including those from the medial prefrontal cortex (mPFC). Activation of the mPFC has been shown to markedly improve depressive moods in humans and reverse depression phenotypes induced in animals. Whereas such "antidepressant-like" effects may be due to direct modulation of 5-HT neuron function by these descending inputs, our limited understanding of the synaptic architecture of the mPFC innervation onto 5-HT neurons in the dorsal raphe nucleus (DRN) complicates the elaboration of a satisfactory framework to explain these findings. Here, we sought to dissect out the functional properties of the mPFC-DRN circuit using a combination of immunohistochemistry, optogenetics and whole-cell recordings. We show that the mPFC inputs to the DRN are: 1) glutamatergic; 2) modulated by 5-HT and; 3) mono-synaptically activate both 5-HT neurons and local GABA neurons located primarily in the lateral wings of the DRN. Activation of the mPFC innervation onto local GABA neurons triggers a di-synaptic inhibition not only of 5-HT neurons, but as well as other local GABA neurons. Future work will be required to fully grasp the dynamical nature and functional implication of this complex array of direct activation and feed-forward inhibition in the DRN triggered by activation of descending cortical inputs implicated in affective disorders.

3-B-32 D1-receptor activation facilitates synaptic transmission in the lateral entorhinal cortex via activation of the cAMP-PKA pathway and elevation of intracellular calcium

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Dopamine modulates synaptic transmission in the lateral entorhinal cortex in a reversible,

concentration-dependent manner. We used voltage-clamp recordings from layer II entorhinal cortex neurons to investigate the intracellular mechanisms mediating dopamine-induced facilitation of EPSCs. Results show that dopamine (1 μ M) activates D1 receptors and the cAMP-PKA pathway to enhance AMPA, but not NMDA, glutamate receptor-mediated currents. Elevations in PKA can lead to direct phosphorylation of AMPA-receptors, and may also enhance synaptic responses by increasing the activity of protein phosphatase 1 (PP-1) inhibitors. The synaptic facilitation was blocked by intracellular application of PKA inhibitors, and was also blocked by inhibition of PP-1, suggesting that dopamine facilitates AMPA currents via PKA-induced inhibition of PP-1. In addition, we found that application of the Ca²⁺ chelator BAPTA blocked the facilitation of EPSCs, indicating that the facilitation is dependent on elevated intracellular calcium. Calcium release from internal stores can be triggered by IP₃ or ryanodine receptor (RyR) activation. Application of either heparin, an IP₃ antagonist, or dantrolene, a RyR blocker, blocked the facilitation of EPSCs, suggesting that Ca²⁺ release from internal stores is essential. Overall, the present results suggest that the dopamine-induced facilitation of AMPA receptor-mediated currents in the entorhinal cortex is mediated via a D1-receptor-dependent activation of the cAMP-PKA pathway, inhibition of PP-1, and elevated intracellular calcium released from internal stores.

3-B-33 Intrinsic theta-gamma coupling properties in the mouse CA1/subiculum area in the complete hippocampus in vitro

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Fast neuronal network oscillations in the gamma range have been involved in a variety of brain functions. Many cognitive operations require dynamic coordination of activity across different groups of neurons. Gamma oscillations are known to synchronize the activity of selected ensembles of cells during hippocampal function. Our group recently reported that theta (4-12Hz) oscillations were elicited concomitantly with low (25-50Hz) and high (150-250Hz) gamma oscillations in the subiculum area in an isolated hippocampal preparation in vitro (Jackson et al., 2011). Blockade of GABAergic receptors using

low dose Bicuculline (2 μ M) reduced the theta power by 68 \pm 5%, low gamma power by 91 \pm 3% and high gamma power by 51 \pm 9%. The Modulation index (a measurement of how gamma amplitude is modulated by theta phase) of low and high gamma were reduced by 88 \pm 4% and 84 \pm 4 % respectively (t-test, n=4, p<0.01). However, it is still remains to be determined how theta gamma coupling are related to theta power and frequency change. Here, using optogenetic techniques, we further examined the properties of theta and gamma oscillations as well as their interactions (Modulation index) in the CA1/subiculum area. By injecting a CRE-dependent AAV2-Cheta-eYFP virus in CamKII-CRE and PV-CRE mice line to selectively activate pyramidal cells or GABAergic PV-positive interneurons, we reliably elicited theta and gamma oscillations with blue light. By varying protocols of stimulus frequency (ZAP) and light intensity (RAMP), we are exploring the coupling properties between theta and low/high gamma oscillations.

3-B-34 Action Potential Induced Dendritic Calcium Responses in Spinal Cord Lamina I Neurons

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Lamina I neurons of the spinal cord, which are responsible for integrating pain information from the periphery and projecting this to the brain, are hyperexcitable in chronic pain conditions. Despite the role of calcium as a second messenger in many cellular processes, calcium dynamics of spinal lamina I neurons have been largely neglected. Here, we develop an approach to measure calcium dynamics in lamina I neurons, and use this to characterize calcium responses evoked by action potential firing. We made whole-cell patch recordings from the soma of visualized lamina I neurons in adult rat spinal cord slices. The neurons were loaded with the calcium indicator Oregon Green Bapta-1 and we performed simultaneous current-clamp recording and two-photon imaging. Action potentials were induced through 200-300pA current injections via the patch pipette. Single action potentials induced fluorescence increases in the soma ($\Delta F/F = 0.07 \pm 0.03$, n= 4 somata), dendrites ($\Delta F/F = 0.17 \pm 0.02$, n= 5 dendrites), and dendritic spines ($\Delta F/F = 0.12 \pm 0.02$, n= 2 spines). The presence of tetrodotoxin abolished both the action potentials and their subsequent calcium responses. Increasing frequency of a burst of four action

potentials within a range of 0.2Hz to 5Hz led to a non-linear rise in fluorescence. These findings suggest that somatically generated action potentials elicit calcium responses in the soma and propagate into the dendritic arbour of lamina I neurons. Additionally, calcium responses to action potentials in lamina I neurons integrate in a non-linear process.

3-B-35 Translational control downstream of initiation during mGluR LTD in cultured hippocampal neurons

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Some forms of synaptic plasticity require rapid, local activation of protein synthesis. It is believed that mRNAs are translationally repressed during transport to synaptic compartments, but the mechanisms underlying this translational silencing, and its de-repression during plasticity, remain largely unknown. We recently showed using field potential recording in hippocampal slices that translational regulation in metabotropic glutamate receptor-mediated long-term depression (mGluR LTD) occurs downstream of initiation, likely via the reactivation of stalled polysomes. Here, we use a cell culture model to study mGluR LTD at the single synapse/cell level to further investigate these mechanisms. Using whole cell recording from hippocampal neurons in dissociated cultures, we show that application of the group I mGluR agonist DHPG produces a significant decrease in the frequency of miniature excitatory postsynaptic currents (mEPSCs) lasting 30 minutes, indicative of mGluR LTD. Application of the translation elongation inhibitor emetine during induction blocks the decrease in mEPSC frequency while homoharringtonine, an initiation inhibitor, does not affect depression of mEPSC frequency. Our results indicate that translational control during mGluR LTD in cultured hippocampal neurons occurs downstream of initiation, consistent with a de-repression of stalled polysomes. Having established a cell culture model of mGluR LTD, we will next use gene knockdown and protein rescue studies to determine the molecular mechanisms underlying translational control in this synaptic plasticity.

3-B-36 Activity induced changes in neuronal excitability in a developing central synapse

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The development of high fidelity synaptic transmission at the calyx of Held-MNTB synapse requires the complex regulation of synaptic glutamate receptors and active intrinsic membrane properties. Previous studies have shown that patterned activity can concurrently activate NMDARs and mGluRs and increase the fidelity of neurotransmission at high rates by translational and post-translational modifications of glutamate receptors. It is however unknown whether or how activity drives the development and maturation in the intrinsic excitability of MNTB principal neurons. We used NMDA and DHPG (100 μ M each) to selectively co-activate postsynaptic glutamate receptors of developing MNTB neurons from the auditory brainstem of prehearing mice. We show that co-activation of NMDAR and mGluR have time-dependent alteration of the action potential (AP) waveform and of the maximum AP frequency during a 100ms current step stimulus. Given that AP waveform is dependent on the biophysical properties of a number of voltage-gated ion channels, we performed whole cell voltage-clamp recordings to determine potential mechanisms underlying observed changes in AP waveform. We observed an increase in current density and an acceleration of kinetic properties of TEA-sensitive potassium current 30 minutes after application of NMDAR and mGluR agonists. These results suggest that selective co-activation of NMDAR and mGluR can regulate intrinsic membrane properties and play a role in shaping action potential waveform and the ability to spike at high-frequencies.

3-B-37 Role of synaptically-induced intracellular acidification on synaptic plasticity

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Synaptic plasticity involves activity-dependent recruitment of proteins to the synapse. This results in a strengthening of the synapse through electro-chemical and local structural changes. Stimuli that evoke synaptic long term potentiation (LTP) at excitatory synapses result in the insertion of glutamate receptors and recruitment of Ca²⁺/calmodulin dependent kinase II (CaMKII) into spines, which can be measured optically as metrics of LTP induction.

These processes are known to depend on NMDA receptor activity and Ca²⁺ influx. However, other ions such as Na and H also increase transiently during glutamatergic transmission. It has been shown that intracellular acidification occurs during neuronal firing and that the accumulated H is extruded from the neuron in a regulated manner with slower kinetics when compared to Ca²⁺ sequestering. Little is known however on the dynamics of pH fluctuations in spines and their potential impact on synaptic plasticity. We set out to measure pH fluctuations, using pH-sensitive fluorescent proteins, in dendritic spines of cultured hippocampal neurons, in conjunction with measurements of either Ca²⁺ fluctuations (GCaMP5) or GFP-aCaMKII translocation. To evoke plastic changes in the cultures, we used a chemical LTP (cLTP) induction protocol, which triggered a global and local transient acidification. Interestingly, disruption of this acidification associated with cLTP induction resulted in a loss of CaMKII clustering suggesting a possible role for protons during synaptic potentiation.

3-B-38 Adaptive role for TNF α -mediated plasticity in cocaine addiction

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Drug addiction is a maladaptive form of learning and memory that is regulated by glutamatergic synaptic plasticity. Tumor Necrosis Factor- α (TNF α) is a pleiotropic cytokine that modulates glutamatergic synaptic plasticity via the trafficking of AMPA-type glutamate receptors (AMPA_Rs) and is a key mediator of homeostatic synaptic plasticity. The contribution of TNF α to drug addiction has never been thoroughly investigated. We examined the role of TNF α on the behavioral and synaptic changes occurring in mice after cocaine administration. First, we observed that chronic cocaine administration increases TNF α levels in the nucleus accumbens, a key brain region that regulates drug addiction. Blocking TNF α signaling both genetically and pharmacologically in mice increased behavioral sensitization to cocaine. This increased behavioral response to cocaine was correlated with an increased synaptic response to cocaine in the nucleus accumbens of TNF α KO mice. Following chronic cocaine treatment, TNF α KO mice also displayed an elevated dendritic spine density in the nucleus accumbens. Overall, these results suggest that

TNF α signaling antagonizes cocaine-induced changes in behavior and synaptic strength. We conclude that TNF α may be an adaptive mechanism to reduce synaptic changes induced in the nucleus accumbens by chronic cocaine.

3-B-39 Active zone proteins RIM1ab are required for normal corticostriatal transmission and striatal-dependent behaviours

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The presynaptic scaffolding proteins RIM1 $\alpha\beta$ (RIM1) coordinate key active zone processes involved in fast neurotransmitter release and mediate various forms of presynaptic plasticity. Genetic deletion of RIM1 results in several behavioural abnormalities and learning deficits. Given the near ubiquitous neuronal expression of RIM1, the specific cell types and projections contributing to the altered transmission and behaviour seen following RIM1 deletion are unknown. Using offspring of Cre-conditional RIM1 knockout mice and Emx1-Cre mice (Emx1:RIM1 KO), we report that loss of RIM1 in cortical pyramidal neurons alters corticostriatal transmission and select striatal-dependent behaviours. Whole-cell recordings of excitatory transmission in medium spiny neurons of the dorsolateral striatum reveal that Emx1:RIM1 KO mice show enhanced paired-pulse facilitation and reduced synaptic depression during 10-Hz trains, indicating impaired release probability at these synapses. Consistent with global RIM1 α KO mice, Emx1:RIM1 KO mice show normal motor learning on the accelerating rotarod, but elevated basal and novelty-induced locomotion. Emx1:RIM1 KO mice also display dramatically enhanced responding for food pellets when trained on random interval schedules. Our results implicate cortical pyramidal cells as important mediators of the behavioural impairments seen in RIM1 α KO mice, and reveal novel physiological roles for cortical RIM1 in normal corticostriatal transmission and striatal-dependent learning.

3-B-40 Cacnb4 regulates cortical fast-spiking basket cells synaptic efficiency: implications for epilepsy and cognitive impairment.

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Background. Mutations in *Cacnb4*, encoding the $\beta 4$ subunit of voltage-gated Ca^{2+} channels, are associated with epilepsy and ataxia in humans and mice. A similar phenotype results from deletions of *Cacna1a*, encoding the $\alpha 1$ subunit of $\text{CaV}2.1$ channels. We recently demonstrated that the targeted deletion of *Cacna1a* in forebrain GABAergic interneurons (INs) results in a selective synaptic impairment of parvalbumin-positive fast-spiking basket cells (PV) and causes epilepsy and cognitive deficits in mice. **Hypothesis.** We propose that *Cacnb4* mutations lead to a similar synaptic impairment of PV INs and that this is sufficient to induce behavioral deficits in mice. **Results.** Using in vitro whole-cell patch-clamp recordings and paired recordings between genetically-labeled PV INs and pyramidal cells, we show a striking reduction of cortical mIPSCs frequency from 33 to 23Hz and an enhancement of synaptic failure rates at PV-mediated perisomatic synapses in *Cacnb4*^{lh/lh};G42EGFP mutants. We also show that $\text{CaV}2.1$ channels mediate the residual synaptic release at PV synapses. Finally, we show that this impairment of cortical perisomatic inhibition is clinically relevant as mutants carrying targeted repression of *Cacnb4* in forebrain GABAergic INs (*Lhx6-Cacnb4*^{kd}) display behavioral abnormalities reminiscent of those of *Cacnb4*^{lh/lh} mutants. **Perspectives.** Our results illustrate the critical role of *Cacnb4* in regulating cortical PV INs synaptic function and its relevance to cognitive dysfunctions in epilepsy.

3-B-41 Imaging of miniature synaptic Ca^{2+} -transients as a readout of synaptic potentiation

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Classical measurements of synaptic plasticity involve electrophysiological recordings which lack information about the location of the synapses that undergo plastic changes. Since synaptic plasticity can be synapse-specific, it is necessary to directly correlate changes in synaptic transmission with local molecular mechanisms. We thus combined imaging of a genetically-encoded Ca^{2+} sensor GCaMP5 and whole-cell patch clamping to record both miniature synaptic Ca^{2+} -transients (MSCTs) and miniature excitatory postsynaptic currents (mEPSCs) in cultured hippocampal neurons. We

observed a robust increase in the number of sites per neuron exhibiting MSCTs, as well as their frequency and amplitude, following a chemical LTP stimulation (0Mg²⁺/Glycine). Even though the amplitudes of MSCTs and mEPSCs at the same synapse should be correlated (Murphy TH et al, 1995), the observed potentiation of mEPSCs was subtle, emphasizing the need to identify the specifically potentiated synapses over the non-potentiated ones. Moreover, the whole-cell configuration decreased basal frequency of MSCTs, suggesting a possible effect of cytosolic washout on the recordings. We observed two types of MSCTs: the Ca^{2+} transients were either restrained to spines or spreading also in short segments of the dendritic shaft. Mg²⁺ or APV application confirmed the NMDAR-dependent nature of MSCTs. We are now using dual-color imaging to assess if the potentiation of MSCTs is correlated with other synaptic events associated with plasticity such as CaMKII translocation, AMPARs insertion and changes in spine size.

3-B-42 Dynamic regulation of TREK-1 gating by polycystin-2 via a Filamin A-mediated cytoskeletal mechanism.

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TREK-1 is a mechanosensitive potassium channel that is widely expressed throughout the mammalian nervous system and implicated in several mechanosensory functions. Our understanding of the mechanisms responsible for TREK-1 mechanosensitivity and the regulation of its gating by accessory proteins is incomplete. In heterologous systems expressing TREK-1 channels, mechanical stimuli induce an outward current. In basal conditions, this current is under a partial inhibition by the F-actin cytoskeleton. Interestingly, in the presence of polycystin-2 (PC2), the inhibitory effect of the F-actin cytoskeleton is enhanced through the recruitment of the actin binding protein Filamin A (FLNa). However, the characteristics of this inhibition are poorly understood. Our hypothesis is that this inhibition is highly dynamic and is mediated by a FLNa-dependent increase in F-actin cross-linking at the cell membrane. Our preliminary results show that the gradual and reversible removal of the F-actin reversibly relieves PC2-mediated TREK-1 inhibition. Interestingly, the rate at which the cytoskeletal inhibition recovers is faster in cells expressing TREK-1 with PC2 than TREK-1 alone. These

results indicate that PC2 recruits FLNa to the membrane, where it cross-links F-actin filaments into a 3D framework that rigidifies the cortical cytoskeleton and inhibits TREK-1 activity. This suggests that conditions that involve a change in cytoskeletal stiffness may affect mechanosensory processes via a modulation of mechanosensitive channel gating.

3-B-43 The microglial activation state regulates migration and invasion

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In response to damage or disease, microglial cells undergo complex activation processes. Recently, there is considerable interest in comparing their pro-inflammatory (e.g., 'classical') and resolving (e.g., 'alternative') activation states. Almost nothing is known about how these activation states affect the ability of microglia to migrate and degrade ECM in order to reach their target sites. In the present study, we exposed primary rat microglia to LPS to evoke classical activation or IL4 to evoke alternative activation. We monitored changes in cell morphology and analyzed their ability to migrate and invade. A panel of inhibitors was used to analyze the contributions of different matrix-degrading enzymes to microglial migration and invasion, and qRT-PCR was used to assess changes in their expression. IL4 increased the migratory capacity of microglia but eliminated the preferential anterior nuclear-centrosomal axis polarity. Microglia degraded fibronectin regardless of treatment, but LPS-treated cells were relatively immobile and IL4-treated cells invaded much more effectively through Matrigel[®]. For invasion, untreated microglia primarily used cysteine proteases, but IL4-treated cells used a wider range of enzymes. In addition, each activation condition up-regulated a different set of enzymes. Microglia migrate during CNS development and after CNS damage or disease. Thus, there are broad implications of the finding that classically and alternatively activated microglia differ in their migratory and invasive capacity, and their usage of ECM-degrading enzymes.

3-B-44 Role of the Synaptotagmin-Dynamin interaction in synaptic vesicle recycling

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Synaptic vesicle protein Synaptotagmin I (Syt I) is a well-studied calcium sensor critical for synchronized neurotransmitter release. Syt I contains a conserved juxtamembrane domain of unknown function. Using pulldown assays with fusion proteins, we have shown that the Syt I juxtamembrane region directly interacts with the endocytic protein Dynamin. The interaction is specific to the Dynamin I isoform and is localized to its membrane-interacting pleckstrin homology domain. This interaction is blocked in vitro using a Syt I phosphomimetic mutation. We hypothesize that this interaction mediates activity-dependent retrieval of synaptic vesicles, and thus may influence short-term synaptic plasticity. To determine if disrupting the interaction affects vesicle retrieval, we are expressing a mutant Syt I that does not interact with Dynamin in hippocampal neurons and neuroendocrine chromaffin cells. In neurons, live-imaging of exogenous membrane tracer FM 4-64 during a pulse-chase assay will determine if compensatory endocytosis is affected. In chromaffin cells, electrophysiological recordings of membrane capacitance will determine how vesicle fission pore kinetics of large dense-core vesicles may be affected. Findings from this work have implications in uncovering a molecular link between synaptic vesicle exocytosis and endocytosis.

3-B-45 Activation of the ERK/MAPK pathway by dimethyl sulfoxide is ameliorated by the 5 α -reduced testosterone metabolite 3 α -androstanediol via GABAergic modulation in SH-SY5Y neuroblastoma cells.

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The mechanisms by which androgens regulate synaptic plasticity in the brain are largely uncharacterized. It has recently been demonstrated that hippocampal synaptic responses to fluctuating androgen levels are more complicated than previously believed, and it is becoming increasingly important to understand the signaling pathways involved. Additionally, it has been suggested that the cognitive effects of testosterone may be largely mediated by conversion to its 5 α -reduced metabolites. Therefore, we investigated the

effects of the testosterone metabolites dihydrotestosterone (DHT) and 5 α -androstane-3 α ,17 β -diol (3 α -diol) on extracellular signal-regulated/mitogen-activated protein kinase (ERK/MAPK) activation in SH-SY5Y human neuroblastoma cells. During our investigation, we found a substantial activation of ERK/MAPK by the dimethyl sulfoxide (DMSO) vehicle, which, even at a final concentration of 0.001%, interfered with any observable androgenic effects of DHT. However, the reduced metabolite of DHT, 3 α -diol, significantly reduced this activation via modulation of gamma-aminobutyric acid (GABA)_A receptor signaling. The importance of these findings is two-fold. First, it appears that the commonly used vehicle DMSO induces activation of ERK phosphorylation, which may interfere with studies of rapid steroid signaling responses. Second, 3 α -diol has the ability to inhibit ERK/MAPK activation through GABAergic modulation. The interaction between GABA signaling and ERK/MAPK activation may have a role in synaptic remodelling.

3-B-46 Decreased activity of the lateral septum in sucrose-overeating rats with a history of repeated food restriction and stress

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The Lateral septum (LS) is interconnected to the limbic, hypothalamic and reward centers of the brain to regulate coordinated behaviors such as stress response, feeding behavior and motivational states. LS lesion escalates sweet taste preference. LS activity is enhanced by stress and attenuated by access to sucrose. Stress responses elicit essential behavioral and physiological changes to ensure survival but chronic stress may become maladaptive. Role and mechanisms by which the LS regulate chronic stress-induced changes in dietary preference are largely unknown. We subjected rats to chronic food restriction with intermittent access to sucrose and weekly foot shock stress session to evaluate the ingestive behavior along with molecular and electrophysiological effects on LS. Treatment leads to increased consumption of sucrose with enhanced lick numbers, lick-cluster number and its duration. It also amplified GAD-67 mRNA expression, decreased firing rate of LS neurons, consequently reducing c-fos mRNA expression in the LS. Additionally, baclofen-induced LS

inhibition increased sucrose intake and decreased anorectic stress effects. Food restriction and chronic stress induced LS inhibition leads to compulsivity and increased incentive value of palatable food, eventually switching rats to a stable sucrose-seeking and -consuming phenotype. This shifted preference towards high palatable food to counteract stress effects comes at a cost of maladaptive feeding behavior and harmful metabolic consequences including diet-induced obesity.

3-B-47 Functional Analysis of Calcium Channel-Synaptic Vesicle Tethering in Live Cryoloated Synaptosomes.

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Temporal synchronization between quantal release and Ca²⁺ influx through a single Ca_v led to the prediction that SVs must be attached by a molecular tether to the channel (Stanley, 1993). A recent biochemical report proposes that tethering is mediated by RIM binding to vesicular Rab3a and its simultaneous domain-specific interactions with the channel C-terminal. Two binding sites were proposed: first, directly via the C terminal tip 'PDZ ligand domain', and indirectly via a more proximal proline-rich domain (Kaeser et al., 2011). We hypothesized that if SV binding to the channel C terminal was blocked by competing peptides then SV recycling should be compromised. However, due to the small size of typical presynaptic terminals, there was no simple method to introduce the peptide blockers. We therefore developed a novel method, termed cryoloading, to introduce any large alien compounds into isolated CNS presynaptic terminals (synaptosomes; SSM). PDZ-ligand domain and proline-rich domain mimetic peptides were cryoloated into the SSMs and SV turnover was assayed using the standard FM, styryl dye method. Cryoloated botulinum light chain was used as a positive control and blocked FM uptake, as predicted. However neither mimetic peptide blocker, used alone or in combination, had any effect on SV turnover. This lack of functional deficit together with complementary biochemical SV binding studies (see Gardezi et al. CAN 2014 Poster) lead us to conclude that SVs tether to Ca_v C terminals by an alternative but unidentified mechanism.

3-B-48 Neurotensin And Anxiety In The Oval Bed Nucleus Of The Stria Terminalis

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Anxiety is both an adaptive and pathological behavioural state that is coordinated by neuronal pathways. Previous evidence, has implicated the Bed Nucleus of the Stria Terminalis (BNST) in anxiety behaviour and responses to contextual stimuli. More recently, a study demonstrated optogenetic stimulation of the oval nucleus (ovBNST), a subregion of the BNST, enhanced anxiety-like behavior. However, the underlying cellular mechanism remains unknown. Neurotensin (NT) is a neuropeptide found both in NT-positive neurons and synaptic terminals of the ovBNST and plays a role in anxiety behaviour. Yet, the neurophysiological role of NT in the ovBNST and its involvement in anxiety remains largely unclear. Our electrophysiology experiments were conducted in male Long-Evans rats using whole cell patch clamping. Our observations show that endogenous and exogenous release of NT potentiates GABAergic inhibitory synaptic transmission in the ovBNST. NT mostly acted pre-synaptically to increase the probability of GABA release since the paired-pulse ratio (PPR) significantly decreased ($p=0.001$). These results suggest NT is a modulator of inhibitory synaptic transmission in the ovBNST. We then investigated whether selective NT-antagonist in the ovBNST had an effect on anxiety-like behaviour. Our preliminary results suggest NT has an anxiogenic effect in the ovBNST.

3-B-49 Hydrogen peroxide potentiation of a tonic GABA current in hippocampal neurons requires extracellular oxidation and the Fenton reaction

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Background: Hydrogen peroxide (H₂O₂), a key reactive oxygen species, contributes to pathological disorders such as ischemia-reperfusion injury and neurodegenerative disease. We recently showed that H₂O₂ robustly increases a tonic GABA current in hippocampal neurons that might contribute to H₂O₂-induced brain dysfunction. The mechanisms underlying such H₂O₂ effects are unclear. H₂O₂ can regulate the function of ion channels via oxidative reaction, as well as Fenton reaction that produces hydroxyl radical

([•]OH) through interaction of H₂O₂ with ferrous irons (Fe²⁺). The aim of the current study is to determine whether H₂O₂ similarly regulates GABA_A receptor-mediated tonic current. Methods: Whole-cell voltage clamp recordings were performed from cultured mouse hippocampal neurons. The antioxidants glutathione (GSH, 1 mM), dithiothreitol (DTT, 1 mM), or the iron chelator deferoxamine (DFO, 100 μ M) were used to prevent the oxidative reaction and Fenton reaction, respectively. An oxygen glucose deprivation (OGD) protocol was used to stimulate the endogenous release of reactive oxygen species, including H₂O₂. Results: The addition of GSH to the pipette solution failed to modify the H₂O₂-induced increase in tonic current. In contrast, the addition of GSH, DTT or DFO to the extracellular solution completely abolished the increase. GSH also completely abolished the increase in tonic current produced by OGD. Conclusion: These results suggest that the increase in tonic GABA current by H₂O₂ results from an extracellular oxidative reaction and the Fenton reaction.

3-B-50 Unique interweaved microtubule scaffold mediates osmosensory transduction via physical interaction with TRPV1

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The electrical activity of mammalian osmosensory neurons (ONs) is increased by plasma hypertonicity to command thirst, antidiuretic hormone release and increased sympathetic tone during dehydration. Osmosensory transduction is a mechanical process whereby decreases in cell volume lead to the activation of transient receptor potential vanilloid type-1 (TRPV1) channels to cause depolarization and increase spiking activity in ONs. However it is not known how cell shrinking is mechanically coupled to channel activation. Using super-resolution imaging we found that ONs are endowed with a unique interweaved scaffold of microtubules in their soma. Microtubules physically interact with the C-terminus of TRPV1 at the membrane surface and this interaction is necessary for channel activation during cell shrinking. Moreover, changes in microtubule stability can bi-directionally modulate osmosensory gain. Microtubules are thus an essential component of

the vital neuronal mechanotransduction apparatus that allows the brain to monitor and correct body hydration.

3-B-51 TRPM2 regulates migration of primary microglia cells

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TRPM2 is a calcium permeable non-selective cation channel that is highly expressed in immunocytes throughout the periphery and in the primary immune cell of the central nervous system: microglia. We hypothesized that TRPM2 may play a role in migration since calcium regulation is important for cell migration. Our results revealed a significant decrease in TRPM2 knockout (-/-) microglia in scratch-wound assays when compared to WT microglia. This work will contribute significantly to our understanding of the role of TRPM2 in neuroinflammatory diseases.

3-B-52 Astrocytes provide steady-state, activity independent control of cerebral blood vessel diameter

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Neuro-vascular coupling encompasses all the signalling events utilized by neurons and astrocytes that instruct the micro-vasculature to change diameter, thereby controlling local blood flow. In the prevailing model, synaptic glutamate release activates glutamate receptors on both neurons and astrocytes, which initiate Ca²⁺ dependent pathways that ultimately signal to contractile vascular smooth muscle cells. Astrocytes, therefore, are believed to be an essential relay cell that communicate to arterioles on behalf of neural activity. This model however, has not been systematically dissected. Studying activity-dependent neuro-vascular coupling in acute brain slices of the neocortex using two-photon microscopy, we observed robust vasodilations and vasoconstrictions that follow increases in neural and astrocytic free Ca²⁺ that initiated rapidly in response to brief electrical stimulation. Interestingly, effective reduction of free astrocytic Ca²⁺ by the intracellular delivery of BAPTA into the astrocyte network, failed to affect activity-dependent diameter changes. However, when BAPTA itself invaded the astrocyte endfeet that were directly apposed to the arteriole, a prominent vasoconstriction was observed. Our data

indicate that synaptically evoked astrocyte Ca²⁺ transients are not necessary to change arteriole diameter in response to a burst of afferent activity, suggesting neural signalling alone is sufficient. Further we propose a novel role for the resting Ca²⁺ concentration in astrocytes in controlling the steady-state tone of vessels when neural activity is quiesce

3-B-53 The cellular mechanisms of neuronal swelling underlying cytotoxic brain edema

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Cytotoxic brain edema is the principal cause of mortality following brain trauma, cerebral infarct and infection yet the mechanisms underlying neuronal swelling are poorly understood. We examined the ionic basis for neuronal swelling, and quantified the intracellular sodium and chloride concentration of individual cortical neurons as they swelled using fluorescence lifetime imaging. We show that sodium entry, triggers a secondary transmembrane chloride influx via a yet unidentified pathway and that this chloride influx is required for neuronal swelling and calcium independent cell death. To identify the novel chloride influx pathway that caused neuronal swelling we first took a pharmacological approach to narrow down a list of candidates followed by siRNA knockdown of these targets using lipid nanoparticle delivery. The identification of cellular targets necessary for neuronal swelling could conceivably lead to therapies to reduce cytotoxic edema by inhibiting the transporter or process required for swelling and subsequent cell death.

3-B-54 Non-uniform dendritic distributions of Ih channels in experimentally-derived multi-compartment models of oriens-lacunosum/moleculare hippocampal interneurons

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Inhibitory interneurons are crucial for generating prominent network rhythms and coordinating information flow in hippocampal microcircuits. The oriens-lacunosum/moleculare (O-LM) cell is an interneuron type in the hippocampal CA1

region that mediates feedback inhibition onto pyramidal cells and gates information flow between sensory input from entorhinal cortex and previously stored associations from the CA3 area. O-LM cells are known to express the hyperpolarization-activated cation current (I_h), but due to experimental constraints it is not known whether these channels are present in dendrites. In previous work, we used ensemble modeling techniques in conjunction with experimental data to show that physiologically realistic multi-compartment O-LM cell models may possess dendritic I_h, but only uniform distributions across the dendritic tree were examined. Here, we tuned the I_h model's kinetics in addition to examining the influence of non-uniform dendritic distributions on the model cells' outputs. We found that different I_h kinetics as well as non-uniform distributions were better able to reproduce experimental O-LM cell responses. Interestingly, this occurred for decreasing conductance densities away from the soma. This is in contrast to pyramidal cells which have higher I_h conductance densities in more distal dendrites.

3-B-55 Radial glia filopodial motility is required for the normal development of excitatory synapses in the optic tectum of *Xenopus laevis*

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Radial glia serve as a migratory scaffold for the neuroblasts in the developing mammalian brain. However, glia, especially astrocytes, are also known to be actively involved in synapse development and maintenance. In the *Xenopus laevis* tadpole, which lacks classical astrocytes, the radial glia extend many fine filopodial processes within the neuropil where they appear to interact with developing synapses, as has been described for astrocytic processes in the mammalian brain. Earlier findings in our laboratory revealed that neural activity induces an increase in glial process motility, dependent on the activation of neuronal N-methyl-D-aspartate (NMDA) receptors and the production of nitric oxide. We have further determined that the increase in motility of these filopodia requires the activation of cGMP dependent protein kinase I (PKGI), known to phosphorylate the small GTPase RhoA. Using genetic manipulations of small GTPase activity to either eliminate the fine glial processes altogether or to "freeze" their movement, we found that both the

amplitude and frequency of the miniature excitatory postsynaptic currents measured in nearby tectal neurons were diminished, suggesting reduced synaptic maturation when glia cannot fully participate. We have also observed that the rates of glial process motility vary with the age of the tadpoles, being highest during the period of greatest synaptogenesis.

3-B-56 Role of Early Acoustic Experience in Development of the Rat Primary Auditory Cortex

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Cortical architecture is established by both genetic and experience-dependent factors. Postnatal sensory experience plays a significant role in the maturation and refinement of cortical sensory fields, such as the primary auditory cortex (A1). Long-term potentiation (LTP) mediates synaptic strengthening in sensory cortices with postnatal sensory exposure. We assessed levels of LTP and short-term plasticity (paired-pulse facilitation/depression; PPF/PPD) in vivo (under urethane anesthesia) in A1 of normally reared rats and rats reared in the absence of patterned acoustic input through continuous white noise exposure (cWN). Rats reared under cWN showed greater LTP of field postsynaptic potentials in A1 compared to controls, indicative of immature, more plastic synaptic connectivity. Both groups showed similar, moderate levels of PPD (25-1000 ms intervals) prior to LTP induction. Importantly, PPD was significantly enhanced after LTP induction, indicative of a presynaptic component of thalamocortical LTP in A1. Ongoing morphological analyses (Golgi-Cox staining) currently do not show clear differences in dendritic complexity of A1 pyramidal neurons (layers II/III) between control and cWN groups. These data indicate that patterned sensory experience results in a reduction of plasticity in A1, indicative of more mature, hard-wired synaptic connectivity. Further, we propose that LTP in A1 in vivo is mediated in part by presynaptic mechanisms, such as increases in transmitter release probability at thalamocortical synapses (supported by NSERC and The Hearing Foundation of Canada).

3-B-57 Calcium-dependent calcium decay explains STDP in a dynamic model of hippocampal synapses

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It is widely accepted that the direction and magnitude of synaptic plasticity depends on post-synaptic calcium flux, where high levels of calcium lead to long-term potentiation and moderate levels lead to long-term depression. At synapses onto neurons in region CA1 of the hippocampus (and many other synapses), NMDA receptors provide the relevant source of calcium. In this regard, post-synaptic calcium captures the coincidence of pre- and post-synaptic activity, due to the blockage of these receptors at low voltage. Previous studies show that under spike timing dependent plasticity (STDP) protocols, potentiation at CA1 synapses requires post-synaptic bursting and an inter-pairing frequency in the range of the hippocampal theta rhythm. We hypothesize that these requirements reflect the saturation of the mechanisms of calcium extrusion from the post-synaptic spine. We test this hypothesis with a minimal model of NMDA receptor-dependent plasticity, simulating slow extrusion with a calcium-dependent calcium time constant. In simulations of STDP experiments, the model accounts for latency-dependent depression with either post-synaptic bursting or theta-frequency pairing (or neither) and accounts for latency-dependent potentiation when both of these requirements are met. The model makes testable predictions for STDP experiments and our simple implementation is tractable at the network level, demonstrating associative learning in a biophysical network model with realistic synaptic dynamics.

3-B-58 Using dynamic clamp to quantify changes in somatosensory afferent excitability associated with neuropathic pain**Petri Takkala¹, Steven Prescott¹**¹University of Toronto

Hyperexcitability in cutaneous primary afferent neurons is a feature of the pathophysiology underlying neuropathic pain. Qualitative changes in excitability, such as a change in spiking pattern, are obvious whereas subtler changes can be difficult to quantify. The switch in spiking pattern occurs abruptly at a tipping point that depends on the relative proportion of sodium and potassium conductances (gNa and gK, respectively). Using acutely dissociated dorsal root ganglion (DRG) neurons, we sought to measure subtle changes in excitability caused

by manipulations like altered extracellular potassium concentration and application of inflammatory mediators. Here we describe a novel methodology in which virtual conductances are applied via dynamic clamp, and the effect of various manipulations on excitability are quantified by the change in the minimal virtual sodium conductance needed to switch the spiking pattern. In other words, we use dynamic clamp to measure the distance of the neuron from its tipping point before and after manipulations that mimic various factors contributing to neuropathic pain. By establishing a novel methodology by which the distance to the tipping point can be assessed, effects of inflammatory mediators and other factors on excitability can be rigorously quantified. Moreover, the susceptibility of different cell types to qualitatively altered excitability can be compared.

3-B-59 Synapse-specific expression of the alpha5 GABAA receptor subunit in hippocampal interneurons and its rapid decline in the pilocarpine model of temporal lobe epilepsy**Elise Magnin¹, Lisa Topolnik¹**¹Université Laval

Synaptic expression of the alpha 5 GABAA receptor subunit (α5-GABAAR) has been reported in hippocampal CA1 inhibitory interneurons (INs). However, the types of synapses that express α5-GABAAR and its functional role have not been investigated. Using a combination of whole-cell patch-clamp recordings, optogenetics and immunohistochemistry, we examined the synapse-specific expression of the α5-GABAAR and its modifications in the animal model of temporal lobe epilepsy (TLE). Our data showed that both the α5-GABAAR and its anchoring protein radixin exhibit a strong colocalisation with a vesicular GABA transporter in hippocampal CA1 oriens-alveus (O/A) INs. Moreover, inhibitory postsynaptic currents evoked in O/A INs by selective activation of calretinin-positive (CR+) interneuron-specific cells in CR-Cre mice were decreased in the presence of the α5-GABAAR inverse agonist L-655,508 (to 65 ± 5.7 % of control; n = 8). Synaptic expression of the α5-GABAAR was revealed in different IN types, including oriens-lacunosum moleculare cells, bistratified cells, basket cells and oriens-oriens interneurons. Finally, a rapid decrease in the α5-GABAAR and radixin expression was observed in pilocarpine

model of TLE, pointing to a significant disinhibition of O/A INs. Our data showed that the $\alpha 5$ -GABAAR is expressed at inhibitory synapses formed at different types of INs by the local inhibitory input from CR+ cells. Moreover, the rapid decline of the $\alpha 5$ -GABAAR in hippocampal INs during TLE may contribute to IN disinhibition and hyperexcitability with consequences for network activity.

3-B-60 The adrenocorticotrophin (ACTH) secretagogue, arginine vasopressin (AVP) reduces the background TREK-1 current in mouse pituitary corticotropes

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Upon stress, the hypothalamic hormones, corticotropin-releasing hormone (CRH) and AVP stimulate ACTH secretion from corticotropes. We showed previously that the resting potential of corticotropes is set by the basal activities of TREK-1 channels. The suppression of TREK-1 current by CRH causes depolarization and activation of voltage-gated calcium channels. AVP acts synergistically with CRH to stimulate ACTH secretion but the underlying mechanism is unclear. We tested the hypothesis that TREK-1 current can be reduced by AVP and this inhibition is additive to that mediated by CRH. Using the green fluorescent corticotropes isolated from POMC-eGFP mice, we found that AVP (200 nM) reduced the amplitude of the TREK-1 current at -70 mV by ~28%; and the addition of CRH (20 nM) in the continued presence of AVP, further reduced the current by 45%. For cells exposed first to CRH, the TREK-1 current was reduced by ~38%; and the addition of AVP in the continued presence of CRH, further reduced the current by ~47%. These results show that the AVP and CRH-mediated inhibition on TREK-1 current are additive. We also examined the effect of AVP and CRH on membrane potential. The co-application of AVP and CRH depolarized the corticotropes by ~33 mV, larger than that evoked by AVP (~11 mV) or CRH alone (~24 mV). Overall, our results suggest that in the presence of CRH, AVP can further suppress the TREK-1 current, resulting in a more robust depolarization. This mechanism may contribute to the synergistic actions of these two hypothalamic hormones on ACTH secretion.

3-B-61 Non-canonical signaling of NMDARs to pannexin-1 in ischemia

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N-methyl-D-aspartate receptors (NMDAR) are critical mediators of excitotoxicity and neuronal death. As such, NMDARs have been the focus of neuroprotection studies for ischemic stroke. Despite over a decade of success in animal models, pharmacologically targeting NMDARs during / post stroke failed clinical trials due to lack of efficacy and / or were poorly tolerated due to side effects. However, important mechanistic insights were gleaned because of the differential efficacy of antagonists with distinct modes of action. Here, we hypothesized that NMDARs can signal to the large-pore channel pannexin-1 (Panx1) without opening as ion channels. To approach this, we performed whole-cell patch clamp recordings, as well as fluorescent calcium imaging to measure excitotoxic events while NMDARs were pore-blocked with either magnesium or MK-801. Pore-blocked NMDARs activated Panx1 channels to induce ionic dysregulation, leading to mitochondrial dysfunction and neuronal death during ischemia. Furthermore, Panx1 antagonism with a Src family kinase (SFK) interfering peptide blocked neuronal death during ischemia / stroke both in vitro and in vivo. We conclude that NMDAR signaling during stroke involves a novel, ionotropic-independent mechanism.

3-B-62 Regulation of NMDA receptors by tyrosine-protein kinase Fyn in human induced pluripotent stem cell-derived neurons

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NMDA receptor (NMDAR)-mediated fast excitatory neurotransmission is implicated in a broad range of physiological and pathological processes in the mammalian central nervous system. The functions and regulation of NMDARs have been extensively studied in neurons from rodents and other non-human species, and in recombinant expression systems. Here, we investigated human NMDARs in situ by using neurons produced by directed differentiation of human induced

pluripotent stem cells (hiPSCs). The resultant cells showed morphological, biochemical and electrophysiological characteristics demonstrating that they are bona fide neurons. In particular, the hiPSC-derived neurons expressed functional ligand-gated ion channels, including glycine receptors, GABAA receptors, AMPA receptors, and NMDARs. The pharmacological and electrophysiological properties of the NMDAR currents indicated that these were dominated by receptors containing GluN2B subunits. The NMDAR currents were suppressed by genistein, a broad-spectrum tyrosine kinase inhibitor, or by PP2, a selective inhibitor of Src family tyrosine kinases. Furthermore, we found that the currents were suppressed by a Fyn-interfering peptide, Fyn(39-57), but not a Src-interfering peptide, Src(40-58), and normalized currents after treated by scrambled Fyn(39-57) or Fyn(39-57) were $88.4 \pm 4.5\%$ and $60.2 \pm 5.1\%$ ($P < 0.01$), respectively. In addition, RT-PCR revealed that the hiPSC-derived neurons expressed mRNA for Fyn. Together, these findings are the first evidence that Fyn kinase regulates the function of NMDARs in the hiPSC-derived neurons.

C - Disorders of the Nervous System

3-C-63 Neuregulin-1 Attenuates Astrogliosis and Glial Scar Formation after Spinal Cord Injury

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Reactive astrogliosis is a key pathophysiological event after Spinal Cord Injury (SCI). Activated astrocytes secrete pro-inflammatory cytokines and chondroitin sulphate proteoglycans (CSPGs) that impede spinal cord repair and regeneration. Our recent evidence shows that dysregulation of Nrg-1 signaling after SCI may underlie the astrocytes reactivity following injury. Here we elucidated the impact of Nrg-1 on astrocyte activation after SCI. Using an in vivo rat model of incomplete compressive SCI and two in vitro models of reactive astrogliosis, we found that availability of Nrg-1 can mitigate astrocyte reactivity. In SCI rats, recombinant human Nrg-1β1 (rhNrg-1β1) was delivered intrathecally and spinal cord tissue was analyzed by immunohistochemistry and stereological techniques 2 weeks post-SCI. For in vitro astrogliosis, primary astrocytes were activated using lipopolysaccharide or

transforming growth factor-beta and then Nrg-1 was added to the cultures. Using immunocytochemistry, spectrophotometry, Western and slot blotting on astrocyte conditioned media and cell lysate, we show that Nrg-1 treatment significantly attenuates several inhibitory aspects of reactive astrogliosis including CSPG production and the release of TNF-α and IL-1β. Moreover, Nrg-1 activation attenuated cell proliferation and nestin upregulation, two cellular characteristics of astrogliosis. Intrathecal Nrg-1 infusion in SCI also resulted in mitigation of astrogliosis in vivo. Evidence in this work and our previous reports suggest the therapeutic potential of Nrg-1 for the treatment of SCI.

3-C-64 Altered muscarinic activation in Perisynaptic Schwann Cells of SOD1G37R mice; implication for the fate of the neuromuscular junction.

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Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease characterized by a progressive loss of motoneurons and consequent skeletal muscle denervation and neuromuscular junction (NMJ) destruction. Furthermore, ALS is a non-cell autonomous disease. Perisynaptic Schwann cells (PSCs), specialized glial cells at the NMJ, regulate morphological stability and integrity and actively participate in the re-innervation of the NMJ, events under the influence of synaptic communication by muscarinic receptors (mAChRs) activation. Hence, we postulate that PSCs muscarinic activation is altered in ALS. NMJs from soleus nerve-muscle preparations of presymptomatic (120 days) and symptomatic (370 - 500 days) SOD1G37R mice and their litter mates were used. We labelled the three synaptic elements (pre-, postsynaptic and PSCs) as an indicator of the integrity of the NMJ. Also, PSCs decoding ability was investigated using calcium imaging of PSCs elicited by endogenous neurotransmitter release and local agonist application. Our data suggest that alteration of PSC mAChRs functions are detectable at presymptomatic stage of ALS (P120), independently of motor unit vulnerability and persist at a symptomatic stage (P363) in a specific motor unit matter. Taken together, these results suggest that the inadequate muscarinic regulation of PSCs could lead to an improper

maintenance and repair of NMJs structure and functions during the course of the disease.

3-C-65 Adeno-Associated Virus Gene Delivery of Fmr1 in Fragile X Knockout Mice

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Fragile X syndrome is caused by a pathological trinucleotide repeat expansion in the FMR1 gene; the expanded repeat causes a reduction or elimination of the gene product, Fragile X mental Retardation Protein (FMRP). Current pharmacological treatments for fragile X syndrome partially ameliorate certain symptoms yet do not address the underlying etiologies. In order to attempt to up-regulate FMRP expression in the central nervous system of the Fmr1-knockout (Fmr1-KO) mouse model of fragile X syndrome, we used single-stranded adeno-associated viral vector (AAV) that expresses the major isoform of FMRP, driven by a neuron-specific promoter. The vector was delivered into the brain via bilateral intra-cerebroventricular injections in neonatal Fmr1-KO mice. FMRP transgene expression and behavioral assessments were conducted at different time points post-injection. Western blotting and immunocytochemical analyses of AAV-FMRP injected KO mice revealed FMRP transgene expression in the striatum, hippocampus, retrosplenial cortex and cingulate cortex. FMRP expression was almost exclusively restricted to neuronal cells and reached approximately 50% of wild-type levels in the hippocampus, striatum and cerebral cortex. The elevated repetitive behavior and deficit in social dominance behavior seen in PBS-injected Fmr1 KO mice were normalized to wild-type values following AAV-FMRP injection. These results provide the first proof-of-principle for gene therapy for reversing specific behavioral abnormalities in the mouse model of fragile X syndrome.

3-C-66 Identification of the optimal time window to enhance NPC survival after a focal ischemic injury.

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Neural precursor cells (NPC) within the subventricular zone (SVZ) respond to an

ischemic insult by proliferating and migrating to the site of injury where they have the capacity to differentiate. The majority of these cells however undergo apoptosis before differentiating and affecting neural recovery. My goal is to promote the survival of NPCs by manipulating the expression of anti-apoptotic proteins. To do this, I am examining the NPC and glial response to an ET-1 focal ischemic injury to identify the optimal time point in which to manipulate the NPCs. BrdU/Dcx double labelling at 1, 3, 5, 7 and 10 days post ischemia revealed a significant increase in neuroblast proliferation at 7 days. Next, immunohistochemistry was performed with antibodies to CD-68, a marker of activated microglia and macrophages and to GFAP, a marker of activated astrocytes to examine the glial response. Microglial and macrophage activation started at 3 days and continued to 14 days post-ischemia. Astrocyte activation spread across the ipsilateral cortex and into the contralateral cortex by 7 days post-ischemia. Currently we are manipulating the expression of anti-apoptotic proteins in NPCs in the first week post-ischemia to identify the optimum time point that would have the maximum effect on neuroblast survival. Acknowledgements: This work was supported by an operating grant from the CIHR, RDC-NL, HSF-Partnership for Stroke Recovery Catalyst grant to JV. RFB is supported by a HSF-Partnership for Stroke Recovery Studentship, and RBR was funded by a Keith Griffiths HSF Student Award.

3-C-67 Temporal evolution of different limbic seizure onset types in the pilocarpine rat model of mesial temporal lobe epilepsy

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Depth electrode EEG studies in patients with mesial temporal lobe epilepsy have identified two types of seizure onset: the low-voltage-fast onset (LVF) and the hypersynchronous onset (HYP). LVF onset consists of a pre-ictal spike preceding low-voltage beta activity, whereas the HYP onset refers to an early, repetitive pre-ictal spiking activity. It has also been suggested that LVF and HYP seizures originate from extra-hippocampal regions and from the hippocampus proper, respectively. Finally, HYP seizures may correlate with hippocampal sclerosis. In this study, we have analyzed the temporal evolution of HYP and LVF seizures in pilocarpine-treated animals. We performed depth EEG recordings in the CA3 region of the hippocampus, entorhinal

cortex, dentate gyrus and subiculum in pilocarpine-treated rats from 4 days to 2 weeks (n = 8 rats, 419 seizures) after status epilepticus (SE) and from 4 to 6 weeks (n = 7 rats, 140 seizures) after SE. Results showed that 63% of LVF started outside of CA3, compared to 12% of HYP. In contrast 88% of HYP involved the CA3 at onset compared to 37% of LVF. HYP seizures predominated during the 2 first weeks following SE but decreased in favor of LVF onset type 5 weeks after (2.3 HYP seizure per days compared to 0.5 LVF seizure per day during the first 2 weeks following SE ($p < 0.05$), 1.3 LVF seizures per days after 2 weeks compared to 0.4 HYP seizure per days ($p < 0.05$)). We are currently evaluating respective neuronal loss in each of the recorded structures using a stereological method.

3-C-68 The effect of striatal pre-enkephalin overexpression in MPTP mouse model of Parkinson's disease

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Midbrain dopamine (DA) cell death in Parkinson's disease (PD) is associated with an upregulation of striatal pre-enkephalin (pENK). Our previous results using the parkinsonian monkeys suggest that increased expression of pENK mRNA in the striatum is a compensatory response to alleviate PD motor symptoms. To determine the role of striatal pENK in motor behavior, and to define whether striatal pENK may have a protective effect against the neurotoxin insults in the MPTP mouse model of PD, viral vector was used to overexpress striatal pENK before DA depletion. One group received saline, and two other groups received striatal injection of AAV2-GFP or AAV2-GFP-pENK two weeks before MPTP injection. Overexpression of striatal pENK associated with an increased level of opioid peptide ENK in striatum, globus pallidus (GP) and substantia nigra. Moreover, mice overexpressing pENK displayed enhanced locomotor activity. Higher density of striatal tyrosine hydroxylase (TH) positive fibers also detected in mice overexpressing pENK in different regions of the striatum compared to control groups. High performance liquid chromatography showed a marked reduction of DA level in the striatum and GP of MPTP-treated groups compared to saline. Remarkably, striatal overexpression of pENK led to two folds

higher DA concentrations and DA turnover in the GP compared to AAV-GFP MPTP. These results suggest that upregulation of striatal pENK could enhance locomotor activity, increase DAergic tone in GP, and may exert protective effect against the MPTP insults at nigrostriatal nerve terminals.

3-C-69 The interplay between astrocytes and microglia shapes the progression of Alzheimer's disease.

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A. Alzheimer was the first to describe dysmorphic microglia and astrocytes aggregated around amyloid plaques. However, exactly how these cells contribute to the onset or progression of AD still remains an open question. Using a new method for high-resolution confocal microscopic analysis on human tissue, we investigated the characteristics of glial organization around amyloid plaques and paired-helical filaments (PHF) aggregates. We observed that glial cells have a remarkable spatial organization around amyloid plaques, with an inner shell of activated microglia and an outer shell of reactive astrocytes. We named this structure "the reactive glial net" (RGN). Using the CRND8 AD mouse model, we monitored the temporal characteristic of RGNs. Their assembly starts with the appearance of amyloid deposits, is dynamic and sequential. RGNs are progressively disrupted in late stages. Furthermore, RGNs form a toxic environment for neurons as neurites trapped within this structure become dystrophic and produce hyperphosphorylated Tau granules in CRND8Tg. This is correlated with high concentrations of PHF within RGNs in human AD brains. We screened for the expression of the cytokines IL1 β and IL6, biomarkers of inflammation in AD and potential promoters of Tau pathology. Astrocytes of RGNs, but not microglia, were found as a main source of IL-1 β and IL6 in mouse and human tissue. Interestingly, their upregulation is graded and first restricted to the astrocytes of RGNs but spreads locally at late stages. We conclude that RGNs are key structures that shape the progression of AD.

3-C-70 Characterizing the effect of maternal immune activation on perisomatic

GABA neurons using a dual recombinase-mediated gene activation strategy.

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The GABAergic system encompasses a functionally diverse network of neurons defined by their structural, electrophysiological and molecular expression profiles. Disruptions to this system have been implicated in animal models of neuropsychiatric disorders, such as maternal immune activation (MIA); however, previous experiments often lack the spatial resolution necessary for defining this relationship to the specific subtypes of GABA cells. We attempt to circumvent these technical limitations by employing a dual-recombinase system (Cre/loxP and Flp/FRT) to drive the expression of a reporter molecule at the intersection of *Dlx5/6* and parvalbumin (PV) or cholecystokinin (CCK) driver genes. For subsequent experiments, we achieved the selective labelling of PV- or CCK-positive GABA neurons with GFP and the remaining subtractive GABA neurons with mCherry. Here, pregnant mice received an i.p. injection of saline or the viral mimetic polyinosinic:polycytidylic acid (poly I:C) at gestational day 9 or 17. Neural tissue was harvested at post-pubertal stage for cell counting and morphological analyses, specifically in the hippocampus and medial prefrontal cortex. Future aims of this study will attempt to characterize the epigenetic profile in these region-specific, genetically labelled populations using fluorescent activated cell sorting (FACS). By combining this intersectional strategy to analyses at both the cellular and epigenetic level, we hope to elucidate the relationship between specific interneuronal classes and the pathophysiological changes in MIA.

3-C-71 Investigation of TNF α signaling before amyloid overproduction in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) develops decades before clinical symptoms arise and can remain undiagnosed for years. For this reason, there is a need to intervene before the development of cognitive symptoms. One possible culprit in the development of AD is tumor necrosis factor- α (TNF α), a pro-inflammatory cytokine. TNF α is

upregulated in both humans with AD and transgenic models of the disease. Although TNF α is a well-known immune mediator, it is recognized as a modulator of learning, memory, and synaptic function. The role of TNF α in synaptic function is thought to be regulatory or neuroprotective at physiological levels, but neurotoxic when found in excess. Since proper synaptic function underlies healthy cognitive processing, it is critical to understand the effect of TNF α on synaptic function in AD. Consequently, the early intervention of TNF α signaling may help prevent the detrimental effects of excess TNF α on synaptic function. We hypothesize that increased levels of TNF α at early stages of AD-like pathology may become pathogenic and lead to deficiencies in synaptic function. We use the TgCRND8 mouse model of AD, which overexpresses a double human mutation of the precursor to amyloid- β (A β), the amyloid precursor protein, to show that 1-month-old mice display both a significant increase in TNF α and alterations in synaptic function before the overproduction of A β or cognitive deficits. Our findings may provide insight on TNF α as a potential therapeutic target in the prodromal stages of AD and will help elucidate the effects of TNF α on synaptic function.

3-C-72 Depletion in pro-inflammatory monocytes/macrophages is neuroprotective in the myenteric plexus but not in the basal ganglia in a mptp mouse model of Parkinson's disease

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A growing body of evidence supports a critical role for inflammation in the dysfunction of neurons in the central nervous system (CNS) and the enteric nervous systems (ENS) during acute and chronic insults. We investigated the role of inflammation in dopaminergic central and myenteric neuronal alterations secondary to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioning. Our results show that mice depleted in pro-inflammatory Ly6Chi monocytes by clodronate liposome treatments were protected against the MPTP-induced loss of tyrosine hydroxylase (TH) expression in the ENS. Furthermore, a strong immune response was observed in undepleted mice 5 days following the first MPTP injection, as demonstrated by the prominent presence of pro-inflammatory monocytes and the production of IL-1 β and IL-6 in all the segments of the gut.

However, in the CNS, both mice depleted and undepleted in pro-inflammatory monocytes were still subjected to 25% of striatal TH loss following MPTP administration. Moreover, the MPTP treatment elicited strong microglial activation in the striatum in both depleted and undepleted animals. Taken together, our results demonstrate a critical role for pro-inflammatory monocytes/macrophages in the gastrointestinal dopaminergic dysfunction in the MPTP model of Parkinson's disease. At the contrary, in the CNS, the immune response was not impaired by the clodronate liposome treatment, suggesting that circulating monocytes do not reach the brain parenchyma and do not contribute to the MPTP-induced toxicity.

3-C-73 Glia-derived tumor necrosis factor alpha promotes retinal ganglion cell death through overexpression of philanthotoxin-sensitive calcium permeable AMPA receptors.

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Tumor Necrosis Factor- α (TNF α) has been proposed to mediate retinal ganglion cell (RGC) death in glaucoma, but its mechanism of action is unknown. Here we tested whether TNF α enhances cell surface expression of calcium permeable AMPA receptors (CP-AMPA) thus increasing the vulnerability of RGCs to glaucomatous damage. Ocular hypertension (OHT) was induced in rats by injecting hypertonic saline into an ocular episcleral vein. TNF α , TNFR1/2, and AMPAR GluA2 subunit expression was examined by RT-PCR, western blot and immunohistochemistry. Expression of CP-AMPA was assessed by cobalt staining assays. Electrophysiological responses were examined by whole-cell patch-clamping. RGC soma and axon density were assessed on flat-mounted retinas or optic nerve cross sections, respectively. Our data demonstrate that TNF α and TNFR1/2 levels increase early after OHT induction and remain elevated with disease progression. TNF α was detected in microglia and Müller cells of glaucomatous retinas. Cobalt uptake through CP-AMPA, observed in RGCs subjected to OHT, was blocked by philanthotoxin (PhTX). While the GluA2 subunit was edited at the Q/R site, the level of GluA2 in RGCs was markedly reduced. Electrophysiological data

confirmed that RGCs in hypertensive eyes are more sensitive to PhTX than those in control eyes, indicative of CP-AMPA upregulation. Robust protection of RGC soma and axons was observed using inhibitors of TNF α or CP-AMPA. Our data support a model in which TNF α -induced upregulation of CP-AMPA plays a key role in RGC damage in experimental glaucoma.

3-C-74 The effects of focal ischemic lesions of the prefrontal cortex on attentional set-shifting behaviour in the rat

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Stroke is one of the leading causes of disability in adults, and has been estimated to cost the Canadian economy 2.8 billion dollars annually. Further, studies examining patients three months post-stroke have found that approximately 25% of stroke survivors suffer from cognitive deficits. Cognitive deficits in stroke survivors may occur because of damage to the prefrontal cortex (PFC), which has been heavily implicated in the control of higher-order cognitive functions in the brain. Therefore, an improved understanding of the role of the PFC in cognitive dysfunction post-stroke is necessary. Utilizing bilateral microinjections (2 x 1 μ l) of the vasoconstricting peptide endothelin-1 (ET-1) (400 pmol) into the medial PFC in adult male SD rats (N=12/group) we produced localized ischemic lesions in the PFC (or sham) and the effects of these lesions on cognition were assessed using a colour-texture discrimination attentional set-shifting task. Following testing, rats were euthanized and lesion location and volume was confirmed via cresyl violet stain. During the set shifting aspect of the test, animals shifting attention to colour from texture reached criterion faster than animals shifting attention to texture from colour. Further analysis revealed a difference in the rate of learning between stroke and sham animals for this aspect of the test. This model shows potential in examining post-ischemic cognitive deficits in rats, and future experiments are required to further refine the model and to test other aspects of higher order cognitive functioning post-stroke.

3-C-75 Spatial characterization of optogenetically induced seizures

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Synchrony of the local field potential (LFP) in addition to single unit - LFP synchrony are characteristic features of epileptic seizures in rodent seizure models and in clinical epilepsy. The role synchronization plays in the initiation and termination of seizures remains unresolved. Here we exploit mouse lines expressing channelrhodopsin-2 (ChR2) in cortical interneurons under the control of the vesicular gamma-aminobutyric acid transporter (VGAT) to induce interneuronal synchrony in the network and investigate the critical transitions that underlie ictogenesis. Cortical brain slices from VGAT-ChR2 mice were stimulated using a 470 nm fiber coupled light emitting diode (LED) and the LFPs recorded using a 60-channel multi electrode array. Seizure like events (SLEs) were reliably initiated with optical stimulation while the slice was perfused with 4-Aminopyridine (4-AP) containing artificial cerebrospinal fluid (ACSF). We show putative pyramidal cells synchronously fire at the onset of both light induced and spontaneous SLEs and their firing patterns appears to be a characteristic feature of seizure initiation. In accordance with seizures observed in humans and rodents, pyramidal cell firing was synchronous with the low frequency LFP. We further demonstrate layer specific activation patterns which rapidly diverge after light induced synchronization. Finally we use of focal 470nm light stimulation to generate SLEs and explore the propagation dynamics of these SLEs within the slices.

3-C-76 USP8 Regulates Mitophagy by Removing K6-linked Ubiquitin Conjugates from Parkin

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Mutations in the Park2 gene, encoding the E3 ubiquitin-ligase parkin, are responsible for a familial form of Parkinson's disease (PD). Parkin-mediated ubiquitination is critical for the efficient elimination of depolarized dysfunctional mitochondria by autophagy (mitophagy). As damaged mitochondria are a major source of toxic reactive oxygen species within the cell, this pathway is believed to be highly relevant to the pathogenesis of PD. Little is known about how parkin-mediated ubiquitination is regulated during mitophagy or about the nature of the ubiquitin conjugates involved. We report here that USP8/UBPY, a deubiquitinating enzyme not

previously implicated in mitochondrial quality control, is critical for parkin-mediated mitophagy. USP8 preferentially removes non-canonical K6-linked ubiquitin chains from parkin, a process required for the efficient recruitment of parkin to depolarized mitochondria and for their subsequent elimination by mitophagy. This work uncovers a novel role for USP8-mediated deubiquitination of K6-linked ubiquitin conjugates from parkin in mitochondrial quality control.

3-C-77 Peptide-mediated degradation of a death-inducing kinase as a therapy for stroke

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Despite the massive socioeconomic toll of the ischemic stroke, there is currently no effective neuroprotectant available for clinical use. Recent efforts have identified death-associated kinase 1 (DAK1) as a key mediator of ischemic cell death. Following an ischemic insult, DAK1 activates and binds to the GluN2B subunit of the NMDA Receptor (NMDAR) and enhances NMDAR-mediated currents and excitotoxicity. DAK1 is also involved in mediating cell-death through non NMDAR-mediated oxidative stress. In an effort to reduce ischemic brain injury, we fashioned a blood brain barrier and cell membrane permeant peptide TAT-GluN2BCTM which, when systemically administered in the MCAo rat model of ischemia, efficiently knocks down active (but not inactive) DAK1 through chaperone-mediated autophagy. A single intravenous injection of 10mg/kg TAT-GluN2BCTM (but not control TAT-GluN2B) 1h post reperfusion specifically knocked down DAK1 only in brain areas affected by the insult, including the ipsilateral striatum and nearby cortex as visualized with hematoxylin and eosin staining. Most significantly, the specific knockdown of DAK1 by TAT-GluN2BCTM was associated with a much more substantial reduction of the infarct area and fewer number of degenerating neurons in comparison with that provided by uncoupling DAK1 from the GluN2B receptor signaling complex with TAT-GluN2B. Together, these data point to DAK1 as a key target for the intervention of neurotoxicity and illustrate peptide-mediated degradation of death-promoting proteins as a new avenue to develop therapeutics for stroke.

3-C-78 Age-Related Changes in Working Memory in the Hebb-Williams Maze in the Triple Transgenic Mouse Model of Alzheimer's Disease

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The 3xTG-AD mouse model of Alzheimer's disease (AD) has three mutations, two associated with AD, APPSwe, PS1M146V, and one with tau pathology, tauP301L. 3xTG mice develop intracellular plaques at 3 months of age, followed by extracellular plaques at six months, and tau tangles by 12 months of age. Previous research using 3xTG mice demonstrated impairments in spatial learning at six months of age. However, there has not been any reported progression of deficits from six to twelve months of age in these mice. We used the Hebb-Williams (H-W) maze to assess spatial learning and memory in 3xTG mice at 7 months of age. The H-W maze is an open field with start and goal boxes located at opposite corners. Barriers of various lengths are arranged to design different mazes that the mouse has to solve in order to find food reward. The H-W maze has multiple levels of difficulty, with 12 maze designs in total, four in each group of easy, intermediate and hard mazes. In previous studies using the H-W maze, mice with limbic lesions performed worse than control mice (Meunier et al., 1986). We found that the 3xTG mice made significantly more errors than wild type mice in only the hard mazes. Our results support the previously reported spatial memory deficit in 3xTG mice at seven months of age compared to their wild type controls. However this difference may be smaller than previously reported. Our results emphasize the value of behavioural tests with multiple levels of difficulty such as the H-W maze, which are more sensitive at detecting smaller cognitive deficits.

3-C-79 RhoA proteolysis: A novel mechanism for RhoA regulation and its possible applications for CNS regeneration

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Traumatic injuries to the central nervous system have devastating and persistent clinical consequences as a result of the failure of injured axons to spontaneously repair themselves. Axon repair is impeded by inhibitory molecules at the injury site, which bind to receptors on axonal surface and signal inappropriate cytoskeletal

remodeling through activation of the RhoA GTPase. RhoA plays a central role in blocking axon regeneration and has been targeted in clinical trials for spinal cord injury. We have found that RhoA is processed through proteolytic cleavage to generate a stable 10kDa amino terminal fragment. We are studying this process to understand how this may affect RhoA activity. Our studies revealed that RhoA proteolysis occurs preferentially for active RhoA and that this process is dependent on prenylation and phosphorylation. Further, inhibitors of Calpains and Caspases stabilize the amino terminal RhoA fragment. We have mapped the RhoA cleavage site and generated a cleavage-resistant RhoA construct. Overexpression of wild type or cleavage resistant RhoA is sufficient to induce stress fiber formation in serum-starved fibroblasts. Overexpression of the amino terminal RhoA fragment induces mild stress fiber formation while overexpression of the carboxy terminal RhoA fragment leads to the formation of nuclear actin rods, a hallmark of cellular stress observed in conditions of neurodegeneration. Our findings describe a novel mechanism for processing RhoA, which affects actin cytoskeletal remodelling. Future work will focus on the role of RhoA cleavage in neurons.

3-C-80 Age-dependent tau hyperphosphorylation and deregulation of PP2B in Huntington mice models

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Background: Huntington disease (HD) is an autosomal dominant neurodegenerative disorder caused by a polyglutamine expansion in the N-terminal region of the huntingtin protein. HD is characterized by proteolytic cleavage, misfolding and aggregation of huntingtin, leading to neuronal death, primarily in the spiny neurons of the striatum, but also in structures involved in cognition. Aggregates of hyperphosphorylated tau proteins are characteristic of a class of neurodegenerative disease called tauopathies, including Alzheimer's disease. HD is not a tauopathy, but there are several articles reporting limited tau pathology in HD patients. These observations prompted us to hypothesized that HD pathology might promote tau hyperphosphorylation. Methods: To test this hypothesis, we used two well-characterized models of HD (R6/2, Q175), and analyzed tau

phosphorylation. Results: We found that before the onset of HD symptoms, mice present a slight elevation of tau phosphorylation compared to controls. After the onset of symptoms, mice displayed tau hyperphosphorylation at multiple epitopes. There was no activation of tau kinases examined that could explain this hyperphosphorylation. However, when we examined tau phosphatases, we found that PP2B was extremely downregulated. Moreover, inhibition of PP2B in cells promoted tau hyperphosphorylation. Conclusion: Our data suggest that, in R6/2 and Q175 mice, mutant huntingtin lead to deregulation of PP2B and consequent tau hyperphosphorylation, and that the mild tau pathology seen in HD might, to some extent, stem from impaired PP2B regulation.

3-C-81 Effects of chronic typical and atypical antipsychotic treatment on mouse brain volume: a longitudinal magnetic resonance imaging study

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Longitudinal MRI studies have consistently shown that patients suffering from schizophrenia exhibit decreases in brain volume over the course of the illness. However, these patients are often treated with antipsychotic (AP) medication. Thus, it is difficult to determine whether the changes are related to the disorder, to the effects of the medication or both. Using animal models allows for a more ethical assessment of the effect of AP treatment on brain volume, without interference of the pathology. Naïve C57BL/6J mice received daily intraperitoneal injections of saline or haloperidol (HAL) (0.5mg/kg/day) for 9 weeks. Animals were scanned using a Brücker 7T small MRI scanner before starting treatment, then at 3, 6 and 9 weeks to assess volumetric changes in the whole brain and predefined subregions. In accordance with previous studies done in rats, this treatment lead to a decrease in pre-frontal cortex volume. With access to D2 and D3 dopamine (DA) receptor knockout transgenic strains, we aim to assess the role that D2-like DA receptors may play in the brain volume changes associated with AP use. The effect of a typical - HAL - and a D2-independent atypical AP - clozapine - in D2KO and D3KO mice will allow us to better understand if the decrease in

brain volume is due to the activity of D2-like receptors, commonly targeted by typical APs, or whether these changes might involve alternate mechanisms. To understand the cellular basis of volume changes, stereological analysis of neuronal and glial populations will be performed in the brain areas showing significant variations.

3-C-82 Reduction of 2-4 Hz coherence between the hippocampus and prefrontal cortex following chronic prefrontal cortex stimulation

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Many psychiatric disorders likely result from a breakdown of communication in particular brain pathways, but others may stem from pathologically high communication levels. Hyperactivity of the ventral medial prefrontal cortex (vmPFC) has been observed in patients suffering from depression. This can be treated, with associated depression symptoms, by focal electrical stimulation ("deep brain stimulation", or DBS). The effects of DBS on communication between the vmPFC and other brain regions remain unknown. The present study examined the effect of DBS on communication between the rat ventral hippocampus and vmPFC by measuring coherence of local field potentials (LFPs). Rats received daily treatments of vmPFC DBS (100 µA pulsed at 130 Hz) or sham stimulation. Recordings were conducted in unrestrained, behaving animals on the day before treatment, 1 to 10 days of treatment, and 10 days after stimulation ended. Coherence between the two regions was high in the 6 to 10 Hz (theta) range, which could be further evoked by auditory stimulation; however, this was not affected by DBS. In contrast, coherence in the 2 to 4 Hz band was reduced following 10 days of DBS ($p = 0.009$). Coherence at 2-4 Hz was not related to rats' movement speed, suggesting that the physiological changes took place independent of behavior. Physiological patterns in the 2 to 4 Hz band have been previously associated with dopamine activity; the data may therefore have implications for how frontal cortex DBS affects the dopaminergic system and its feedback on hippocampal-prefrontal processing.

3-C-83 Abnormal Myelination during Brain Development in Fragile X Mice

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Myelination in the mammalian brain is mainly a postnatal process. It begins in the brainstem and cerebellum and then proceeds rostrally over several weeks in rodents, and over years in humans. Fully intact and mature myelin sheaths are obligatory for the normal firing and differentiation of neurons. Thus, myelination, neuronal firing, and neuronal differentiation are intimately linked to each other, and impaired myelination may impact negatively on neuronal development. We have reported that FMRP co-localizes with oligodendrocyte precursor cells in the immature CNS, and that myelination in the Fmr1 fragile X knockout mouse cerebellum is delayed compared to wild-type mice (Pacey et al., 2013). Markers for myelination eventually reach normal (wild-type) levels around 3-4 weeks after birth in the Fmr1 mouse. The consequences of this delay in brain maturation are not understood. Our recent studies have focussed on assessing which brain regions (in addition to the cerebellum) are affected by abnormal myelination, whether other types of glia show abnormalities during maturation, and whether Fmr1 mouse myelination responds differentially to stress compared to control mice. We have observed that astrocytes are "activated" in the immature brain, and that this appears to be a permanent feature of the Fmr1 mouse brain. Further studies are being conducted to examine the relationship between these parameters (oligodendrocytes, astrocytes, and stress) in the mouse model of fragile X syndrome.

3-C-84 Endoplasmic reticulum stress, TDP-43, FUS and progranulin involvement in models of Huntington's disease

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Introduction: Cytoplasmic accumulations of TDP-43 and FUS are observed in multiple late-onset pathologies including Huntington's disease. However, how these pathological accumulations occur remains poorly known. **Objective:** The study of TDP-43 and FUS contribution and the impact of progranulin on

neurodegenerative phenotypes associated with Huntington's disease allow identifying mechanisms involved in this disease. **Methodology:** Genetic models of polyglutamine (polyQ) toxicity and lines having mutations in orthologous genes for TDP-43, FUS and progranulin in *C. elegans*, polyQ striatal cell lines with 111 repeats and the polyQ Q175 mouse model were used. **Results:** In *C. elegans*, we observed that mutations leading to loss of function for orthologous genes TDP-43 and FUS reduced behavioural deficits and neurodegeneration caused by polyQ toxicity. Moreover, we found that TDP-43 and progranulin genetically interact for regulating polyQ toxicity and that progranulin protects from toxicity associated with mutant huntingtin in *C. elegans*. Furthermore, in the Q175 mice, we observed an increase of TDP-43 levels and decreases of FUS and progranulin levels. Also, quantitative PCR revealed changes in expression of genes involved in the endoplasmic reticulum stress response. **Discussion/conclusion:** TDP-43 and FUS seem to be key actors in polyQ toxicity and progranulin could act play a crucial role, enabling a potential therapeutic target of Huntington's disease. **Funding:** CIHR (AP, CJ), CIHR-Huntington Society of Canada (AT), CHUM Foundation, ALS Canada.

3-C-85 Ceramidase Activity is Required for the Neurotoxic Effects of a Lipid Second Messenger Molecule Elevated in Alzheimer's Disease

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Alzheimer's disease (AD) is associated with distinct changes to the lipid profile of the brain. The consequence of these apparent alterations in lipid metabolism upon neuronal cell function and viability are not well understood. Previously we have reported that elevating intraneuronal concentrations of the AD-associated glycerophosphocholine second messenger, PC(O-16:0/2:0) or C16 platelet activating factor (C16 PAF), is sufficient to induce endoplasmic reticulum stress and neurotoxicity. Subsequent studies in *Saccharomyces cerevisiae* have suggested that the toxic effects of PC(O-16:0/2:0) are, in part, due to a disruption in ceramide metabolism. Utilizing the genetic tractability of *S. cerevisiae* we have now

identified the alkaline ceramidase orthologues, YPC1 and YDC1, as critical mediators of the PC(O-16:0/2:0)-induced changes in ceramide metabolism and toxicity in yeast which led us to examine whether similar changes may occur in neurons. Profiling of the ceramide lipid species by mass spectrometry revealed a significant increase in most species following a 24 h treatment of terminally differentiated hNT cells. Furthermore, a small molecule inhibitor of ceramidase (Ceranib-2), but not ceramide synthase activity (Fumonsin B1), protected neurons from the neurotoxic effects of PC(O-16:0/2:0) over a 24 h period. In conclusion, our data support an unappreciated role for ceramidase activity in mediating neuronal toxicity associated with pathological levels of PC(O-16:0/2:0).

3-C-86 Elevated microglial activation in PD patients expressing a polymorphism for high- affinity binding for [18F]-FEPPA in striatal and extra-striatal regions: A PET study

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Microglial activation has been implicated as a potential mechanism for the disease progression and producing non-motor symptoms of PD. It can be quantified in vivo using PET radioligands targeting for 18kDa translocator protein (TSPO). Second-generation TSPO radioligands including [18F]-FEPPA may present three binding affinity phenotypes: High affinity binders (HABs), low affinity binders (LABs) and mixed-affinity binders (MABs). These phenotypes can be predicted by genotyping one polymorphism (rs6971) in the TSPO gene. Here, we have investigated the role of microglial activation in PD pathology using [18F]-FEPPA. Forty subjects were included in the study. Total distribution volume (VT) values, obtained using an unconstrained two-compartment kinetic model were compared between PD and healthy controls (HCs) matched for the binding affinity phenotypes. There were 25 HABs (13 PD and 12 HCs), 12 MABs (6 PD and 6 HCs) and 3 LABs (3 PD). PD patients showed significantly elevated VT values compared with HCs in the HAB group while no difference in VT value was observed in the MAB

group. In the HAB group, the elevated VT occurred in the striatum, putamen, thalamus, as well as left frontal, left temporal, and occipital cortices. This is the first study to demonstrate that only PD patients expressing the genotype for high affinity binding for [18F]-FEPPA presented elevated microglial activation compared to HCs. Our preliminary data suggest that this radioligand may provide new evidence of an interaction between TSPO genotype and microglial expression in PD pathology.

3-C-87 Investigation of neurotransmission and synapse maintenance in cultures from G2019S knock-in mice

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Parkinson's disease (PD) is the second most common neurodegenerative disease, with mutations in the Leucine-rich repeat kinase 2 (LRRK2) protein being the most common known cause. The LRRK2 G2019S mutation alone accounts for up to 30% of all cases in some populations. Whilst the physiology of LRRK2 is poorly understood, it has been implicated in synaptic transmission and dendritic morphology. Previous findings suggest that LRRK2 mediates glutamate release in simple model systems, but this has yet to be examined in a LRRK2 mutant mouse model. We investigated synaptic transmission in primary cortical cultures from non-transgenic and G2019S knock-in (KI) mice. Patch clamp recordings were conducted in mature neuronal cultures. We measured miniature excitatory/inhibitory currents to estimate receptor numbers/responsiveness (amplitude) and release probability (frequency). Staining for pre- and postsynaptic markers was used to determine synaptic density. Western blotting was also performed to identify changes in levels of neurotransmitter receptors, synaptic markers, and LRRK2 itself. We observed alterations to both excitatory and inhibitory transmission in the presence of the G2019S mutation. Our results suggest that mutant LRRK2 disrupts neural transmission within cortical networks, highlighting the benefit of further characterization of this model. We hope to determine the mechanistic defect conveyed upon LRRK2 by this mutation, and the potential consequences for PD. Subsequent research will

focus on LRRK2 in synaptic connectivity and plasticity in corticostriatal co-cultures.

3-C-88 Elevated NKG2D and NKG2D ligands expression in a mouse model of multiple sclerosis (EAE)

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Multiple sclerosis (MS) is an inflammatory disease of the central nervous system. Hallmarks of MS lesions include injury to oligodendrocytes and axonal loss. NKG2D is an activating (co)receptor expressed by numerous immune effector cells. Our laboratory has previously shown that: 1) oligodendrocytes express ligands of NKG2D (NKG2DL) in MS lesions but not in controls; 2) CD8 T cells in MS lesions are detected in close proximity to NKG2DL expressing cells. We have also previously established that disruption of the NKG2D-NKG2DL interaction inhibits killing of human oligodendrocytes by immune cells. These results imply that NKG2D-NKG2DL interaction can contribute to cytotoxic response mediated by immune effector cells in the inflamed CNS, as observed in MS. Our goals are to characterize the in vivo role of NKG2D and its ligands in experimental autoimmune encephalitis (EAE) mice. To determine qualitatively and quantitatively NKG2D/NKG2DL expression during the development of EAE we use flow cytometry, qPCR and immunohistochemistry. We observed that NKG2D ligands are upregulated in the CNS during the development of EAE. Microglia and neurons from EAE animals express elevated levels of NKG2DL compared to controls. Moreover, a greater proportion of T cells that have infiltrated the CNS express NKG2D compared with cells from other organs. Our results suggest that NKG2D and its ligands could play a role in the development of EAE. We will use different strategies to block NKG2D at different disease states to clearly establish whether NKG2D is a relevant therapeutic target.

3-C-89 The role of neuroligins in the generation of abnormal rhythmic theta discharges in EEG recordings

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Autism spectrum disorders (ASD) are a class of life-long neurodevelopmental disorders. Their diagnostic criteria consist of impaired social interactions, restricted interests, and repetitive behaviours. In addition to the diagnostic features, patients with ASD are found to have increased risk for seizure. For example, while 30% of ASD cases are reported to develop seizure, epileptiform activity is reported in 60% of patients with ASD during sleep. Despite the high risk of seizure development in ASD, its molecular basis remain elusive. Alterations in the excitability of neuronal networks has been proposed as a key contributor to ASD as well as seizure. Neuroligins (NL), which are a family of postsynaptic cell adhesion proteins, were shown to be important for the proper maintenance of neuronal excitation/inhibition balance, and their mutations have been linked with ASD. The present study aims to examine the role of neuroligins in seizure development. We observed that deletion of NL2 is linked with an occurrence of abnormal rhythmic theta discharge that resembles electrographic seizures.

3-C-90 The role of NOD-like Receptor Nlrp12 in Multiple Sclerosis

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Multiple Sclerosis (MS) is a disease that affects the central nervous system as well as the spinal cord resulting in the presence of demyelinating plaques. Several molecular pathways have been identified as potential targets for therapeutic interventions in MS such as NF- κ B and nucleotide-binding leucine-rich repeat-containing proteins (Nlrs). Nlrs are regulatory proteins of the immune system. Once activated, these proteins trigger pro-inflammatory pathways for example activation of NF- κ B pathways. Newly discovered Nlrp12 has been shown to inhibit inflammation by suppressing NF- κ B activity. To evaluate the hypothesis that Nlrp12 plays an anti-inflammatory role in MS, we used a well-characterized mouse model of MS, Experimental Autoimmune Encephalomyelitis. Immunization of C57BL/6 female mice with MOG35-55:CFA, demonstrated Nlrp12 knockout (KO) mice to have an aggravated form of the disease. Also, the disease started earlier in KO and was characterized by higher clinical scores compared to Wild-Type (WT) mice. An increase in the expression of pro-inflammatory genes (mip3 α , Cox2, IL-1 β Ccr5) were seen in KO

compared to WT mice. In vitro stimulation of primary microglial cells with LPS demonstrated an increase in the expression of iNOS in KO mice. Griess reagent assay showed more nitrates secreted in the media from KO mice. Additionally, purified microglia from KO mice treated with LPS for 12 hours demonstrated significantly more pro-inflammatory cytokines TNF- α and IL-6. Moreover, photomicrograph of spinal cord showed enhanced astrogliosis in KO compared to WT mice.

3-C-91 Nucleus accumbens DNA methylation states determine cocaine craving

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Cocaine addiction is characterized by a pattern of cyclic binges. Indeed, a withdrawal period following a cocaine abuse phase, progressively leads to high craving and resumption of the drug. Usually, relapse, which is the major clinical problem in the treatment of cocaine addiction, is elicited during this incubation period by re-exposure to a cue previously associated with cocaine intake. Mechanisms underlying progression to high craving during the withdrawal period are poorly understood. We show using a rat model that genome-wide DNA methylation modifications in the nucleus accumbens are associated with cocaine self-administration. DNA methylation changes increase over time during a withdrawal period in the absence of any exposure to cocaine, paralleling the increase in craving. Interestingly, application of an environmental cue, paired with cocaine self-administration, reverses the DNA methylation changes associated with the withdrawal period. Moreover, we found that intra-cerebral injections of DNA methylation modifying drugs modulate cocaine craving. These results suggest that DNA methylation modifications define progression to cocaine addiction and that it might be possible to reverse craving in humans using epigenetic modulators following long withdrawal periods.

3-C-92 Development of a Novel Lumbar Spinal Cord Injury Model to Examine the Therapeutic Potential of Transplanting Neuronally Induced Neural Stem/Progenitor Cells.

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Introduction: Patients with injuries to the thoracolumbar region of the spinal cord often lose neurons essential for locomotion. Our focus is on replacing lost circuitry by transplanting adult spinal-derived neural stem/progenitor cells (NSPCs) that have been differentiated into neurons in vitro. We hypothesize that optimal differentiation of NSPCs in vitro towards a neuronal lineage will promote transplant survival and functional recovery after transplantation in the injured lumbar spinal cord. **Methods:** NSPCs were treated with 1mM dibutyryl-cyclic AMP (dbcAMP) to enhance neuronal differentiation and stained with BIII tubulin to confirm neuronal character. 40 (Wi) rats were injured with a 26g clip and split into 4 treatment groups: (1) dbcAMP treated cells + Rolipram injection (RI) post-op, (2) dbcAMP treated cells + saline injection (SI), (3) untreated cells + RI, (4) media injection control + SI. Four hundred thousand cells were transplanted 1mm rostral and caudal to the injury site in the subacute phase of injury. **Results:** DbcAMP robustly differentiated NSPCs towards BIII positive neurons: 72% \pm 6.3. In the transplant study, rats in the double treatment group (dbcAMP cells + RI) improved to a statistically significant ($p < 0.05$) average of 5.16(\pm 3.2) compared to dbcAMP + saline = 2.2(\pm 1.5), untreated cells + RI = 1.1(\pm 1.07) or control = 2.2(\pm 1.3). **Conclusion:** We have generated a novel pre-clinical lumbar spinal cord injury model and displayed that transplant of neuronally differentiated stem cells is associated with an increase in functional recovery.

3-C-93 Decreased mTOR signaling via p70S6K/eIF4B is associated with loss of the excitatory postsynaptic marker PSD-95 in autism

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Defects in the establishment of neuronal networks are believed to be responsible for the clinical symptomatology of autism. However, the molecular mechanisms underlying the abnormal cortical circuitry seen in autistic brains remain to be elucidated. We previously found imbalances in TrkB isoforms and decreased upstream components of the mTOR pathway in postmortem brains of autism versus control subjects. mTOR downstream signaling pathways p70S6K/eIF4B and 4E-BP1/eIF4E are involved in regulation of dendritic spines which form excitatory postsynapses. Thus, we now

aimed to examine whether mTOR-mediated signaling pathways are disrupted in autism and whether their disruption is associated with changes in PSD-95, a marker of excitatory synapses. Phospho-mTOR, mTOR, p70S6K, eIF4B, 4E-BP1, eIF4E and PSD-95 were measured by Western blotting in postmortem fusiform gyrus of 11 autism and 13 control subjects. Significantly decreased phospho-mTOR, mTOR, p70S6K, eIF4B and PSD-95 protein levels were observed in autism versus control fusiform gyrus. Surprisingly, no significant changes in 4E-BP1 and eIF4E protein expression were found. Our findings show decreased mTOR expression and activation and down-regulation of mTOR downstream pathway p70S6K/eIF4B in autism which might result in reduced protein translation at spines. Spine protein translation deficits are likely to adversely affect spine density as suggested by decreased PSD-95 in autistic fusiform gyrus. Changes in spine density might perturb cortical circuitry and thus contribute to autism's cognitive and behavioural deficits.

3-C-94 Chemical genetic screens of TARDBP modifiers in *C. elegans* and zebrafish

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Mutations in the TARDBP gene (coding for TDP-43) have been reported to cause Amyotrophic Lateral Sclerosis (ALS) and related dementia, but little is known about the neurotoxic mechanisms. We have generated *C. elegans* and zebrafish models expressing wild-type or mutant human TDP-43[G348C] that reflect aspects of ALS. To explore the potential of our models in identifying chemical suppressors of mutant TDP-43 neuronal toxicity, we tested a set of compounds with potential neuroprotective properties. We performed motility assays in zebrafish and lifespan and stress response assays in worms. We observed that TARDBP have roles in the response to oxidative and osmotic stress. The expression of mutant TDP-43 in worm motor neurons produces robust, adult onset motility defects and in both models this was caused by motor neuron deficits. We isolated a number of chemical suppressors of mutant TARDBP toxicity. Under normal conditions TDP-43 regulates specific aspects of

the cellular stress response. The transgenic models allowed us to isolate chemical suppressors of motor defects. In particular, several neuroleptics protected against development of the motor phenotype and one is currently in clinical trial. Together these data provide clues to help unravel the mechanism for TDP-43 toxicity that should also provide leads for early drug discovery.

3-C-95 Alteration of Spreading Depolarization During Infarct Maturation

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Introduction: We studied the influence of initiators of spreading depolarization (SD) in mouse brain slices taken immediately and 12-hours after 30-min middle cerebral artery occlusion (MCAO). **Method:** Coronal slices (350 µm, C57BL6/J, n=107) were harvested immediately or 12-hr after 30-minute MCAO and superfused with oxygenated aCSF at 34°C. Oxygen-glucose deprivation (OGD), 9.7mM [K⁺] and 500 µM glutamate solutions replaced control aCSF during imaging and SD events were detected as light transmittance changes. Synaptic communication was assessed with field recordings evoked with a bipolar stimulating electrode (1 Hz, 0.25 ms) in Layer VI and recorded with a micropipette in Layer II/III in overlying cortex. **Results:** Immediately after MCAO, OGD-initiated SD in the ischemic hemisphere in 29/29 slices, [K⁺] (3.2 mM and 9.7mM) initiated SD in 12/20 slices and glutamate initiated SD in 0/11 slices. In contrast, 12-hr following 30-min MCAO, OGD-initiated SD in the ischemic hemisphere in 5/12 brain slices, [K⁺] (3.2 mM and 9.7 mM) initiated SD in 3/9 slices and glutamate initiated SD in 0/10 slices. All 12 slices harvested immediately after MCAO displayed evoked field potentials in the ischemic brain territory, whereas only 3/8 slices displayed evoked responses following 12-hr of reperfusion (Mann Whitney U, p < 0.05). **Conclusion:** Both SD susceptibility to OGD or elevated [K⁺] and synaptic communication are intact immediately following MCAO but diminish following 12 hr of infarct maturation. Elevating glutamate does not promote SD at either timepoint.

3-C-96 Effect of 6-hydroxydopamine and resveratrol on Nur77 nuclear to cytoplasmic translocation in PC12 cells

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Nur77, a member of the nuclear receptors superfamily, plays an important role in dopamine neurotransmission and in mediating the effects of antiparkinsonian and neuroleptic drugs. As well, Nur77 survival and apoptotic roles depend largely on its subcellular localization. Resveratrol (RESV), a natural polyphenol, is known for its neuroprotective properties, as demonstrated in vitro and in vivo. However, its action on Nur77 translocation pertaining to neuroprotection has not been investigated yet. The aim of our study was to perform a kinetic study on the effect of neurotoxic 6-hydroxydopamine (6-OHDA) and RESV on the subcellular localization of Nur77 with reference to the modulation of apoptosis in PC12 cells. PC12 cells, a well-known catecholaminergic paradigm, were pre-treated with or without RESV for 3 hours, then treated with or without 6-OHDA for 0, 3, 6, 9, 12 or 24 hours. Leptomycin B, a specific inhibitor of CRM1-mediated nuclear export, was used to confirm the effect on Nur77 cytoplasmic translocation. Our results demonstrate that 6-OHDA significantly enhances cytoplasmic translocation of Nur77 after merely 3 hours while concomitantly precipitating apoptosis. Interestingly, pre-treatment with RESV delays Nur77 accumulation in the cytoplasm and postpones apoptosis induced by 6-OHDA. Our findings demonstrate an important role for Nur77 subcellular localization in 6-OHDA-mediated apoptosis. Our data also highlight a novel mechanism for RESV neuroprotection in this catecholaminergic paradigm, relevant to the development of new therapeutic avenues in Parkinson's disease.

3-C-97 TAU-INDUCED DOWN-REGULATION OF BDNF IN TRANSGENIC MOUSE MODELS OF TAUOPATHY

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Tau is a microtubule binding protein that can be altered by various post-translational modifications to result in the primary toxic species of neurodegenerative diseases known as tauopathies. In one such tauopathy, Alzheimer's disease (AD), soluble tau is hyperphosphorylated and eventually aggregates

to form insoluble neurofibrillary tangles. The toxic mechanism of tau is not well understood. We hypothesize that tau's neurotoxicity is due to its ability to decrease trophic support for affected neurons. Brain-derived neurotrophic factor (BDNF) supports the survival of neurons that are vulnerable in aging and in AD. Here we have used 8c-het and hTau transgenic mice to examine the effect of excess tau on BDNF expression. 8c-het mice over-express wild-type human tau on a wild-type mouse tau background, and while they exhibit increased tau phosphorylation and more abundant 3-repeat tau compared to normal human brain, they do not develop neurofibrillary tangles. On the other hand, hTau mice which over-express wild-type human tau on a null mouse tau background exhibit neurofibrillary tangles similar to those found in AD. We have found that 8c-het mice but not hTau mice show significant down-regulation of BDNF mRNA compared to non-transgenic mice, as quantified by qRT-PCR. This BDNF down-regulation in 8c-het mice is specific for transcript IV, the same transcript that is decreased in AD. Our results demonstrate that excess tau alone is capable of down-regulating BDNF, show that a mutation in tau is not required, and suggest that soluble tau may be the toxic form.

3-C-98 Effect of mild traumatic brain injury on sleep structure and sleep molecular markers in mice

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Introduction: Subjects who experience mild traumatic brain injury (mTBI) often complain about sleep-wake disturbances. However, sleep serves brain recovery, and sleep disturbances may thus be deleterious to recovery after brain injury. This study aims to evaluate the impact of mTBI on sleep architecture and to verify that sleep loss is detrimental to brain recovery following mTBI. Methods: Adult male C57BL/6J mice were submitted to mTBI or sham surgery. EXP 1: Sleep was recorded by electroencephalography starting 14-16 hours post-mTBI and vigilance states were analyzed for a first 24 hours. EXP 2: Mice were submitted to 2 consecutive 6 hours sleep deprivation (SD) during the light period, and their brain was then sampled for quantification of mRNA levels of genes associated to sleep regulation or plasticity (Arc, Homer1a, Hif1A, Bdnf, Fos, EfnA3, EfnB3, EfnB2, Dnajb5, Fgf1, EphA4, EphB2) in the

hippocampus and a region covering the thalamus and hypothalamus. Results: Mild TBI increased slow wave sleep and decreased wakefulness in the first 24 after mTBI. In the thalamus/hypothalamus, SD decreased the expression of Fos in both mTBI and sham mice, and of Homer1a only in mTBI mice. In the hippocampus, SD decreased the expression of EfnB3 in all animals but decreased the expression of Arc and EfnA3 only in mTBI mice. Conclusion: The results suggest that mTBI affects the duration of sleep without having a major effect on gene expression in the thalamus/hypothalamus. Gene expression will next be quantified in the cerebral cortex.

3-C-99 Generation of a novel mouse model of the neuronal isoform Kif1a/25b to study hereditary sensory & autonomic neuropathy type II

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Problematic: Hereditary sensory & autonomic neuropathy II (HSANII) is an early-onset, autosomal recessive disorder characterized by a loss of perception to pain, touch, and heat that results in severe debilitating complications. Our group reported truncating mutations in HSANII patients of a KIF1A nervous-system specific isoform; KIF1A/25B (Kinesin Family member 1A/Isoform 25B). This report was the first identifying a pathology associated with truncating mutations of KIF1A/25B, from which the functional mechanism leading to HSANII remains unknown. We hypothesized that truncating mutations in KIF1A/25B trigger degeneration of myelinated axons in the peripheral nervous system, leading to nociception defects in affected patients. Methods: To investigate the effect of KIF1A/25B truncating mutations in HSANII development, we will generate a mouse model harbouring the equivalent human truncating deletion. The model will be generated by insertion of a single T deletion at the conserved position in the mouse genome (Kif1a/25bdelT/delT). Standardized nociception tests will be performed to evaluate the development of a sensory phenotype on 20 animals of different age (1, 3, 6, and 12 months) for each genotype (wild-type and Kif1a/25bdelT/delT). Significance: The mouse model generated in this project has the potential to inform us on the biology of a truncating

mutation on Kif1a/25b isoform, from both a fundamental and pathological perspective. Furthermore, the mouse model generated in this project will serve as a paradigm for testing future HSANII therapeutics treatment.

3-C-100 The Effects of Sepsis on the Neurons of the Osmoregulatory Pathway

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The osmoregulatory pathway of the hypothalamus is a vital system for the maintenance of plasma osmolality. Hyperosmolality stimulates the osmosensor organum vasculosum of the lamina terminalis (OVLT), which in turn stimulates the supraoptic and paraventricular nuclei (SON; PVN) to induce release of vasopressin (antidiuretic hormone) into the bloodstream. In sepsis (a systemic immune response to a severe bacterial infection), this pathway is severely disturbed by hypovolemia and hypotension which leads to organ failure and death. Several studies have shown that vasopressin levels do not appropriately adjust in septic shock despite severe hypotension or hyperosmolality, and that osmotic thirst sensation is defunct. We hypothesized that septic shock irreversibly impairs the osmosensory activity of OVLT neurons, thereby disrupting the osmotic information pathway. We used an acute model of the cecal ligation and puncture (CLP) surgery to induce sepsis in male rats. Extracellular single unit recordings from hypothalamic explants indicate a prominent loss of spontaneously active neurons ($p < 0.005$) and response to hyperosmotic stimulation in the septic OVLT ($p < 0.05$). Whole cell patch clamp recordings indicate that a change in the excitability of these neurons is responsible for this outcome, and not a loss of the neurons themselves. We show that despite a deficit in the OVLT, SON neurons are more spontaneously active ($p < 0.01$), and are more excitable. Understanding the mechanisms of this paradox has great implications for the treatment of severe sepsis.

3-C-101 Role of the lipid transcription factor SBP-1 and its down-stream genes in

dopaminergic neurons degeneration in *C. elegans*

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The human transcription factor srebp-1 regulates lipid levels within cells, partially by controlling the expression of desaturase enzymes. Defects in the regulation of this gene network have been linked to Parkinson's disease and it may represent one of the factors leading to the degeneration of dopaminergic neurons within the brain. The nematode *C. elegans* has a homologous gene network consisting of sbp-1 (srebp-1) and three desaturases: fat-5, fat-6 and fat-7 whose expression is regulated by SBP-1. To study the effects of the sbp-1 regulatory network on dopaminergic neurons (DA), transgenic strains of *C. elegans* were produced. Each transgenic line overexpressed either SBP-1 itself or one of its different transcriptional targets, specifically in DA neurons that were visualized using a GFP reporter (Pdat-1::GFP). Overexpression of sbp-1, the regulatory gene of the network, caused degeneration in a significant number of the worms. Two of the genes whose transcription is upregulated by SBP-1: fat-5 and fat-6 did not cause significant neuronal degeneration. Conversely, fat-7, another target of SBP-1-mediated transcription, was found to cause neurodegeneration in every worm which overexpressed it. Previous examination of the fat genes in the context of lipid metabolism and embryonic development has suggested that they have overlapping functions in *C. elegans*. Our results indicate a difference in function not previously described for these enzymes.

3-C-102 Glucose influences aging, proteotoxicity and stress response in *C. elegans*

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In developed countries, it is believed that over consumption of carbohydrates and fat is responsible for many metabolic disorders, including obesity, type 2 diabetes and coronary diseases. These disorders exact enormous costs on health systems and research into mechanisms and therapeutic approaches are of obvious importance. Conditions that reprogram

metabolism, like dietary restriction, have become active areas of investigation. Work from yeast to primates has demonstrated that dietary restriction may not only increase lifespan, but also more importantly, maintain healthspan. Aging societies are also burdened by the increasing incidences of age-related diseases including late onset neurological disorders including Alzheimer's disease, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). Metabolic dysfunction in neurodegeneration, and in particular the role of glucose metabolism is not completely understood. To investigate the role of glucose metabolism in aging and proteotoxicity, we used *C. elegans* transgenic models expressing human protein TDP-43 in the motor neurons. Mutations in TDP-43 are causative for ALS leading to the loss of motor neurons in patients. Our transgenic TDP-43 worms display motility defects leading to age-dependent paralysis and the degeneration of GABAergic motor neurons. Surprisingly, we have observed that glucose has the capacity to rescue age-dependent proteotoxicity and perhaps global protein homeostasis

3-C-103 Changes in the histone code - a loss of function mechanism resulting from cytoplasmic redistribution and aggregation of FUS

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Fused in sarcoma/translated in liposarcoma (FUS/TLS) is a heterogenous nuclear ribonuclear protein (hnRNP) with major roles in RNA metabolism. FUS mutations have been linked to ALS (ALS6) and FTL. FUS traffics between the nucleus and cytoplasm, but normally appears concentrated in nuclei and at synapses; however, in ALS6, as well as in many other familial forms and sporadic ALS, FUS can accumulate in cytoplasmic inclusions. Protein Arginine Methyltransferase 1 (PRMT1) is responsible for 85% of asymmetric dimethylation of arginine residues. This post-translational modification modulates various cellular functions such as gene transcription, signal transduction and nucleocytoplasmic shuttling of RNA-binding proteins, including FUS. Using cultured motor neurons, we have shown that PRMT1 interacts with and methylates FUS, and influences its intracellular localization (Tradewell et al., 2012). In those studies we noted that PRMT1 redistributes with FUS. This study investigated

the consequences of PRMT1 nuclear depletion on methylation of its nuclear substrates, specifically Arginine 3 on Histone 4, and downstream effects on transcription. In cultured motor neurons, nuclear depletion of PRMT1 was accompanied by decreased H4R3 methylation and H3 acetylation, a downstream consequence, and decreased RNA synthesis.

3-C-104 Motor unit specific synaptic changes at the neuromuscular junction in an ALS mouse model

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Amyotrophic lateral sclerosis is a late-onset neurodegenerative disease that leads to paralysis and eventually death in 2 to 5 years after the diagnosis. The loss of the neuromuscular junction (NMJ) is the first event in the disease process. NMJs show temporal patterns of denervation in ALS depending on the motor unit (MU) type: NMJs from fast-fatigable (FF) MUs are the first to denervate, followed by the fast resistant and finally the slow MUs. However, no study has taken into account the different MU types when investigating neurotransmission at the NMJ in ALS. We therefore hypothesized that NMJ function would be altered in a MU type specific manner. We used electrophysiology and immunohistochemistry to study synaptic properties in two nerve-muscle preparations of SOD1G37R mice and their wild-type (WT) littermates: the slow-twitch Soleus and the fast-twitch extensor digitorum longus (EDL). At a presymptomatic stage (P140), synaptic strength was already altered in SOD1 mice where FF NMJs of the EDL and slow NMJs of the Soleus had respectively a lower and a higher quantal content compared to WT. Long-term synaptic plasticity was also reduced in the EDL. At a symptomatic age (P380), differences in quantal content were still present in innervated NMJs of both muscles. Taken together, these results reveal that NMJ physiology is altered in ALS according to MU selective vulnerabilities. This study provides insights for a better understanding of NMJ function during the disease that is essential for the development of a proper NMJ-targeted treatment in ALS.

3-C-105 Vesicular glutamate transporter 3 expression in Raphe serotonin neurons: evidence for transmitter phenotype

segregation and for a role in serotonin neurons survival

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A subset of serotonin (5-HT) neurons has been shown to release glutamate as a cotransmitter due to specific expression of the vesicular glutamate transporter 3 (VGLUT3). VGLUT3 enhances 5-HT vesicular loading through a functional synergy with VMAT2, the vesicular monoamine transporter, in a common pool of vesicles. A recent study suggested a functional role of glutamate corelease by dopamine neurons in promoting their growth and survival. Whether VGLUT3 plays a similar role in 5-HT neurons is undetermined. It is also unclear if glutamate is released by all or only a subset of 5-HT terminals. Using primary mouse Raphe cultures, in which 5-HT neurons develop highly arborized axonal processes, we first tested the hypothesis of partial axon terminal segregation. Using immunostaining and confocal microscopy, we first found that contrary to 5-HT, the 5-HT reuptake transporter (SERT) was only present in a subset of axonal terminals; while 25 % of terminals were SERT-positive at day 1 in vitro, 48% expressed SERT at day 7. Moreover, only a subset of SERT- and 5-HT-positive axonal varicosities expressed VGLUT3, with SERT and VGLUT3 being mostly segregated in different axonal domains. Finally, using a VGLUT3 knockout mouse, we found that VGLUT3 gene deletion did not impair the axonal and dendritic growth of cultured 5-HT neurons but reduced their survival by approximately 15%. We conclude that Raphe 5-HT neurons express SERT and VGLUT3 in segregated axonal terminals and that VGLUT3 may regulate the vulnerability of these neurons.

3-C-106 Developmental abnormalities in the cerebellum of spinocerebellar ataxia type 6 mice

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Spinocerebellar ataxia type 6 (SCA6) is a neurodegenerative disorder caused by a polyglutamine tract expansion in the P/Q-type calcium channel that eventually causes degeneration of cerebellar Purkinje cells. Although P/Q channels are required for the

proper refinement of climbing fiber synapses on Purkinje cells in the developing cerebellum (Miyazaki et al., 2004), disease onset occurs in adulthood in SCA6. To determine if cerebellar development is altered in SCA6, we used a recently described SCA6 mouse model containing a hyperexpanded polyglutamine tract in P/Q channels (SCA684Q; Watase et al., 2008). We made whole-cell patch clamp recordings from Purkinje cells in acute sagittal slices from cerebellar vermis from juvenile SCA684Q and litter-matched wildtype (WT) mice, and evoked excitatory post-synaptic currents (EPSCs) in Purkinje cells by stimulating climbing fibers extracellularly. At a developmental stage when the majority of WT Purkinje cells normally receive input from only one or two climbing fibers (P10-13; 15% of WT cells innervated by > 2 climbing fibers, N = 19), significantly more Purkinje cells from SCA684Q mice were multiply innervated by climbing fibers (47% innervated by > 2 climbing fibers, N = 17; P<0.005, Wilcoxon rank sum test). Our findings show that circuit alterations occur in the cerebellum during development in SCA6 mice long before the pathophysiology is observed.

3-C-107 Role of angiogenesis in the development of hippocampal atrophy in the pilocarpine rat model of temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is a form of focal epilepsy often resistant to treatment. It is known to be associated with hippocampal sclerosis. We are investigating whether blocking angiogenesis immediately following the initial insult alters the temporal pattern that leads to the initiation of chronic epilepsy and hippocampus atrophy. A rat model of chronic TLE (i.e., the pilocarpine model) was used. A 60-minute status epilepticus (SE) (initial brain injury) was induced by intraperitoneal injection of pilocarpine in adult Sprague-Dawley rats. Angiogenesis was blocked pharmacologically by sunitinib for a total of 14 days in some pilocarpine animals. Control animals were included; some of them were also treated with sunitinib. Animals were sacrificed 3 weeks after SE, and their brains were extracted. Hematoxyline & Eosin staining was performed on the brain sections; areas of bilateral hippocampi and ratios to the whole hemisphere were measured for each animal. Rats with pilocarpine-induced seizures had hippocampi

significantly smaller, compared to controls. In the pilocarpine rats treated with sunitinib, the hippocampi sizes were not anymore significantly different, compared to controls. Of note, the control animals treated with sunitinib had significantly smaller hippocampus areas, compared to controls. Ratios to the whole hemisphere follow the same trends. Blocking angiogenesis immediately following the initial insult may prevent the development of hippocampal atrophy in this animal model. However, using this medication in normal animals may have deleterious effects.

3-C-108 A new animal model of spontaneous autoimmune peripheral polyneuropathy: implications for Guillain-Barré syndrome

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Background: Spontaneous autoimmune peripheral neuropathy including Guillain-Barré Syndrome (GBS) represents as one of the serious emergencies in neurology. Although pathological changes have been well documented, molecular and cellular mechanisms of GBS are still under-explored, partially due to short of spontaneous and translatable models. Results: We demonstrated that B7.2 Tg/CD4^{-/-} (L31/CD4^{-/-}) mice exhibited both motor and sensory deficits, including weakness and paresis of limbs, numbness to mechanical stimuli and hypersensitivity to thermal stimulation. Pathological changes were characterized by massive infiltration of macrophages and CD8 T cells, demyelination and axonal damage in peripheral nerves, while changes in spinal cords could be secondary to the PNS damage. In symptomatic L31/CD4^{-/-} mice, the disruption of the blood neural barriers was observed mainly in peripheral nerves. Interestingly, the infiltration of immune cells was initiated in pre-symptomatic L31/CD4^{-/-} mice, prior to the disease onset, in the DRG and spinal roots where the blood nerve barrier is virtually absent. Conclusions: L31/CD4^{-/-} mice mimic most parts of clinical and pathological signatures of GBS in human; thus providing an unconventional opportunity to experimentally explore the critical events that lead to spontaneous, autoimmune demyelinating disease of the peripheral nervous system.

D - Sensory and Motor Systems

3-D-109 The role of intrinsic contextual cues and extended training in facilitating concurrent reach adaptation to opposing visuomotor rotations

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When reaching towards objects, the human central nervous system (CNS) can actively compensate and adapt to two different perturbations simultaneously, though this does not simply occur upon presentation. In fact, the CNS requires distinctive contextual cues to differentiate between adaptive states. Furthermore, not all contextual cues are effective in facilitating dual adaptation. Here, we investigated the efficacy of contextual cues which are intrinsic to the CNS including hand and body posture, and extended training in adapting to two opposing visuomotor rotations concurrently. Using a virtual reality paradigm, participants manipulated a projected hand-cursor using a digitizing tablet. A 30° CW and CCW rotation was associated with 2 distinct hand postures respectively in the first experiment and 2 distinct body postures respectively in the second experiment. Also, because the learning rate in dual adaptation is not as steep as that of single adaptation, we implemented an extended training set in the first experiment to examine the effect of greater practice. We found that how people held the tool or oriented their body while reaching is sufficient for recalling an adaptive state such that over time, reach errors decrease despite being presented both perturbations in a randomized, concurrent manner. Extended practice did not provide additional benefits suggesting that dual adaptation training reaches a saturation point. Our results suggest that intrinsic cues which produce distinct muscle synergies are effective at facilitating dual adaptation.

3-D-110 Analgesic effect of NMP-7, a novel mixed T-type calcium channel/cannabinoid receptor ligand

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T-type calcium channels and cannabinoid receptors are known to play important roles in chronic pain, making them attractive therapeutic

targets. We recently reported on the design, synthesis and analgesic properties of a novel T-type channel inhibitor (NMP-7) that also shows mixed agonist activity on CB1 and CB2 receptors in vitro. Here, we analyzed the analgesic effect of systemically delivered NMP-7 (i.p. or i.g. routes) on mechanical hypersensitivity induced by Complete Freund's Adjuvant (CFA). NMP-7 produced dose-dependent inhibition of mechanical hyperalgesia of mice treated either by i.p. or i.g. routes in the CFA model without altering spontaneous locomotor activity in the open-field test at the active dose. Neither i.p. or i.g. treatment reduced peripheral inflammation per se, as evaluated by examining paw edema and myeloperoxidase activity. The analgesia produced by NMP-7 in the CFA test was abolished in CaV3.2 null mice, confirming CaV3.2 as a key target. The analgesic action of intraperitoneally delivered NMP-7 was not affected by treatment of mice with CB1 antagonist AM-281, suggesting CB1 receptors are not involved in NMP-7 mechanism of action in vivo even though this compound acts on these receptors in vitro. Overall, our work shows that NMP-7 mediates a significant analgesic effect in a model of persistent inflammatory pain. This effect is dependent on T-type channels, but not CB1 receptors. Our approach provides a novel therapeutic approach for chronic inflammatory pain treatment via a novel class of compounds.

3-D-111 Frequency Response Of Correlated Motion Discrimination In Humans

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Humans effortlessly perceive visual inputs that move together in a correlated fashion. Previous work has shown that there are limits over which these temporal correlations cannot be perceived. For example, the temporal frequency limit of visual motion correlation is ~10 Hz (Maruya, et. al 2013, Holcombe 2009). Our goal was to describe more than just the temporal limit, but to measure the full frequency response of human motion correlation perception. Subjects (N=9) were shown two non-overlapping patches of randomly moving dots. The correlation between these patches was varied from 100% correlated to 100% anti-correlated. With gaze fixed on a central point, subjects viewed a 500 ms presentation of the stimulus and then reported if the motion was correlated

or anti-correlated. Stochastic stimulus motion patterns allowed the application of signal processing techniques to elucidate the frequencies linked to the behavioural choice using second order interactions of the stimulus patterns. Results show that there was a steady reduction in the correlation between stimulus frequency and behavioural report, with the limit at ~10 Hz. Additionally, the initial portion of the stimulus was more correlated with perception than the latter portion. A model, which low passed filtered the motion pulses before computing the correlation of the motion time series, emulated both the psychometric and frequency characteristics of our human subjects. Our results suggest a steady roll-off with lower frequencies contributing more to the perception of temporal correlation than higher frequencies.

3-D-112 Differential encoding of self-generated and externally produced head tilt by the vestibular and fastigial nuclei

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The ability to distinguish sensory inputs that are a consequence of our own actions from those that result from changes in the external world is essential for perceptual stability and accurate motor control. We have previously shown that neurons at the first central stage of the vestibular processing and in the cerebellum robustly encode passively applied head rotation/translation in the horizontal plane while their responses are attenuated during comparable self-generated head motion. However, natural head movements are not restricted to one plane and create more complex vestibular stimuli because of the presence of the gravity. Therefore, we hypothesized that the brain uses an internal model that includes gravity to distinguish between self- versus externally- generated head motion. Here we tested this proposal by performing single unit recording experiments in alert macaques during passive and active 1) head-on-body tilts and 2) head-on-body translations. Interestingly, responses related to actively-generated tilts were significantly attenuated relative to passively applied tilts. Moreover, this attenuation was comparable to that observed for active versus passive head translations (67 vs 74%, $p > 0.05$). Thus, our findings show that the neuronal coding of natural self-motion comprises an elegant computation of an internal model of active head motion that accounts for gravity. Taken together our results have

important implications for understanding the brain's ability to ensure accurate postural and motor control, as well as perceptual stability, during active self-motion.

3-D-113 Neural substrates for allocentric-to-egocentric conversion of remembered target location for reach

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Allocentric cues can be used to encode target location in visuo-spatial memory and the allocentric representation is converted into egocentric representation at the first possible opportunity for reach (Chen et al., 2011). However, neural substrates for allocentric-to-egocentric conversion have not been explored yet. We used fMRI to investigate brain areas involved in this conversion for memory-guided reach. Ten participants reached toward a remembered target location represented in allocentric coordinates. Participants fixated a central point while a target was presented along with an allocentric cue. This was followed by a delay phase (6s), after which an auditory instruction ("Same cue" or "Different cue") instructed participants that the allocentric cue would re-appear at the same location, allowing for a conversion of target location from allocentric to egocentric, or at a different location, requiring participants to wait for the re-appearance of the cue for reach. A second delay phase (10s) followed the auditory instruction. Next, the allocentric cue re-appeared and was followed by reaching toward the remembered target relative to the location of the re-displayed allocentric cue. We found that during the second delay the "Same cue" condition elicited higher activation as compared to the "Different cue" condition in bilateral precuneus, left angular gyrus and bilateral inferior frontal gyrus. Our results suggest that posterior parietal cortex and frontal areas play a critical role in allocentric-to-egocentric conversion of target representation for reach planning.

3-D-114 Multi-Sensory Integration following Mechanical Perturbations

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An important challenge for the brain is to combine information from distinct sensory

modalities into a single percept. Previous work emphasizes that the nervous system integrates different sensory signals by weighting them according to their reliability, such that the resulting estimate is more reliable than each source taken independently. Although this model captures many features of decision-making and motor planning in static conditions, it remains unclear how multi-sensory integration is performed in real-time given differences in sensorimotor delays associated with distinct sensory modalities. We address this problem by asking participants to track their fingertips while mechanical loads were applied on their arm. We also used visual perturbations in which the cursor followed a trajectory similar to their hand motion, without any load applied on their limb. We used the first saccade following the perturbation as a proxy of participants' estimation of their fingertip motion. We show that saccade latencies are substantially faster following mechanical perturbation than following visual perturbations. In addition, tracking performances following mechanical perturbations were similar regardless of whether the fingertip was lit or not. These results indicate that state estimation following perturbations is rapidly performed based on limb afferent feedback. We suggest that multi-sensory integration during online movement control may be more heavily driven by proprioception, which, although less accurate, presents the advantage to be affected by shorter sensorimotor delays.

3-D-115 Vestibular neuronal ensemble coding during self-motion

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Neurons in the vestibular nuclei that receive direct afferent input mediate postural reflexes and contribute to the perception and computation of motion. Behavioral studies have shown, however, that single neurons' motion detection thresholds are not as good as those observed for perception. Thus, we have hypothesized that pooling the activity of many neurons better matches the output of the vestibular nuclei with behavior. To accurately model this ensemble output, it is important first to determine whether the responses of individual neurons are independent. Accordingly, we recorded from pairs of semicircular canal or otolith afferents and pairs of neurons in the vestibular nuclei 1) in the absence of stimulation (resting rate) and 2) during self-motion (driven).

We then computed noise correlations between each pair's activities for the two conditions. We found that the magnitude of resting rate noise correlations for all pairs was not significant. This revealed that in the absence of stimulation, afferent inputs did not synchronize neurons in the vestibular nuclei. We then discovered that pairs of afferents exhibited increased synchrony during motion. Their noise correlations, however, were not significantly different between the resting and driven conditions. The same was true for the vestibular nuclei. Thus, while common afferent input increases synchrony between vestibular nuclei neurons during motion, their outputs remain largely independent. These results provide evidence that the brain pools signals from many neurons to achieve accurate computation of self-motion.

3-D-116 The characterization of Calretinin expressing V3 interneurons in the mouse spinal cord

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V3 interneurons (INs) in the spinal cord are a major group of excitatory commissural INs that are essential in establishing a robust and balanced locomotor rhythm during walking. During development V3 INs, as marked by the expression of the transcription factor Sim1, exit from the most ventral progenitor domain, p3, at embryonic day (E) 9.5 in the mouse spinal cord. In order to better understand the embryonic development of V3 INs, the expression of calretinin (CR) in V3 INs has been investigated. CR is a calcium binding protein that has been implicated as a neuronal sub-population marker in specific areas of the developing nervous system. V3 INs were identified by their expression of tdTomato fluorescent protein in Sim1Cre/+; Rosafoxstop26TdTom mice. Approximately 20-30% of V3 INs begin to express CR at E14.5 in the lumbar and sacral spinal cord. Of these cells two subpopulations of CR expressing V3 INs are identified. The first is a group of ventral and intermediate V3 INs that transiently express CR from E14.5 to P0. The second is a cluster of V3 INs forming a subpopulation at the intermediate region of the L6-S2 spinal cord. Their expression of CR lasts into adulthood. We further studied the effect of sim1 on CR expressing V3 INs during development. We found that in Sim1 null mutant mice, CR positive V3 INs significantly decreased in the transient-expressing group, although the total number of V3 INs didn't change. Sim1 did

not affect the V3 subgroup that persistently expressed CR. Further investigation of these cells function in spinal neural networks is needed.

3-D-117 Topographic analysis of cortical connections of the primary motor cortex (M1) in a New World monkey (*Cebus apella*).

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Premotor areas have extensive cortical connections with the primary motor cortex (M1). To date, the precise topographic location of these connections within M1 has only been studied for the ventral premotor cortex (PMv). PMv is specifically interconnected with the hand and proximal representations in the rostralateral portion of M1. These data suggest that premotor areas are not uniformly interconnected with M1 but instead are preferentially connected with specific subregions in M1. In the present study, we examined the topographic specificity of M1's connections with PMv as well as the dorsal premotor cortex (PMd), supplementary motor area (SMA) and primary somatosensory cortex (S1). Intracortical microstimulations and multiunit recordings techniques were used to define the forearm and hand area in M1, PMd, PMv, SMA and S1. Neuronal tracers were injected within the hand representation of PMd, PMv, SMA and S1. Following incubation and perfusion, the cortex was flattened and cut tangentially. The distribution of labeled cell bodies in the ipsilateral hemisphere was reconstructed and coregistered with the electrophysiological data. We found that PMd has more connections with the rostromedial part of M1; SMA has connections distributed throughout the medial part of M1; and S1 is more intensely interconnected with the caudal part of M1. Our results indicate that subregions in M1 have different patterns of cortical connections. This anatomical specificity may allow areas within M1 to play different roles for the control of hand movements.

3-D-118 Role of Mechanosensitive Channels in Osteoarthritis Pain

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Osteoarthritis (OA) is a chronic debilitating disease that affects millions of Canadian and US

citizens. Though there are many well characterized symptoms of OA, the pain associated with its progression is poorly understood. The pain usually manifests as hypersensitivity to mechanical stimuli (i.e. joint palpation, movement) and is linked to dysfunction in pain-sensing neurons innervating the joint (nociceptors). This dysfunction can be the result of modulation of mechanosensitive ion channels (MSCs) and/or voltage-gated ion channels, responsible for the transduction and transmission of peripheral stimuli, respectively. Importantly, direct evidence for sensitization of the transduction process has not yet been reported. We hypothesize that an important contributor to the mechanical hypersensitivity in OA pain is sensitization of MSCs found in nociceptors. Using the monoiodoacetate (MIA) mouse model of knee OA pain, we performed single channel electrophysiological recordings from MSCs in knee-innervating nociceptors from naïve and OA mice. Our preliminary data shows that in OA nociceptors, the mechanical activation threshold of MSCs is reduced, and the current generated by these channels tends to be greater than those from the contralateral knee or mice injected with control solution. Our data indicates that MSCs sensitization may underlie mechanical hypersensitivity in OA patients. We intend to explore the functional relevance of this sensitization and whether blockade of MSCs will attenuate mechanical hypersensitivity.

3-D-119 Repetitive Transcranial Magnetic Stimulation Disrupts Prediction of Spatial Location of the Limb

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Limb movement prediction has been previously hypothesized to allow differentiation between self-induced sensory information arising from limb from external somatic information. This process of forward modeling has been used to explain many sensory cancellation processes in the body, however the cortical regions responsible for this process in human limb movements are still unclear. In our current experiment, we applied repetitive transcranial magnetic stimulation (rTMS) to 3 different cortical locations (posterior parietal cortex (PPC), dorsal premotor cortex (dPMC), and visual area V4) while subjects performed a vibrotactile temporal order judgement task (TOJ) under moving or stationary conditions. Under moving conditions, participants were instructed to cross their arms as soon as they heard a

tone. Previous work has shown that under stationary conditions, error rates increase when participants have their arms crossed or are about to cross his or her arms. Under stationary conditions, we observed an increase in TOJ error after rTMS was applied to the PPC, but not when it was applied to the dPMC compared to pretesting or to control. However under moving conditions, after rTMS to the dPMC, error rates were decreased relative to pre-testing. This trend was not observed after area V4 was stimulated, and TOJs increased slightly after PPC stimulation. This pattern of results suggests that the brain generates predictions about spatial state of the limb using dorsal premotor cortex in concert with spatial information from the parietal cortex.

3-D-120 The role of cannabinoid receptors in monkey retina

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The expression patterns of cannabinoid receptors CB1 and CB2, and GPR55 are well described in the vervet monkey retina. Yet, there are no reports on the exact role of these receptors in retinal function. Therefore, in order to study the neural correlates that accompany the disruption of endogenous endocannabinoid signaling in the retina, we recorded the neural activity by electroretinographic (ERG) recordings. We evaluated ERG changes after administration of specific antagonists or agonist of these receptors. Photopic or scotopic ERGs were recorded in 15 normal adult vervet monkeys before and shortly after intravitreal injections of CB1 antagonist (AM251), CB2 antagonist (AM630) and GPR55 agonist (LPI). Pre- and post-injection ERG waves were compared. The intravitreal injection of AM251 and AM630 resulted in a significant increase of the b-wave component of the ERG in photopic conditions. Since GPR55 is exclusively expressed in rod photoreceptors, we evaluated the effects of the natural agonist (LPI) of GPR55 on the scotopic ERG. Twenty minutes following the LPI injection, the scotopic b-wave was increased in amplitude in all treated eyes. There was no statistical difference in the latency of the ERG waves and the intraocular pressure before and after injections. These results indicate that CB1 and CB2 receptors in primates are involved in retinal function under photopic conditions, whereas GPR55 is involved in scotopic vision.

3-D-121 Orientation Plasticity in Mouse Primary Visual Cortex

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Information processing in the visual system is shaped by recent stimulus history, such that prolonged viewing of an adaptor can alter the perception of subsequently presented stimuli and modify the visual response properties of neurons in multiple brain areas. In the tilt-aftereffect, the perceived orientation of a grating is often repelled away from the orientation of a previously viewed adaptor grating. A potential neural correlate for this tilt-aftereffect has been described in cat and macaque primary visual cortex (V1), where adaptation produces repulsive shifts in the orientation tuning curves of V1 neurons. In these animals, columns of V1 neurons are clustered into iso-orientation domains and pinwheel centers, and there is evidence that orientation adaptation is more prevalent near pinwheel centers. We investigated orientation adaptation in mouse V1 with the aim of establishing a genetically tractable model to study this form of plasticity. We were also curious about how orientation adaptation might differ in a species known to lack pinwheel columnar organization and have a lower proportion of orientation tuned neurons. We used stimulus protocols that were readily comparable with previous studies, and found that orientation adaptation caused repulsive shifts in the majority of orientation tuned neurons, and a general decrease in firing in neurons that were not selective for orientation.

3-D-122 C-fos study of vestibular activity in neonatal opossums, *Monodelphis domestica*, a marsupial model for sensorimotor development

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The opossum is born very immature. It nonetheless crawls, unaided, from the mother's birth canal to a nipple where it attaches to pursue its development. Of the senses proposed to guide the newborn to a nipple and trigger its attachment, audition, vision and olfaction are insufficiently developed, but the vestibular and trigeminal systems are good candidates. Anatomical studies in newborn opossums have shown that the utricle is the least immature

component of the labyrinth, it is innervated by vestibular afferents projecting to vestibular nuclei, and the latter to the cervical spinal cord. It has also been shown that low intensity electrical stimulations of the vestibulospinal area induce forelimb movements in in vitro preparations. To further test the functionality of the vestibular system, conscious neonatal opossums were subjected to sinusoidal acceleration along the three planes for 60 minutes. Control animals were left in the same condition but without stimulation. The animals were then anesthetized by hypothermia, decapitated and the heads fixed by immersion in paraformaldehyde to be sectioned and processed immunochemically to reveal c-fos. This protein is used as a marker of activity as it is expressed following stimulation in neurons. We did not observe c-fos labeling in the vestibular ganglion and nuclei before P15. These results do not support an influence of the vestibular system in the locomotion of newborn opossums. Instead, the vestibular system shows activity before the opossum starts to detach from the nipple, around 21 days after birth.

3-D-123 Comparison of the reference frames for encoding translational self-motion in the vestibular and rostral fastigial nuclei

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The vestibular sensors provide self-motion information in a head-centered reference frame. Thus, to contribute appropriately to estimates of body or spatial motion, vestibular signals must be transformed from a head- to a body- or world-centered reference frame. Previous studies provided evidence for such a transformation toward body-centered coordinates in the rostral fastigial nucleus (rFN) by showing shifts in the spatial tuning properties of rFN cells when the head was statically repositioned relative to the body in the horizontal plane. In contrast, rostral vestibular nucleus (VN) neurons remained head-centered. However, because these studies were limited to the horizontal plane they could neither confirm a full three-dimensional (3D) transformation towards body-centered coordinates in the rFN, nor examine the evidence for a gravity-dependent transformation towards world-centered coordinates in the VN. To address these questions we characterized the 3D spatial tuning of rFN and VN neurons in two rhesus monkeys during sinusoidal translational motion

(0.5 Hz, ± 9 cm, ± 0.09 G) with the head upright, after vertical plane head reorientation (in pitch/roll) and, whenever possible, after horizontal plane reorientation. Tuning shifts were observed in the rFN after both vertical plane and horizontal plane head reorientations, consistent with the requirements for a distributed 3D transformation towards body-centered coordinates. In contrast, rostral VN cells showed no evidence for such shifts, consistent with an encoding of vestibular signals in head-centered coordinates.

3-D-124 Changes in stimulus envelope reveal two classes of peripheral electrosensory neurons

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Natural sensory stimuli are characterized by time varying moments such as mean (first-order) and variance (second-order). While psychophysical studies have shown that second order attributes (the envelope) are critical for perception, how they are encoded in the brain remains largely unknown. Here we focused on envelope coding by peripheral electrosensory neurons (P-type afferents) in the weakly electric fish, *Apteronotus leptorhynchus*, using narrowband noise stimuli that were modulated by sinusoids in the frequency range between 0.05 and 10Hz. Envelopes are an essential feature of natural electrosensory stimuli. When two fish come within close proximity of one another, each animal will experience an amplitude modulation of its own electric signal that oscillates at the difference between the individual EOD frequencies. The envelope varies in time as the distance between two fish changes, and recent studies have shown that it primarily contains low temporal frequencies (< 1 Hz). While primary afferents always increase their firing rates in response to an increase in EOD amplitude, we show that they can either increase or decrease their firing rates in response to an increase in the envelope provided that this increase is greater than their threshold. However, the gain and phase of afferent responses are independent of envelope frequency. Therefore, this study gives important insights as to how neural heterogeneities can influence responses to envelopes and might provide an answer as to why primary afferent baseline firing rates vary over such a large range.

3-D-125 Contribution of presynaptic inhibition to balance control reactions in healthy subjects

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Background: During unexpected perturbations, sensory inputs are integrated in the central nervous system to generate appropriate postural responses. Presynaptic inhibition is thought to play an important role in modulating such sensory transmission. Here, we assessed the modulation of presynaptic inhibition during postural perturbations. Methods: Subjects (n=9, 27±7 yrs) stood upon a platform that was randomly tilted forward/backward. 1) Modulation of SOL H reflex was assessed by stimulating the tibial nerve at the popliteal fossa at different delays prior and during the perturbations. 2) To estimate presynaptic inhibition, we quantified: A) the facilitation of SOL H-reflex induced by femoral nerve (FN) and B) the depression of SOL H-reflex (D2 inhibition) by common peroneal nerve (CPN). Results: 1) During backward tilt, decreased H-reflex amplitude was observed from 75ms after tilt onset, which preceded SOL EMG decrease (149 ±20 ms). During forward tilt, facilitation was first observed at 100ms and preceded SOL EMG facilitation (162±18 ms). 2) During standing, SOL H-reflex depression by CPN was 81±9% of the test reflex value, and the SOL H reflex facilitation by FN was 105±8%. During forward tilt, H-reflex depression from CPN is decreased (103±6%) and H-reflex facilitation from FN is increased (118±8%), suggesting a decrease in presynaptic inhibition. Similar changes were observed during backward tilt at 75 ms (120±16% for CPN and 196±37% for FEM). Conclusions: Preliminary results suggest that presynaptic inhibition is modulated during balance control reactions.

3-D-126 Characterization of central vestibular neuron activity during electrical stimulation delivered by a vestibular prosthesis

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Ongoing research is focused on developing a vestibular prosthesis as a treatment option for these patients suffering from bilateral vestibular deficiency. This device consists of a gyroscope, which senses the movement of the head, and electrode arrays implanted in each semicircular

canal, which stimulate the vestibular nerve to send head motion information to the brainstem. Behavioral studies have shown that vestibular prosthetic stimulation produces vestibulo-ocular reflex eye movements although these responses remain smaller than what would be expected from normal vestibular function. To optimize stimulation parameters and improve behavioral performance it is imperative to discover how the brain responds to this type of stimulation. Thus, to better understand the effect of prosthetic stimulation protocols on vestibular processing, we recorded the responses of neurons in the vestibular nuclei receiving direct input from the vestibular nerve in alert rhesus monkeys while delivering prosthetic stimulation. We found that each neuron's spiking activity was time locked to pulses delivered through the prosthesis suggesting that the vestibular afferent population was synchronously activated by prosthetic stimulation, which in turn drove synchronous central responses. We hypothesize that this synchronicity is in part the cause for the low behavioral performance obtained using the vestibular prosthesis thus far, and predict that desynchronizing afferent inputs will enhance the response of central vestibular neurons to prosthetic stimulation thereby improving behavioral performance.

3-D-127 Generalization of Reach Adaptation and Proprioceptive Recalibration to Different Distances of the Workspace

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Studies have shown that adapting one's reaches in one location in the workspace generalizes to other novel locations. Generalization of this visuomotor adaptation is influenced by the location of novel targets relative to the trained location. Reaches made to novel targets that are located far from the trained target (i.e. ~22.5°; Krakauer et al. 2000) show very little generalization compared to those closer to the trained direction. However, the generalization is much broader when reaching to novel targets in the same direction but at different distance from the trained target. Reach training with a misaligned cursor not only leads to changes in movements, but also to changes in hand proprioception (i.e., proprioceptive recalibration (Henriques & Cressman, 2012). In this study, we investigated whether proprioceptive recalibration, like motor adaptation, generalizes

to different distances of the work space. Subjects adapted their reaches with a rotated cursor to two-target locations. We then compared changes in open-loop reaches and felt hand position to novel targets located in the same direction as the trained targets but either at a closer or farther distance, with those to the trained targets. We found motor changes generalized to novel, closer and farther targets to the same extent as they did to the trained distance. In contrast, the changes in hand proprioception was significantly smaller for far-novel compared to trained location. From these and earlier results from our lab, we can infer that proprioceptive recalibration may arise independently of motor changes.

3-D-128 Activation pattern of the primary visual cortex elicited by electrical stimulation of the prefrontal cortex in mice

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The prefrontal cortex (PFC) is involved in visual attention which results in enhancement of the neurons response to the attended stimulus in the primary visual cortex (V1). The objective of this study was to determine the pattern of V1 activation following electrical stimulation of different subregions of the PFC. The contribution to V1 activation of the basal forebrain (BF) cholinergic neurons, which are also involved in visual attention, was tested, to evaluate whether they could be a relay in the PFC-V1 interaction. Three PFC subregions stimulation sites were selected; the anterior cingulate (Cg1), the prelimbic (PrL) and the infralimbic (IL) cortices. Electrical stimulation (100 Hz bursts for 0.3 s every 2 seconds at 50 μ A) was performed during 15 or 30 mins to quantify neuronal activity in V1 and BF by thallium autometallography (staining of potassium reuptake) or c-Fos immunoreactivity, respectively. Neuronal activation of layers II/III and V following PrL and IL stimulation was significantly increased in the stimulated hemisphere compared to the non-stimulated one as shown by c-Fos immunoreactive neurons ($p < 0.01$) or thallium optical density values ($p < 0.05$). Cg1 did not elicit neuronal activation in V1. BF was not activated by any of the stimulated PFC sites as shown by both techniques. Moreover, lesions of BF cholinergic neurons using mu-p75 saporin did not eliminate the induced c-Fos expression in V1. This suggests a functional link between the PFC and

V1 but this function is not supported by HDB cholinergic neurons.

3-D-129 Visual remapping is more impaired in patients with unilateral parietal lesion than in hemidecorticate patients as revealed by novel version of the double step task

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Studies of remapping abilities in human patients with distinct cortical lesions are inconclusive. Patients with parietal lobe lesions, primarily of the right side, tested on the classical double-step task have a particular deficit in generating an ipsilesional saccade if it follows a contralesional saccade (Duhamel et al, 1992 & Heide et al, 1995). This deficit has been explained as an inability to generate/ interpret corollary discharge for saccades elicited by the lesioned hemisphere. Recent studies, however, have called this finding into question. A review has re-interpreted the data from these earlier publications, suggesting that these results are actually evidence of right-hemisphere dominance in human visual remapping (Pisella et al, 2011). Several studies of patients with right parietal lesions have determined that ipsilesional but not contralesional eye movements can result in a deficiency in remembering spatial information from previous fixations (Vuilleumier et al, 2007 & Russel et al, 2010). We tested hemidecorticate subjects on a novel version of the double-step task, adapted because our patients are hemianopic. We found that they do not have any impairment in remapping in either direction. We have tested a right parietal patient with this novel double-step task and found that he is unable to generate a contralesional saccade when it follows an ipsilesional saccade, in opposition to the findings of Duhamel et al, 1992. We are in the process of testing more patients with our novel paradigm to provide further insight into the saccadic remapping system in humans.

3-D-130 The effects of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) inhibition on motor map expression in rats

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Motor maps are the topographical representation of movements in the motor cortex and are derived by stimulating pools of layer 5 pyramidal neurons that give rise to the cortical spinal tract. Previously it has been shown that motor map expression can be altered by the quantity and type of neurotransmitters present in the neocortex. The balance between cortical excitation and inhibition determines motor map expression. Endocannabinoids are retrograde neurotransmitters that regulate neurotransmitter release at many different types of synapses and may therefore modulate cortical excitability. Endocannabinoids and the cannabinoid (CB) receptors are found in layer 5 of the neocortex, the source of the cortical spinal tract. Previously we examined the role of CB1 receptors on the expression of cortical forelimb motor maps. It was discovered that activation of CB1 receptors in the motor cortex causes a reduction in forelimb map size. We now determined which endocannabinoid (anandamide or 2-arachidonoyl glycerol) is responsible for this effect. We made the following 2 predictions: 1) inhibition of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) will increase movement thresholds and decrease map size 2) inhibition of MAGL will have a greater effect than inhibition of FAAH. Adult rats underwent high-resolution ICMS to map forelimb (digit, wrist, elbow, shoulder) movement representation areas. Once the initial map was derived a FAAH or MAGL inhibitor was placed on the cortex and 15 minutes later all forelimb responsive sites were re-visited.

3-D-131 Modelling Altered Adaptive Processes for Motor Learning in Aging

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Motor learning is often studied by examining how people adapt their motor output when moving grasped objects with novel dynamics. Such adaptation involves a fast process that adapts and decays quickly and a slower process that adapts and decays more gradually. The fast process is thought to involve declarative memory whereas the slow process is linked to procedural memory. Importantly, each component is characterized by a learning rate that specifies the rate at which motor output is updated based on previous errors, and a retention factor determining the rate of trial-to-trial memory decay. Declarative memory processes decline with age suggesting that the

fast process could be particularly affected in later adulthood. However, aging is also associated with increased rates of forgetting that could translate to changes in retention for either the fast or slow process. To investigate changes in these parameters in aging, we compared the performance of healthy younger ($M = 23$ years) and older ($M = 70$ years) adults when adapting their reaching movements to novel loads, or force fields, applied by a grasped handle. We found that retention of the slow process was most altered in the older adults. However, for older adults who performed poorly on an independent explicit memory task the retention of the fast process was also diminished. These findings demonstrate that aging results in impaired retention of procedural aspects of adapted motor responses, and that declines in the retention of the fast, declarative component are limited to older adults with poor explicit memory.

3-D-132 Stereological analysis of spinal cord neurons involved in primate forelimb motor control

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Neurons within the spinal cord grey matter of non-human primates were quantified to provide a more robust anatomical framework for modelling upper limb motor control. Transverse sections from spinal cords of three rhesus monkeys (*Macaca mulatta*) were examined from segments C5 to T1. Three areas of interest were identified within each section using laminar boundaries in order to compare regions with varying populations of motor neurons and interneurons. The first of the areas contained lamina IX while the remaining two areas contained groups of laminae (V-VI and VII-VIII). Stereological methods were used to estimate neuron density and the total number of neurons in these specific areas of the grey matter. Segments C3 and C4 were also examined using the same criteria in one primate in order to further study the changing populations between segments. Neuron density (neurons per mm^3) from C5-T1 was determined to be 6610 ± 80 (IX), 11770 ± 50 (VII-VIII) and 28030 ± 180 (V-VI) while total number of neurons was calculated to be 36800 ± 800 (IX), 84400 ± 1700 (VII-VIII) and 88800 ± 500 (V-VI). The increase in both density as well as overall number of neurons in laminae V through VIII suggests that a high degree of motor processing can occur in numerous interneurons before activation of a

comparatively smaller population of neurons within lamina IX. These results provide a numerical baseline which can be used to further understand and model the processing that takes place within a system of neurons in order to generate a motor command.

E - Homeostatic and Neuroendocrine Systems

3-E-133 Effects of the cannabinoid receptor 1 antagonist, AM251, on vGluT2 and CRH expression, neuronal injury and anxiety following global cerebral ischemia

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Endocannabinoids can play a modulatory role in emotional brain circuitry and neuroendocrine stress activation. In the current study, we assessed the neuroprotective actions of type 1 cannabinoid receptor (CB1) antagonist, AM251, on neuronal injury, dopaminergic transmission changes, excitotoxicity and stress response, and behavioral deficits post ischemia. 4 groups of male Wistar rats (n=10 per group) were pretreated with AM251 (2mg/kg, i.p.) or saline 30 minutes prior to sham or global cerebral ischemia. After 5 days of recovery, animals were exposed to the open field and the elevated plus maze tests. Rats were killed 7 days post ischemia and immunohistochemical detection was performed. Our findings support a partial or full reversal by AM251 of ischemia-induced reduction in dopamine transmission (tyrosine hydroxylase and dopamine D1 receptor expression) in mesolimbic brain regions including the ventral tegmental area and basolateral amygdala (BLA). Interestingly, AM251 significantly reduced vesicular glutamate transporter 2 expressions in the BLA and paraventricular nucleus, independent of the ischemic/sham condition. Compared to all sham groups, elevated corticotropin-releasing hormone (CRH) expression in the ischemic condition was reduced by AM251. Finally, AM251 reduced CA1 neuronal injury, and prevented ischemia-induced changes in anxiety. AM251 pretreatment prior to global cerebral ischemia has beneficial effects on neuronal survival and behavior, alleviates reduced dopaminergic transmission, and regulates CRH expression in brain areas related to anxiety and stress.

3-E-134 A novel peptide-based mechanism for the regulation of glucose homeostasis in hypothalamic neurons

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The regulation of glucose homeostasis is achieved primarily in the hypothalamic arcuate nucleus. While several peptides such as insulin, GLP-1, etc... are described to be involved in this function, they are not evolutionarily conserved. Recently, the Teneurin C-terminal Associated Peptide family consisting of TCAP-1 to -4 have been discovered to be ubiquitously expressed in metazoans. TCAP-1 administration in vitro results in increased ATP production, decreased lactate accumulation and increased glucose transporter relocation to the plasma membrane of hypothalamic neurons. These findings indicate that the primary role of TCAP may be to regulate metabolic optimization in the brain by increasing the efficiency of glucose transport and energy utilization. I hypothesize that TCAP-1 plays a significant role in regulating energy metabolism of the organism. Preliminary results in vivo indicate that TCAP-1 results in a 15-20% decrease in plasma glucose levels in rats one week after administration. TCAP-1 also induced 3H-deoxyglucose transport into hypothalamic neurons via an insulin-independent manner. We used a previously deduced pathway by which TCAP-1 signals in vitro to establish a link between the MEK-ERK1/2 pathway and glucose uptake as well as a connection between the MEK-ERK1/2 and AMPK pathways. We speculate that the role of TCAP-1 in regulating glucose metabolism could be useful in neuroprotection. TCAP-1 may represent an ancient and conserved mechanism for glucose homeostasis in animals.

3-E-135 Loss of STAT-3 Signaling in Dopamine Neurons Enhances Locomotor Activity and Running Reward

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Leptin modulates neural circuits regulating appetite, locomotion, reward and emotion through the long-form of its receptor (LepRb) via signaling pathways including Jak-Stat3, -PI3K and -Erk. Signal Transducer and Activator of

Transcription 3 (Stat3) is the predominant signal whereby leptin regulates gene expression and LepRb-Stat3 signaling plays an important role in the control of food intake and body weight. Dopaminergic (DA) neurons of the midbrain are part of a neural circuit involved in reward and motivation. To determine the role of Stat3 in this circuitry, we inactivated this signaling molecule in DA neurons of mice (DA-specific Stat3 KO mice). Lack of Stat3 in DA neurons resulted in no changes in feeding, increased locomotion, running-wheel exercise and running reward. Moreover, KO mice exhibited impairments in learning an appetitive operant conditioning task accompanied by reduced DA biosynthesis and DA receptor protein expression in the striatum. Taken together, these results suggest that Stat3 mediates the action of leptin in the midbrain to decrease locomotion and exercise and to increase DA availability, but not to decrease feeding behavior, implying that different LepRb signaling molecules regulate distinct behavioral and biochemical actions of midbrain leptin. Additionally, Stat3 signaling in DA neurons modulates the rewarding aftereffects of running and leptin facilitation of learning. Supported by CIHR and doctoral awards to MFF from Université de Montréal and Canadian Diabetes Association (CDA).

3-E-136 Na⁺ channel expression in rat subfornical organ is regulated by fasting and AMP kinase

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The subfornical organ (SFO) is a sensory circumventricular organ that plays a key role in detection of circulating satiety signals and regulation of energy balance. Previous work using Affymetrix microarrays revealed patterns of gene expression in rat SFO and identified a twofold change in over 600 transcripts after a 48h fast. Included in the expressed and downregulated transcripts was the Nav1.3 voltage-gated Na⁺ channel, a major contributor to electrical excitability of SFO neurons. Quantitative PCR confirmed that following a 48h fast, Nav1.3 levels in SFO were reduced by 54%, compared to sated controls, a result which was paralleled by application of the AMPK activator AICAR to SFO explants. Immunohistochemistry revealed the pattern of expression of Nav1.3 within the SFO. In order to investigate changes in electrical properties associated with the reduction of Nav1.3

transcript, we carried out patch clamp experiments on acutely dissociated SFO neurons from 48h fasted rats and sated controls and quantified action potential and Na⁺ channel properties. After 48h fast, the threshold, height and duration of action potentials showed a significant change. We also observed a concomitant decrease in Na⁺ current density and a depolarized shift in voltage dependence of Na⁺ current activation and inactivation. The 48h fast-induced change in excitability was consistent with altered Na⁺ currents. These data demonstrate food restriction alters Nav1.3 expression level and also electrical properties of SFO neurons, reflecting the notion that SFO is a dynamic sensor for energy balance.

3-E-137 Developmental responses of the lateral hypothalamus to leptin and ghrelin in rat pups.

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Food intake is regulated by a close communication between the homeostatic system in the hypothalamus and the hedonic system (including the mesocorticolimbic pathway). A key region for this communication is the lateral hypothalamus (LH), also known as the "feeding center" of the brain. Both systems are still developing during the late fetal and early post-natal periods in the rat, and they are known targets for peripheral metabolic hormones such as leptin and ghrelin. Here we examined the onset of leptin and ghrelin responsiveness in the LH, and the activation of cellular signaling molecules in identified neurons on postnatal day (PND) 10 and 16. Leptin significantly activated pERK and pSTAT3 in the LH on PND16, while on PND10, only sparse pSTAT3 cells were identified and the activation of pERK did not reach significance. Double-immunofluorescence staining showed that the majority of pERK-activated neurons are orexin-positive neurons. However, leptin-induced pSTAT3 was clearly observed in another population of neurons, possibly neurotensin-positive. The development of ghrelin responsiveness in the LH is currently being investigated using immunostaining for Fos activation. Our results suggest that the development of metabolic hormone responsiveness in the LH is only partially achieved prior to the onset of independent feeding on PND16, and that the maturation of functional connections between the LH and the

ventral tegmental area (VTA - a major center of the hedonic system) might play a crucial role in independent feeding initiation. Supported by CIHR to CDW.

3-E-138 Brainstem noradrenergic afferents excite hypothalamic neurons through glutamate co-release

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The paraventricular nucleus of the hypothalamus (PVN) integrates inputs from diverse stress-sensitive brain areas to regulate the hypothalamus-pituitary-adrenal axis. Tyrosine hydroxylase (TH)-expressing neurons in the caudal medulla densely innervate the PVN, release noradrenaline (NA), and increase the excitability of PVN neurons. Interestingly, subpopulations of caudal medulla TH neurons co-express vesicular glutamate transporter 2, raising a possibility that the excitatory effects of these inputs may also rely on glutamate transmission. To address this question, we used an optogenetic approach. Cre-dependent AAV vector carrying channelrhodopsin 2-enhanced yellow fluorescent protein (eYFP) was stereotactically injected into the caudal medulla of mice that express Cre under the control of TH promoter. We observed that eYFP axons innervating the PVN were TH immunopositive, verifying targeted expression. Using whole-cell voltage clamp recordings from PVN neurons, blue light illumination (473 nm, 5 ms) evoked inward postsynaptic currents (PSCs), with short latency (4.3 ± 0.2 ms) and large amplitude (-93 ± 11 pA). In current-clamp mode, the light stimulation evoked a rapid postsynaptic depolarization, which occasionally generated action potentials. The light-evoked PSCs were still evident in the presence of adrenergic receptor antagonists. They were also unaffected by a GABAA receptor antagonist but were completely abolished by ionotropic glutamate receptor blockade. These results are consistent with the hypothesis that NAergic terminals in the PVN co-release glutamate.

3-E-139 Acute intracerebroventricular administration of relaxin-3 induces sex-specific effects on food intake in rats

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¹CRIUCPQ

Relaxin-3 is a newly discovered neuropeptide which is notably known to produce an orexigenic effect in rats. Previous studies have shown that relaxin-3 intracerebroventricular (ICV) injection increases feeding in male rats but its effect in females remains unknown. Moreover, our previous study shows that chronic stress and food restriction sex-specifically regulates relaxin-3 expression in rats and this neuropeptide could thus have a different effect in female versus male rats. The aim of the present study is to investigate relaxin-3 effects on food intake after its ICV injection at different doses in male and female rats. Twenty male and female Sprague-Dawley rats received once a week, over three weeks, 50, 200 and 800 pmol of relaxin-3 or vehicle in within-subject counterbalanced design. Food intake (standard chow) was measured every 30 minutes during two hours after injection. Results show that post injection food intake significantly increased by low (200 pmol) and high (800 pmol) doses of relaxin-3 in female and male rats, respectively. Moreover, this feeding response appeared earlier in female (30 minutes post injection) than in male rats (60 minutes post injection) and gradually increased during two hours of post-injection in female rats while it reached a plateau in male rats after 90 minutes post injection. In conclusion, because females demonstrated earlier feeding response to a lower dose that persisted longer than in males, this study shows that acute ICV administration of relaxin-3 induces sex-specific effects on food intake in rats.

3-E-140 Repeated Maternal Separation and Fragmented Maternal Care Differentially Modulate Neonatal Neuroendocrine Activation in Response to a Novel Psychological Stressor

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Early-life stress exerts profound effects on the maturation of the neuroendocrine stress axis. However, the effects of early-life stress on the neuroendocrine response to novel psychological stressors during the neonatal stage are unknown. We sought to determine whether two commonly employed paradigms of early-life stress (daily 3-hr maternal separation [MS] or limited access to nesting/bedding material [LB]) would facilitate or dampen the neuroendocrine response to a novel psychological stressor. In the first study, pups exposed to repeated 3-hr

MS from postnatal day (PND) 2-9 were subjected to either a familiar stressor (60 min MS) or a novel stressor (60 min immobilization) on PND10 and corticosterone (CORT) and adrenocorticotropin hormone (ACTH) were measured either 0 or 60 min post-stress. Pups exposed to daily MS displayed enhanced ACTH and CORT secretion in response to immobilization (but not in response to MS) compared to non-stressed pups. No significant differences were observed with respect to basal ACTH and CORT secretion. Next, separate litters were given restricted access to nesting materials (which engenders fragmented maternal care) from PND2-9 and challenged on PND10 with a 60-min immobilization stressor. Preliminary analyses indicate that pups reared in LB conditions exhibited higher baseline CORT secretion and a lack of response to immobilization stress, contrary to what was observed in study 1. Thus, repeated MS and fragmented maternal care exert fundamentally different effects on neuroendocrine responses to a novel stressor during the neonatal period.

3-E-141 Central blockade of type 1 CRH receptors prior to transient forebrain ischemia attenuate delayed basal and stress-induced corticosterone secretion in male rats.

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¹University of Ottawa

We have previously demonstrated that transient global cerebral ischemia is associated with dysregulation of the hypothalamic-pituitary-adrenal axis (HPA) activity leading to important changes corticotropin-releasing hormone (CRH), glucocorticoid receptor and CRH type 1 receptor (CRH-R1) expression in distinct brain regions post ischemia. The current study aimed to examine time-dependent changes in basal and stress-induced corticosterone secretion following 10 min global cerebral ischemia in rats and the effect of pre-treatment with the CRH-R1 antagonist Antalarmin (2  g/2  l; icv). Our findings demonstrate significant inhibition of ischemia-induced CORT morning secretion up to 7 days post ischemia. Effects of Antalarmin pre-treatment on attenuation of CORT levels were maintained in ischemic animals exposed to a 15 min restraint stress 27 days following reperfusion. On the 30th day post ischemia stress-sensitive organs including the seminal vesicles, thymus and adrenal glands were collected and weighted. Analyses show a

significant reduction of organ's weight on ischemic rats and a protective effect of Antalarmin on seminal vesicles for ischemic rat. The findings support that overactivation of CRH-1 receptors associated with global cerebral ischemia plays a significant role in immediate and remote HPA reactivity and/or CORT secretion. The finding that regulation of endogenous regulators of the stress response can exert delayed effects on physiological responses is important to consider when determining therapeutic approaches to stress related disorders.

3-E-142 Conservation of   N-TRPV1 osmosensitive channel in osmoregulating animals.

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Transient receptor potential (TRP) channels have been involved with the complex ways in which metazoans sense stimuli around them. They are implicated in vision, thermosensation, olfaction, hearing and mechanosensation among many others. Several are activated by more than one different stimulus, which is then integrated in the gating step that allows the ions to pass through the channel's pore. The superfamily is divided in 6 subcategories, and even though one member has been described in yeasts, the majority of them appear in more complex multicellular organisms, such as nematodes, insects and all kind of vertebrates. Recently, we have cloned and described a variant form of TRPV1, a member of the Vanilloid subfamily, from rat Supraoptic Nucleus (SON). This variant lacks part of the N-terminus (  N-TRPV1). The transcript forms a channel that allows the non-selective permeation of cations when the cells are stimulated with hypertonic fluids or negative pressure. Based in multiple alignments of TRPV1 sequences, we have predicted the conservation through vertebrates of   N-TRPV1. This hypothesis has been corroborated by the detection and cloning of five variant homologs, introduced in this work, in fish, amphibians, birds and mammals including human. The high conservation of   N-TRPV1 on osmoregulating animals and the evidence pointing for this specific role detecting extracellular fluid osmolality changes, suggests that this variant is a key piece in the machinery that has allowed animals to adapt to and conquest dry land.

F - Cognition and Behavior

3-F-143 Saccadic eye movements in children with and without dyslexia performing a letter naming speed task

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Naming speed (NS) deficits, impaired timing mechanisms that affect reading fluency, are characteristic of reading difficulty from the early stages of reading into adulthood. NS tasks measure how quickly and accurately subjects can name a set of highly familiar stimuli (e.g., letters) randomly presented in a visual array. We used a letter NS task and three variants that were either phonologically and/or visually similar while participants' eye movements and articulations were recorded. We examined how these manipulations influenced performance and whether there were differences with increased reading acquisition from ages 6-10 and between dyslexic and average readers. Participants were in three groups (n=15/group): dyslexics (age 9-10), chronological-age (CA) controls (age 9-10), and reading-level (RL) controls (age 6-7). For all groups NS manipulations were associated with specific patterns of behavior and saccade performance which were influenced by visual rather than phonological similarity. When the task was both visually and phonologically similar all groups made longer naming times and fixation durations, more naming errors, more frequent and shorter saccades, and had shorter eye-voice spans. Compared to CA controls, dyslexics performed more like RL controls and were less efficient, had longer articulation times, pause times, and fixation durations, shorter eye-voice spans, and made more errors, saccades, and regressions. Overall there were developmental changes in performance in normally achieving children from ages 6-10 that appear to occur more slowly for dyslexics.

3-F-144 Noise correlations in macaque areas 46/8a reflect target selection and strength of distractors

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Visual attention selectively enhances neuronal responses to behaviorally relevant stimuli while suppressing irrelevant ones. However, it is unclear how attention modulates interactions

between neurons. We examined such interactions in simultaneously recorded pairs of neurons in the dorsolateral prefrontal cortex (dlPFC) of a macaque monkey while it performed an attentional task. While fixating the center, the animal had to indicate a change in one of two peripherally presented stimuli, ignoring the other. The target was determined by the stimulus color. ~20% of the neurons showed a preference for the target's location (ipsi- versus contralateral hemifield). We analyzed noise correlations (r_{noise}) during the allocation and maintenance of attention. We found differences in r_{noise} based on spatial preferences with positive correlations between similarly tuned neurons and negative correlations between neurons of opposite tuning. Interestingly, the size of r_{noise} varied between epochs for contralateral neurons but not for ipsilateral neurons. Correlations also depended on task difficulty: Similarly selective neurons were more positively correlated for easier trials than more difficult ones. Likewise, r_{noise} between pairs of neurons with opposite preferences were more negative for easier stimulus pairs. Finally, we used different classification tools to explore the importance of noise correlations on stimulus decoding. Together, these results suggest that neuronal interactions contribute to the amount of information carried by neuronal populations within dlPFC areas 46/8a.

3-F-145 The Effects of Acute Nabilone Administration on Resting State EEG in Healthy Participants

Ashley Beaudoin¹, Sara de la Salle¹, Joelle Choueiry¹, Dylan Smith¹, Danielle Impey¹, Renee Nelson¹, Jasmit Heera¹, Lawrence Inyang¹, Vadim Ilivitsky¹, Jakov Shlik¹, Verner Knott¹

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Long-term use of cannabis, the most widely used illicit drug, has been associated with the appearance of psychotic symptoms and cognitive impairments, similar to those found in schizophrenia (SZ). Although the exact mechanisms are unclear, CB1 receptors in the dorsolateral prefrontal and anterior cingulate cortices are often increased in SZ patients, which may suggest that CB1 receptors play a role in cannabis precipitated psychosis and associated neurobiological dysfunction. In SZ, cerebral dysfunction is evidenced by disturbed patterns of electroencephalographic (EEG)

activity, which is also modulated by acute and long-term cannabis use. The main objective of this study was to examine the EEG correlates of acute selective CB1 agonist treatment in a healthy population. EEG power spectrum measures were acquired during resting state in 20 healthy, non-smoking, non-cannabis using males in a randomized, double-blind procedure, with participants receiving the selective CB1 agonist nabilone (0.5 mg) in one session and placebo in the other. Single dose administration of nabilone is expected to mimic the effects of cannabis in EEG and possibly result in transient frequency changes similar to those seen in SZ. The findings of this study may lead to a clearer depiction of the role of the CB1 receptor in the regulation of electrocerebral activity and may help to elucidate the contributing role of cannabis use in the etiology of schizophrenia symptoms.

3-F-146 Strategies to rescue cognitive deficits due to SYNGAP1 haploinsufficiency

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We have previously shown that heterozygous loss-of-function mutations in SYNGAP1 cause nonsyndromic intellectual disability (NSID) with or without epilepsy and autism. SYNGAP1, which codes for a Ras GTPase-activating protein (GAP), is a component of the NMDA receptor complex. Knockdown of SYNGAP1 results in hyperactivation of Ras, an increase of ERK phosphorylation, and an excess number of GluR1 at the surface of post-synaptic membrane, which correlate with altered spine morphologies and impaired long term potentiation. We propose to use Syngap1 ^{-/-} mice to test strategies to rescue the cognitive deficit phenotype. Specifically, since SYNGAP1 exerts its synaptic function through inhibiting Ras, we propose to test whether using Ras inhibitors in Syngap1 mutant mice could rescue the associated behavioral and learning deficits. First, we subjected Syngap1 ^{-/-} mice to a battery of behavioral tests. We showed that they exhibited hyperactivity, appeared less anxious, displayed deficits in working memory, had slight impairments in long term spatial memory and displayed deficit in social recognition, consistent with previously reported observations. Currently, we are testing whether the administration of the well-characterized Ras inhibitor, lovastatin, is able to rescue the behavioral and cognitive defects of Syngap1 ^{-/-} mice. This Ras inhibition

strategy was previously successfully used to reverse the learning deficits in mice caused by the heterozygous knockout of another Ras-GAP gene, neurofibromin 1 (Nf1).

3-F-147 Response selection to emotional stimuli in adults with ADHD: a fMRI study using the Affective Spatial Compatibility task.

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The response selection to stimuli with different emotional valences was investigated in Attention-Deficit/Hyperactivity disorder (ADHD). The stimulus-response compatibility effects and inhibitory mechanisms involved with response selection were assessed using fMRI and manual responses. Method: 20 ADHD male adults underwent the task while been scanned (3-T, TR/TE=2s/30ms). Stimuli were figures of the most popular soccer teams of São Paulo, Brazil presented to the left/right of fixation. Participants informed his Favorite and Rival teams, and for the task, they were instructed to press in one block of trials the key which is ipsilateral to Favorite team hemifield (compatible) and the contralateral key for Rival team (incompatible) and the reverse arrangement in the other block. Blocks were counterbalanced across participants. Results: The behavioral data showed that Favorite-Compatible(FC)/Rival-Incompatible(RI) block were faster than Rival-Compatible(RC)/Favorite-Incompatible(FI) and that FC were faster than RC and FI while RI was faster than FC. fMRI findings are summarized in Fig 1. An ANOVA reveals an interaction between right dorsal-lateral prefrontal cortex (DLPFC) and left posterior parietal cortex (PPC) activations (A). Similar areas were found for the contrasts between RI-RC (B) and RI-FI (C). The bilateral DLPFC, left PPC, thalamus and left basal ganglia areas were found for the RI-FC contrast. Conclusion: RI condition in ADHD recruited areas of the attentional network involved with negative emotional processing. Grants: 2011/19179-5; 2011/09946-9 FAPESP/CNPq/NARSAD

3-F-148 Acute Effects of Nabilone on Attentional Processing in Healthy Participants: A Brain Event-Related Potential Study

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Cognitive impairments are prominent in schizophrenia (SZ) and are a strong predictor of the patient's functional status. While antipsychotics efficiently relieve positive symptoms (i.e. hallucinations), they exert no significant benefits over cognitive impairments. A strong association has been shown between long-term cannabis use and emergence of SZ-like cognitive impairments in healthy individuals. In addition, increased levels of cannabinoid receptors (CB1) have been observed in the dorsolateral pre-frontal and anterior cingulate cortices of SZ patients. Objective: To examine the implication of the CB1 receptor in cognitive processing by applying an acute dose of the selective CB1 agonist nabilone. Methods: Brain even-related (ERP) potential indices of pre-attentive (P3a) and attention-dependent (P3b) processing were assessed in 20 non-smoking males during an auditory odd-ball paradigm within a randomized, double-blind, placebo controlled design involving the administration of 0.5 mg of nabilone. Compared to placebo, nabilone is expected to mediate: 1) lower P3a amplitudes (poorer pre-attentional novelty detection capacity) and, 2) lower P3b amplitudes (poorer attention-dependent performance). Implications: Enhancing our understanding of the CB1 receptor involvement in attentional processing is important for both clinical and scientific aspects, as greater cannabis use is observed in SZ patients compared to healthy individuals and acute cannabis effects on P3a indexed novelty detection/attentional switching has not yet been examined.

3-F-149 The Benefits of Environmental Enrichment on Hippocampal Neurogenesis and Behavior Require Synaptic Zinc Signaling.

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Exposing animals to an enriched environment (EE) increases adult hippocampal neurogenesis and improves spatial learning and memory. Synaptic zinc is highly concentrated in the neurogenic region of the hippocampus, the dentate gyrus, yet its role in hippocampal neurogenesis and behaviour remain unclear. The aim of the current study was to determine whether synaptic zinc is important for modulating adult neurogenesis and, consequently, hippocampal-dependent behavior. By comparing neurogenesis in adult zinc transporter 3 (ZnT3) wild-type (WT) and knockout (KO) mice, which lack synaptic zinc, we found that synaptic zinc is necessary for EE-induced neuronal proliferation and survival in the hippocampus. Further, utilizing two tests of spatial memory, the Spatial Object Recognition and Morris Water tasks, we show that mice lacking synaptic zinc show no improvement in spatial memory following EE. The neurogenic and cognitive effects of EE can be mediated, at least in part, by increased levels of brain-derived neurotrophic factor (BDNF). We demonstrate that EE-dependent increases in BDNF require synaptic zinc signaling, as hippocampal levels of BDNF in ZnT3-KO mice in standard housing were not significantly different from KO animals placed in EE. These experiments show that synaptic zinc is essential for the modulation of adult hippocampal neurogenesis, and hippocampus-dependent behaviour, supporting a role for zincergic neurons in modulating experience-dependent plasticity in the hippocampus. Acknowledgments: Funded by NSERC

3-F-150 GABAA receptor blockade prevents nicotinic reversal of crossmodal object recognition impairment in ketamine-treated rats: implications for cognitive deficits in schizophrenia

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The neural bases of multisensory integration impairments in schizophrenia are not well understood. Rats treated sub-chronically with NMDA receptor antagonists (e.g., ketamine), which model symptoms of schizophrenia, are impaired on a tactile-to-visual crossmodal object recognition (CMOR) task; this deficit is reversed by systemic nicotine. Furthermore, cortical gamma oscillations mediated by parvalbumin-containing GABAergic interneurons (PV-INs) may be deficient in schizophrenia, potentially contributing to aberrant multisensory

processing. PV-INs contain nicotinic acetylcholine receptors (nAChR). The current study assessed the receptor specificity of nicotine's ameliorative effect in the CMOR task and the interaction between nAChRs and GABA. Male Long-Evans rats were treated sub-chronically for 10 days with ketamine or saline and then tested on the CMOR task after a 10-day washout. Systemic nicotine given prior to the sample phase of the CMOR task reversed the ketamine-induced impairment, but this effect was blocked by co-administration of the GABA_A receptor antagonist bicuculline at a dosage that otherwise did not induce impairment. Selective $\alpha 7$ and $\alpha 4\beta 2$ nAChR agonists were also tested, with only the latter reversing the ketamine-induced impairment. These results suggest that nicotine, likely through stimulation of the $\alpha 4\beta 2$ subtype of nAChR, ameliorates CMOR deficits in ketamine-treated rats via stimulation of the GABAergic system. These findings may have important implications for understanding the nature and potential treatment of cognitive impairment in schizophrenia.

3-F-151 Acute Effects of Nabilone on Sensory Memory in Healthy Participants: A Brain Event-Related Potential Study

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Long-term cannabis use has been associated with the expression of psychotic symptoms and cognitive impairments in vulnerable individuals. However, the mechanisms by which cannabis use precipitates these effects is unclear. It has been suggested that certain neuroadaptive changes, such as an increase in cannabinoid-1 (CB1) receptors in the dorsolateral prefrontal and anterior cingulate cortices, may be associated with the pathology of schizophrenia (SZ). Additionally, cannabis use in SZ can exacerbate symptoms and trigger relapses. It has also been demonstrated that in healthy individuals, cannabis use can induce a full-range of transient SZ-like positive, negative, and cognitive symptoms. The objective of this study was to assess the acute effects of the selective CB-1 receptor agonist nabilone on the mismatch negativity (MMN) event-related potential. Previous studies have shown that an acute

administration of a CB-1 receptor agonist decreased MMN amplitudes in healthy controls, and that MMN amplitudes are reduced in long-term and heavy cannabis users. Studies have also consistently shown marked MMN amplitude reductions in SZ. 20 male non-smokers and non-cannabis-users were examined within a randomized, double-blind, placebo controlled design. MMN amplitudes were expected to decrease (vs. placebo) with an acute dose of nabilone (0.5 mg). The implications for these findings may be a better understanding of the role of the CB-1 receptor in cognitive impairments and may help to further elucidate the process by which cannabis use leads to psychosis and cognitive impairments.

3-F-152 Rewards and movement-related costs shape fast decision-making in a human target foraging task

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Real-world tasks typically consist of a series of target-directed actions and often require choices about which targets to act on and in what order. Such choice behaviour can be assessed from an optimal foraging perspective whereby target selection is shaped by a balance between rewards and costs. Here we evaluated such decision-making in a rapid movement foraging task. In a given trial, participants were presented with 15 targets of varying size and value and were instructed to harvest as much reward as possible by either moving a handle to the targets (hand task) or by briefly fixating them (eye task). The trial duration (3.25 s) enabled participants to harvest about half the targets, ensuring that total reward was due to choice behavior. We developed a probabilistic model to predict target-by-target harvesting choices that considered rate of reward and costs associated with target distance and size for up to five future harvests in succession. Because the time and energy costs are greater for hand than eye movements, we predicted that in the hand task, in comparison to the eye task, target choice would be more strongly influenced by movement-related costs and would take into account a greater number of future targets. The model results confirmed both predictions. In a version of the hand task in which choices could only be based on target positions, participants consistently chose among the shortest

movement paths. Our results demonstrate that optimal foraging theory offers a useful framework for understanding choice behaviour in target-directed movements.

3-F-153 Decline in Cognitive Function and Risk of Elder Self-Neglect: Finding from the Chicago Health Aging Project

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Background: This study aimed to examine the longitudinal association between decline in cognitive function and risk of elder self-neglect in a community-dwelling population. **Methods:** Prospective population-based study of those participated in the Chicago Health Aging Project (CHAP). Of the 5,519 participants, 1,017 were reported to social services agency for suspected elder self-neglect from 1993-2005.

Measurements: The primary predictor was decline in cognitive function assessed using the MMSE, the Symbol Digit Modalities Test (Executive Function), and both immediate and delayed recall of the East Boston Memory Test (Episodic Memory). An index of global cognitive function scores was derived by averaging z-scores of all tests. Outcome was elder self-neglect as confirmed by social services agencies. Logistic and linear regression models were used to assess these longitudinal associations. **Results:** After adjusting for potential confounding factors, decline in global cognitive function, MMSE or episodic memory was not independently associated with increased risk of reported and confirmed elder self-neglect. Decline in executive function was associated with increased risk of reported and confirmed elder self-neglect. Decline in global cognitive function was associated with increased risk of greater self-neglect severity (PE=0.76, SE=0.31, p=0.014). **Conclusion:** Decline in executive function was associated with increased risk of reported and confirmed elder self-neglect. Decline in global cognitive function was associated with increased risk of greater self-neglect severity

3-F-154 Escalated sucrose intake is accompanied by increased value

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With 24 h periods of every 3rd day access (E3DA), rats markedly escalate their intake of a

4% sucrose solution, whereas with continuous, every day access (EDA), the same solution is consumed at lower, stable levels. If sucrose solution access is then switched to a common E2DA schedule, rats with E3DA experience continue to consume more solution than rats with EDA experience over an extended period of time. Here, we ask if the E3DA/EDA difference reflects a change in sucrose solution value. Rats were first provided with a 4% sucrose solution paired with one flavour (counterbalanced), for 12 E3DA exposures or 36 days of EDA. Rats with E3DA escalated their intake to consume almost twice as much sucrose solution as EDA rats (326 vs. 173 grams). Next, a series 24h, 2 bottle preferences tests were performed on an E2DA schedule. On each test day, preference was measured for the standard solution against ascending concentrations (2-32%) of a second sucrose flavour (the alternate). The point at which standard and alternate solutions were isohedonic occurred at a higher alternate concentration (rightward shift) for rats with E3DA relative to EDA experience. Furthermore, both preference for the standard, as well as overall consumption of standard and alternate combined, were always higher after E3DA experience. Preference testing was followed by 12 E2DA exposures to the standard only. Again, rats with E3DA experience consumed more than rats with EDA experience. These findings suggest that E3DA relative to EDA can increase the value of a sucrose solution over a longer term.

3-F-155 The action of vitamin K in brain during aging is linked to the pAKT/AKT and apoptotic caspases-3, -8, -12 signaling pathways.

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Vitamin K (VK) participates in brain function through sphingolipid metabolism and activation of proteins S and Gas6. We have previously provided evidence that long-term low VK intake leads to cognitive deficits in aging rats and to a concurrent accumulation of ceramides in certain brain regions. To gain further insight on the mechanisms of action of VK in brain, we investigated the phosphatidil-inositol-3-kinase/Akt and apoptotic caspases-3, -8, -12 cell signaling pathways, as well as DNA damage, in the brains of 21-mo female Sprague Dawley rats which had been fed either a low (L: 80 µg K1/Kg diet) or an enriched (E: 2000 µg K1/Kg diet) VK

diet since weaning, or a L (ages 2-13.9 months) followed by an E diet (ages 14-21 months). Lifetime intake of an enriched VK diet was associated with a significant increase in cell survival based on the pAKT/AKT ratio and significant decrease in DNA damage as assessed by transcription protein p53. Strong trends were also observed for decreased apoptosis. The systematically higher pro-form of caspases-3, -8, -12 suggests that less of these proteins were being activated, an assumption that was verified with caspase-12 where both the pro and active forms were assessed. In conclusion, long-term consumption of a VK rich diet appears to offer cellular protection in brain and could contribute to the maintenance of cognitive functions during aging. Supported by CIHR.

3-F-156 Validation and comparison of 2-day and standard-length MWM protocols: Analysis of procedural sensitivity to detect visuo-spatial learning and memory in the 5xFAD mouse model of Alzheimer's disease.

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The Morris Water Maze (MWM) is one of the most popular measures of visuo-spatial learning and memory in mice. Standard MWM protocols take 6-8 days to complete. Gulinello et al. (2009, *Behav Brain Res* 196(2)) attempted to validate a 2-day MWM protocol that used one day of visible platform trials, followed by one day of hidden platform trials. Although they were able to detect a deficit in the 3xTG mouse model of Alzheimer's Disease (AD) at 18-19 months of age, two questions arise: (1) is the task as sensitive as standard protocols, and (2) are the mice using spatial strategies to locate the platform. Therefore, we compared the standard and 2-day MWM protocols with a hybrid 2-day and conventional protocol in the 5xFAD mouse model of AD (10M, 10F) and their wild-type (WT) controls (10M, 10F) at 4-6 months of age. All mice showed improvement across days in latency and swim distance in all phases of the experiment but there were no significant genotype effects in latency or distance in either protocol. In the 2-day phase, 5xFAD mice had more thigmotaxis than WT mice, with females exhibiting more thigmotaxis than males. Female mice swam significantly longer distances and swam faster in the acquisition stage of the standard MWM. Lastly, female 5xFAD mice spent significantly less time in the target quadrant and made fewer annulus crossings

than WT females in the probe trial. This experiment indicates that the standard MWM may be more sensitive than the 2-day MWM because it detected modest deficits in spatial learning in female mice that were not detected in the 2-day version.

3-F-157 The supraoptic nucleus neuron-glia structural plasticity and the social behavior responses to salt loading in rodents are regulated by EphA4

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The hypothalamic supraoptic nuclei (SON) display marked structural changes after salt loading (2% NaCl in drinking water): astrocytic leaflets enveloping oxytocin (OT) neurons retract and are replaced by new synapses. We here examined whether EphA4, which regulates cell motility and adhesion, is involved in this neuronal-glia structural plasticity (NGSP). Using in situ hybridization (ISH), immunohistochemistry and electron microscopy (EM), we assessed the expression and localization of EphA4 in the SON of naïve and salt-loaded rodents. We also used EM to quantify the effect of salt loading on NGSP in the SON of wild type (WT) and EphA4 KO mice. ISH showed selective expression of EphA4 in the SON and other hypothalamic nuclei exhibiting NGSP. Counting silver grains showed a significant increase in EphA4 mRNA in the SON of salt loaded vs. naïve WT rats. By EM, there was a significant increase in the number of EphA4-labeled dendrites in salt loaded vs. WT mice, but no change was detected by Western blotting of EphA4 from rat SON. In addition, NGSP did not occur in salt loaded KO mice. Since OT influences social behavior, we tested KO and WT mice in the 3-chamber social interaction test after salt loading. Compared to untreated KO mice, salt loaded KO mice exhibited a significant decrease in the total duration of their interactions with all targets combined. In contrast, salt loading in WT mice tended to increase the total duration of interactions. These results support a role for

EphA4 in mediating NGSP of OT neurons in the SON, and thereby influencing social behaviour.

3-F-158 Acquisition of schedule-induced polydipsia: hippocampal and striatal dissociation in the Y maze

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In rodents, excessive non-physiological drinking can be induced by intermittent presentation of food in the presence of a drinking spout to a hungry animal (schedule-induced polydipsia; SIP). Acquisition of SIP can be modified by lesions to the hippocampus and by drugs targeting the dopamine (DA) systems of the striatum. We previously observed that repeated amphetamine exposure shifted rats' learning strategies from the hippocampus to the striatum and also significantly increased water-drinking (SIP). It is currently unknown whether the animals that develop SIP have impaired hippocampal functioning, augmented DA functioning in the striatum or other neurological changes that predispose them to compulsive drinking behaviors (SIP). We attempted to elucidate some of the neurological underpinnings of SIP development. We examined each rat's learning strategy in the Y-maze: animals were food-restricted and trained in a Y maze to find food pellets placed in a baited arm to determine if the animals were response learners or place learners. Following this, all animals were exposed to the SIP paradigm for a period of 21 days to determine if rats that went on to develop SIP had a learning-strategy preference. Following SIP acquisition, histological staining for FosB & c-fos examined neuronal activation elicited by SIP. Rats characterized as response learners on the Y-maze demonstrated greater SIP than rats characterized as place learners. Immunohistochemical data in the caudate putamen, prefrontal cortex & Ca1 area of the hippocampus will be reported.

3-F-159 The development of a rat model of chronic cerebral ischemia: effects on motor and cognitive functions

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Introduction: Cerebral ischemia is one of the leading causes of death and long-term disability in aging populations. Chronic cerebral ischemia can induce the accumulation of reactive oxygen species (ROS) and nitric oxide (NO), which lead to neuronal injury in selective, vulnerable regions of the brain, especially the hippocampus and cerebral cortex, leading to cognitive impairment and some motor dysfunction. **Aims:** In the present study, the effect of chronic cerebral ischemia induced by permanent, bilateral occlusion of the common carotid arteries (2VO) on locomotor activity and learning and memory processes were evaluated at different time points following the occlusion. **Method:** Male Sprague Dawley rats (200-300g) were subjected to 2VO or sham-operated surgery and tested 1, 2, 3 and 4 weeks following the ischemic insult. **Results:** The results showed that 2VO significantly reduced step-through latency in a passive avoidance task at all time points when compared to the sham-operated group. Further, 2VO rats showed significant increases in escape latencies during training in the Morris water maze, as well as a reduction of the percentage of times spend in target quadrant of the maze at all time points following the occlusion. Importantly, there were no significant changes in locomotor activity between 2VO and sham-operated group. **Conclusion:** The present data suggest that the 2VO procedure effectively induces behavioral, cognitive symptoms associated with cerebral ischemia and, consequently, provides a valuable model to study ischemia and related neurodegenerative disorder.

3-F-160 Identifying TrkA receptor mediated mechanisms affecting hippocampal-dependent memory

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The molecular and cellular mechanisms by which memories are formed, stored and retrieved are poorly understood. Also, no effective medications exist to halt the neurodegenerative process leading to cognitive impairment in Alzheimer's disease (AD) and

ageing. Here, we present an in vivo pharmacological model where memory can be manipulated; and through which we hope to gain insights into the mechanisms of memory formation. We also might validate a therapeutic target to relieve memory-impairing pathologies. The in vivo model involves manipulation of the activity of the receptor for nerve growth factor (NGF), called TrkA. TrkA activation by NGF promotes neurogenesis, neuronal differentiation and survival and plays a role in hippocampal dependent memory. Reduced TrkA receptor density and function correlates with progression in AD, ageing, and Down syndrome patients and rodent models. Our laboratory designed D3, a specific peptidomimetic partial agonist of TrkA. D3 improved learning and long-term memory (LTM) in aged rats with memory impairment. D3 also improved short-term memory (STM) in the amyloid precursor protein AD mouse model. Paradoxically, D3 impaired LTM in wild-type young mice, without effects on STM or evidence of toxicity. Our preliminary results in vitro and in vivo suggest that this LTM impairment was due to D3 increasing hippocampal neurogenesis, and a corresponding change of several signaling proteins in the hippocampus. This work may validate TrkA as a therapeutic target for memory-impairing pathologies.

3-F-161 What prevents us from engaging in self-unfit behaviors: the putative role of the frontal N400 event-related potential in their preconscious inhibition

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Across a lifespan, one can embody various social roles. The mechanisms by which one decides which are conceivable with respect to oneself still remains largely unknown. The present research aims at exploring one possibility as to these mechanisms. This possibility is based on extrapolations of results of grounded semantics and affordance studies. It rests on the idea that names of social roles automatically and preconsciously activate the behaviors associated with them. In a self-fit task in which participants have to decide whether or not they would endorse the role, the behaviors that are deemed unfit to the self could then be inhibited. Roles that are rejected should thus induce more inhibition. According to the idea that the N400 event-related brain potential indexes a preconscious inhibition, these rejected roles should elicit a larger frontal N400 (fN400)

than the accepted roles. This prediction has been confirmed by our recent works. Here, we test whether this additional fN400 activity actually indexes the inhibition of self-unfit behaviors. Participants were shown 216 names of social roles in three tasks. First, they performed the self-fit task. Then, they named all the behaviors associated with the role. Finally, they named only the first behavior that came to mind. In accordance with the hypothesis, fewer behaviors were named for the social roles that were rejected and the dampening effect of stimulus repetition on the N400 amplitude was eliminated between the 2nd and 3rd task, as should be the case given a greater need of behavior repression in the third task.

3-F-162 Systems consolidation and reconsolidation in the thalamus

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The anterior thalamic nuclei (ATN) and the intralaminar/lateral thalamic nuclei (ILN/LT) play different roles in memory processes. The ATN are believed to be part of an extended hippocampal system, and the ILN/LT have strong connections with the medial prefrontal cortex. Recently, it was shown that the ILN/LT were involved in systems consolidation. However, it could not be determined clearly whether the ILN/LT were necessary only for the process of systems consolidation, or for retrieval as well. The role of the ATN in retrieval was also left unclear. We therefore used an immediate-early gene imaging approach and reversible inactivations to further investigate the role of these nuclei in recent and remote memory retrieval, in a contextual fear conditioning task in rats. As the ATN are believed to be part of an extended hippocampal system, we also assessed whether the ATN were involved in reconsolidation and systems reconsolidation. Results confirmed a differential role of the ATN and ILN/LT in systems consolidation, and pinpoint for the first time which specific nuclei are involved in this process: the anterodorsal nucleus (AD), and the mediodorsal nucleus (MDL). The results also show for the first time the involvement of the ATN in recent but not remote memory retrieval. In addition, we show that the ATN is not involved in reconsolidation or systems reconsolidation, suggesting that these nuclei do not store memory but provide essential feedback to the hippocampus during recent memory retrieval.

3-F-163 Developmental high fat diet exposure induces long-term changes in anxiety behaviour and glucocorticoid signaling in the brain

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Maternal obesity and a diet high in saturated fat during pregnancy carry significant risks for health problems in offspring that manifest later in life. For example, maternal high fat diet consumption is a risk factor for heart disease, type 2 diabetes and the metabolic syndrome in offspring, in addition to less obvious effects on the risk for affective disorders. A high fat diet also increases systemic inflammation and recent studies show that it directly influences local inflammatory expression in the brain. However, few studies have examined the effect of maternal high fat diet on brain areas linked to anxiety behaviour. We investigated the influence of maternal high fat diet in male and female offspring in Long-Evans rats. We measured anxiety behaviour in the open field, elevated plus maze and light dark transition tasks in adolescent and adult offspring. In addition, we examined levels of corticosterone, a major stress hormone, by radioimmunoassay and gene expression by quantitative PCR in the brains of adolescent and adult offspring. High fat diet-exposed offspring show age-specific alterations in anxiety behaviour and corticosterone levels. High fat diet-exposed offspring also show sex-specific alterations in the expression of corticosterone receptors and inflammatory genes in the hippocampus and amygdala, brain areas known to regulate anxiety behaviour and the response to stress. The data suggest that developmental high fat diet exposure alters anxiety and the response to stress at least in part by long-term changes in glucocorticoid signaling pathways in the brain.

3-F-164 Effects of heroin dependence on yohimbine-induced reinstatement of heroin seeking and startle reflex in rats

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Heroin dependence alters responses to stressors, but it is not clear if these effects can be observed after dissipation of withdrawal symptoms. The current study explored whether heroin dependence alters the action of

yohimbine (YOH) on goal-directed and reflexive behaviors. Male Sprague-Dawley rats self-administered 0.05 mg/kg/inf heroin for 10 sessions (1 session x day, 3h each). Four hours after each session, rats received 3 injections (SC) of vehicle or heroin, 2h apart. The dose of these injections escalated from 3 to 24 mg/kg/day. Withdrawal precipitated by naloxone (NAL; 0.1 mg/kg, SC) was measured after the last session of self-administration (SA) on a progressive ratio schedule. After 9 sessions of extinction, rats were pre-treated with 0 or 0.5 mg/kg injection (IV) YOH and tested for reinstatement. In a separate study, rats received SC injections of heroin as described above. Following a 13-day drug-free period, rats received two tests of startle separated by 24 h: one test following an injection of vehicle, and the other after 0 or 2.5 mg/kg YOH (IP). It was found that rats injected with heroin during SA displayed greater signs of NAL-precipitated withdrawal, and greater YOH-induced reinstatement. YOH also amplified startle reactivity, but this was not affected by previous heroin dependence. These data suggest a possible dissociation between the actions of heroin dependence on stress-induced behaviors. Specifically, with chemical stressors i.e., YOH, dependence may amplify the effect of stress on goal-directed behavior, without altering reflexive stress responses.

3-F-165 Optogenetic modulation of cholinergic neurons in the medial septum-vertical limb of the diagonal band of Broca (MSvDB) and its effect on hippocampal activity

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Cholinergic neurons in the medial septum-vertical limb of the diagonal band of Broca (MSvDB) are known to have an important role during normal hippocampal functions and theta oscillations. Although previous studies have focused on understanding how cholinergic neurons from the MSvDB neurons fire to pace hippocampal theta oscillations, a significant portion of MSvDB neurons are slow-firing and thus may not have the capacity to pace theta oscillations. The function of these neurons, especially their role in modulating hippocampal activity, remains unknown. Here, we combined optogenetics with our recently developed septo-hippocampal preparation in vitro to answer this question. For this study, we used ChAT-

mhChR2-YFP BAC transgenic mice expressing channelrhodopsin-2/EYFP fusion protein (mhChR2:YFP) targeted selectively to cholinergic neuronal populations. Characterization of septo-hippocampal neurons showed photocurrents of 190 \pm 50 pA (steady state: 160 \pm 50 pA) and depolarizations of 25 \pm 3 mV. Cholinergic neurons followed light stimulation of 1 to 20 ms pulse duration, firing efficiency increased with pulse size and stimulation response ratio reached value of 1 - 5, 10 and 20 ms, suggesting that ACh neurons could be finely activated with light. Surprisingly, optogenetic activation of cholinergic neurons caused a scopolamine sensitive reduction in theta power in the CA1 area in the intermediate hippocampus. Together, these results suggest that the role of slow firing septo-hippocampal neurons contribute to oscillation power modulation in the hippocampus.

3-F-166 Endocannabinoid System Involvement in Impulsivity and Decision-Making

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Recent studies suggest that dysregulation of the endocannabinoid system may lead to impaired decision making. For example, chronic cannabis use is associated with impaired learning of the optimal strategy in the Iowa Gambling Task. The present study sought to elucidate the role of endocannabinoids in impulsivity and decision-making in laboratory rats. Food-deprived adult male CD 1GS rats (n=10) were trained in a food-reinforced 5-choice serial reaction time task (5-CSRTT) until accuracy exceeded 80%. In subsequent drug-naïve test sessions, administration of cocaine (15 mg/kg, i.p.) significantly decreased choice accuracy relative to saline control treatments, primarily by increasing the number of premature responses. The fatty acid amide hydrolase (FAAH) inhibitor URB597 (0.03, 0.3 or 1.0 mg/kg, i.p.), which leads to an accumulation of the endogenous cannabinoid anandamide, had no significant effects on choice accuracy or premature responding. Experiments in progress are examining the ability of the CB1 cannabinoid receptor antagonist/inverse agonist rimonabant to attenuate cocaine-induced premature responding. Future experiments using a rat analog of the Iowa Gambling Task (the rGT), and a newly developed operant slot machine task will be used to further examine the role of

endocannabinoids in problem gambling-related decision making.

3-F-167 Effects of parent removal on parents and offspring in a typically biparental songbird, *Taeniopygia guttata*

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Stressful events during childhood, such as having a single parent, shape brain and behaviour later in life. Previous work modelling the effects of developmental stress have primarily used a rodent model, however because only mothers provide care for the young, meaning the potential contribution of fathers to offspring rearing, and the effects of single vs. biparental care cannot be examined. Our model uses a typically biparental songbird, the zebra finch, so we can examine how removal of one parent affects both the remaining parent and the offspring in terms of feeding behaviours, begging behaviours, and corticosterone levels. In addition, we compared song learning in males reared by single fathers to those reared by both parents to see if song learning was compromised. Preliminary results show that offspring reared by single parents did not weigh less than offspring reared by both parents, however, males reared by a single father had lower similarity scores (when comparing their own song to their fathers) compared to males reared by both parents, indicating that having a mother present during song development is beneficial, even when she does not contribute to song learning directly.

3-F-168 Mesocortical dopamine depletion reverses blunted responses to amphetamine in dcc haploinsufficient mice

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The netrin-1 receptor, dcc, determines a) the extent of dopamine (DA) innervation to the medial prefrontal cortex (mPFC), b) the organization of local circuitry, and c) individual vulnerability to effects of drugs of abuse. Adult dcc haploinsufficient mice have a selective increase in mPFC DA fiber innervation and DA release in comparison to wild-type littermates. Furthermore, adult dcc haploinsufficient mice show blunted DA release in the nucleus

accumbens (NAcc) and reduced locomotor activity when challenged with drugs of abuse such as amphetamine (AMPH). Because mPFC DA transmission in the mPFC can negatively regulate DA release in the NAcc in response to drugs of abuse or stressors, we hypothesized that the blunted effects of AMPH in dcc haploinsufficient result from increased mPFC DA innervation. We therefore examined the effects of selective mPFC DA depletion on AMPH-induced locomotion in dcc haploinsufficient and wild-type mice. Adult mice received bilateral mPFC injections of 6-hydroxydopamine (lesion) or vehicle (sham) 10 days before an i.p. AMPH challenge. In wild type mice, AMPH-induced locomotion was similar between lesion and sham groups. Remarkably, mPFC DA lesions in dcc haploinsufficient mice reversed their blunted response to AMPH. These findings demonstrate that the protective phenotype of adult dcc haploinsufficient results from the effects of DCC on the organization of mPFC DA connectivity.

3-F-169 Structural correlates of language abilities: surface-based region-of interest morphometry study

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Intro: Examination of the relation between brain structure and behaviour offers novel insights into the neural underpinning of language and cognitive processes. Objective: Explore the relation between cerebral morphometry and performance on language tasks. Method: MRI images were acquired from 21 healthy adults (25±4.4 yrs, 10 men) who also underwent 2 language tasks: 1) verbal fluency and 2) sentence generation. Using Freesurfer, MRI images were edited to segment gray matter into multiple regions. For each cortical region, cortical thickness (CT), surface (SF) and volume (VOL) were calculated; for subcortical regions, the VOL was calculated. Analyses of correlation were conducted between morphometric data and the scores on the 2 language tasks (number of words produced and percent of correct sentences). Results: Fluency scores correlated positively with the triangular part of the left inferior frontal gyrus SF and right frontomarginal gyrus TH; and negatively with the orbital part of the left inferior frontal gyrus and inferior frontal sulcus THs. Sentence generation scores

correlated positively with the left postcentral sulcus SF and VOL, and the right frontomarginal sulcus TH, SF and VOL; and negatively with the left supramarginal gyrus TH. No correlations were found with the subcortical regions.

Conclusion: The structure of several frontal and parietal regions was associated with language performance, mainly in the left hemisphere. The presence of both negative and positive correlations highlights the complex relation between language and its anatomical correlates.

3-F-170 Attentional oscillation as a clocking mechanism for timing rhythm intervals

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An important goal in neuroscience is to understand how the brain computes the passage of time. We wanted to understand the neural mechanisms responsible for predicting the time of future events based on observed stimulus periodicities. We used a visual rhythmic motion pulse stimulus (200ms coherent random dot motion pulses, separated by 350ms random motion), and trained two Macaque monkeys to detect a dysrhythmia created by omitting a pulse and presenting it slightly later. After the last motion pulse and around omission time, the animals viewed only 0% coherent motion. We recorded 64 multiunits from the Middle Temporal area (MT) of the visual cortex while the monkeys performed the task. In randomly interleaved trials, we matched the direction of the pulses to either the preferred or the null direction of an isolated unit. Peri-omission neural activity was informative of the animals' speed of response and detection performance in a manner consistent with top-down attention. These neural-behavioral correlations increased in time in expectation of the omitted pulse, suggesting that attention was entrained to the regular motion pulses. Our results therefore imply that the brain may use attentional oscillation for clocking intervals in subsecond rhythms. This in turn suggests that perception of time does not arise from the activity of a specialized module in the brain, but is derived from actively-generated internal dynamics.

3-F-171 Von Economo neurons may be responsible for vocal acquisition and language

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Von Economo neurons (VENs) can be good targets for research to solve the mystery of origin of language. Vocal learning is a substrate for spoken language, the ability to modify acoustic or syntactic sounds, or ability to acquire vocalizations through imitation rather than instinct. It is a rare trait, and has only been detected in a few groups of mammals- humans, bats, cetaceans, pinipedes (seals and sea lions), elephants, macaques monkey, goats and zebras and three distantly related bird groups including songbirds, parrots, and hummingbirds. Surprisingly almost all vocal learner groups of mammals have VENS. The emergence of VENs in great apes coincides with the evolution of the planum temporale, a region that is important for language comprehension considering the language comprehension abilities of great apes. VENs are reported to be altered in patients with distinct symptoms affecting language such as frontotemporal dementia, autism and schizophrenia which also associated with the role of VENs in origin of language; But these cells have not been noticed to date in any bird taxa of equivalent behavioral correlates. Here we first report the localization and neuroarchitecture of VENs by Nissl and Golgi method in vocal counterparts of parrot (*Psittacula krameri*) brain and discuss the role of VENs in vocal learning and origin of language. we also compare VENs and brain areas of parrots and other vocally imitative animals to reveal how evolution shapes neural mechanisms for complex social communication which provide new insight into role of VENs in evolution of language.

3-F-172 Awake reactivation of association-related neuronal ensemble patterns in rat medial prefrontal cortex

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During periods of rest or immobility, hippocampal neurons are reactivated in a sequence corresponding to a specific spatial trajectory regardless of whether it was experienced or not (Foster and Wilson, 2006; Gupta et al., 2011; Pfeiffer and Foster, 2013). It remains unknown whether a similar phenomenon is observed during the processing of non-spatial information in other brain regions. Neurons in the medial prefrontal cortex

drastically change their firing rate to a sensory stimulus (CS) when paired with a salient outcome, such as an eyelid shock (US). This results in a neural ensemble pattern unique to the CS-US association. Here, we examined whether this association-related ensemble pattern was reactivated during periods of quiet wakefulness. Rats received daily pairings of an auditory CS and US (100 trials, TEBC) followed by pairings of a visual CS and US (100 trials, VEBC). The trials were separated by an interval ranging from 20 to 40 seconds. Prefrontal neuron ensemble formed two distinct ensemble patterns for the auditory CS and visual CS. These ensemble patterns were reactivated during the inter-trial intervals at an apparently random timing. The reactivation of two patterns was equally robust during both TEBC and VEBC and was observed from the first day of conditioning. Thus, prefrontal neurons are spontaneously organized into groups whose synchrony is driven by an internal mechanism, rather than an immediate experience. The internal dynamics during off-line periods may influence which group of neurons participates in the encoding of external stimuli.

3-F-173 Unilateral cerebellar haemorrhage combined with early systemic inflammation alters neonatal mouse sensorimotor reflex development

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Background: Extreme preterm infants are exposed to multiple stressors during their postnatal development including perinatal cerebellar haemorrhage (CBH) and postnatal infection, two major risk factors for neurodevelopmental impairments. In order to understand how cerebellar development is altered by those insults, we developed an animal model using a cerebellar haemorrhagic insult combined with an early or late inflammatory event in mice pups. Methods: Unilateral intraparenchymal CBH was induced by using local injection of bacterial collagenase (0.15U) at postnatal day 1 (P1) combined with intraperitoneal lipopolysaccharide (LPS) injection (300µg/kg) concomitantly (early inflammatory state, EIS) or at P5 (late inflammatory state, LIS). Mice were behaviourally tested on a daily basis and cerebellar tissues were collected at P2, P7 and P15. Results: Sensorimotor reflexes were altered in the treated mice. There was a 24hr

delay in forelimb grasp acquisition in the collagenase-treated group compared to vehicle. At P15, grip strength in the CBH EIS group was lower compared to vehicle (0.055 ± 0.002 vs 0.033 ± 0.002 , $p=0.0013$). However, no significant difference in the forelimb strength was measured in the LIS groups at P15. P2 preliminary histological data shows an increase in microglial cells surrounding the haemorrhagic site along with a massive ipsilateral inflamed subarachnoidal space. Conclusions: Unilateral cerebellar haemorrhage combined with early systemic inflammation delayed grasping reflex acquisition and altered forelimb strength in neonatal mice pups.

3-F-174 Maternal care effects on ATRX expression and long-term neurobehavioural development

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Mounting evidence indicates that the maintenance of chromatin architecture is essential for normal human development and cognitive function. The ATRX gene, which is essential for normal growth and cognitive development, encodes a chromatin-remodeling protein that is expressed in developing neural structures, including newly-born cortical and hippocampal neurons. We have shown that maternal care influences gene programmes, including forebrain ATRX gene expression, and is associated with stable individual differences in learning and memory and anxiety-related and social behaviour, as well as cortical and hippocampal function in adult rodents. These results suggest the possible involvement of ATRX in somatic behaviour in response to maternal care. Since disruption of ATRX impairs cognition and motor functions, inhibits learning in mouse pups, and contributes to developmental silencing of imprinted genes that shape somatic growth and brain, we hypothesize that the effects of mother-offspring interactions during the first week of postnatal life on ATRX expression influences epigenetic programmes that underlie cognitive and emotional development. The elucidation of the mechanisms involved in the effects of maternal behaviour addresses perhaps the most challenging question in development: How are experiences occurring in early life rendered permanent? In the case of sustained changes in gene expression in brain cells, we can begin to understand the neurobiological basis for individual differences in personality and

cognition as well as related risk for neuropsychiatric disease.

3-F-175 Neurons are recruited to a memory trace based on relative neuronal excitability at the time of training

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Memories are thought to be sparsely encoded over a distributed memory network. How are neurons that become integrated into the memory trace chosen? We, and others, showed that it was possible to bias the subpopulation of neurons selected to participate in a memory trace by manipulating levels of the transcription factor CREB (cAMP/Ca² responsive element binding protein) in individual LA neurons, although the underlying mechanism is unknown. Neurons overexpressing CREB are highly excitable. We used 3 ways to examine if increasing intrinsic excitability in a small population of LA neurons is sufficient to bias their inclusion in a memory trace. First, we manipulated KCNQ2, a voltage-dependent K channel which mediates afterhyperpolarization currents. A dominant negative dnKCNQ2 (hQ2-G279S) co-assembles with native KCNQ2/3 subunits, suppresses their activity and increases neuronal excitability. Second, to gain temporal control of neuronal excitability, we virally expressed the DREADDs (designer receptors exclusively activated by designer drug) hM3Dq. Activation of hM3Dq receptors by the synthetic ligand clozapine-N-oxide (CNO) leads to depolarization and increased action potential firing. Increasing intrinsic excitability minutes before training enhanced memory and these neurons were more likely to be included in the memory trace. Finally, we used optogenetics to show that increasing excitability seconds before training similarly enhanced memory formation. Together, these data suggest that neurons are recruited based on their relative excitability at the time of training.

3-F-176 Morphology and patterns of the anterior intermediate parietal sulcus of Jensen in the human brain

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The anterior intermediate parietal sulcus of Jensen (aipsJ), also called the sulcus of Jensen, emerges out of the inferior bank of the intraparietal sulcus in the human brain between the first and second caudal terminations of the superior temporal sulcus and runs for some distance at the posterior end of the supramarginal gyrus (Petrides, 2012). Because the aipsJ often approaches the first caudal superior temporal sulcus and may appear to merge with it, there has been considerable confusion in its identification. The aipsJ was examined in forty magnetic resonance images of human brains in order to define its relations to the intraparietal sulcal complex and the caudal terminations of the superior temporal sulcus. The results demonstrate that the aipsJ forms a number of patterns with the nearby sulci. In the majority of cases, the aipsJ emerges out of the main stem of the intraparietal sulcus as an inferiorly directed side-branch. In a smaller number of cases, the aipsJ is a small shallow sulcus attached to the main stem of the intraparietal sulcus and the connection between the two sulci is observed only in a few horizontal sections. When the aipsJ is underdeveloped, it appears as a notch on the surface of the brain. The aipsJ appears to mark the boundary between an anterior section of the intraparietal sulcus related to the supramarginal gyrus (areas PF and PFG) and a more posterior part related to the angular gyrus (area PG).

G - Novel Methods and Technology Development

3-G-177 Two-photon optogenetics and FRET sensors for studying the role of cGMP in living neurons

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Cyclic GMP (cGMP) is a second messenger with a variety of physiological functions, including synaptic plasticity in the nervous system. However, its role in activity-dependent dynamics at the synapse is poorly understood due to a lack of visualization and manipulation techniques. Here we report the development of genetically encoded cGMP sensors, in combination with an optogenetic approach using two-photon microscopy, to study the role of cGMP in dendritic spines during synaptic

plasticity. Optogenetics is a powerful tool to non-invasively manipulate cellular functions by light. We utilized Blgc, a mutant photoactivatable adenylyl cyclase (PAC) that produces cGMP by binding GTP instead of ATP. We demonstrated cGMP production by activating Blgc by various light sources in vitro. To apply this optogenetic approach to target subcellular regions, we optimized two-photon laser excitation light in vitro. For visualization of cGMP dynamics at the single synapse level in living hippocampal neurons, we evaluated and optimized genetically encoded cGMP sensors utilizing two-photon FRET (Förster resonance energy transfer) both in vitro and in vivo. To avoid photobleaching of the cGMP sensors during the optogenetic light stimulation, we modified the cGMP sensors for FRET/FLIM (fluorescence lifetime imaging microscopy), and demonstrated that Blgc produced cGMP in response to light activation in living hippocampal neurons. This combination of two-photon optogenetics and live imaging techniques will provide new tools for studying the cGMP signaling pathway in living tissues.

3-G-178 A Dynamic Model of the Potassium Chloride Co-transporter KCC2 in Regulating Efficacy of Inhibitory Neurotransmission

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The potassium-chloride co-transporter KCC2 plays a critical role in neuronal chloride (Cl⁻) homeostasis, and therefore regulation of inhibitory neurotransmission. Post-translational modifications of KCC2 such as phosphorylation and oligomerization in the membrane may have significant effects on KCC2 transport function, which modifies the strength of GABAergic inhibitory synaptic transmission. Phosphorylation of specific residues in the intracellular domain have been shown to play an important role in KCC2 membrane stabilization, and implicated in the recruitment of additional KCC2 molecules for oligomerization, which has been suggested to be critical to proper transport function. In this study we hypothesized that phosphorylated KCC2 is a more efficacious transporter than its unphosphorylated equivalent, in part because this phosphorylation promotes oligomerization. We test this

hypothesis using experimentally-constrained computational modeling, first creating a single compartment model to simulate event probability of KCC2 transport based on phosphorylation state. We compared simulation data to both biochemical and electrophysiological data obtained from hippocampal neurons to verify the model. We then expanded our model to include morphometric data from a CA1 pyramidal neuron, allowing us to assess the effects of KCC2 phosphorylation on transport function in distal dendrites. This model allows us to observe these effects in real time, and draw conclusions about Cl⁻ regulation in distal dendrites, which have been previously experimentally intractable.

3-G-179 Quantification of Protein Levels in Single Cells

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Accurate measurement of the amount of specific protein a cell produces is important for investigating basic molecular processes of the cell. The current methods for determining protein amounts have poor cellular resolution and are inherently destructive to cells, limiting the accuracy and relevance of the measurements. We have developed a technique that allows for quantitation of protein levels in single living cells. This Protein Quantitation Ratioing (PQR) technique uses a genetic tag that produces a stoichiometric ratio of a fluorescent protein reporter and the protein of interest during protein translation. The fluorescence intensity (i.e., brightness of the cell) is directly proportional to the number of molecules produced of the protein of interest, and thus is used to determine the relative protein amount within the cell. Using quantitative imaging and electrophysiology, we demonstrate that PQR can produce stoichiometric separations and linear relationships between different genes. Using the circadian system, we demonstrate cyclical changes in fluorescence in small lateral ventral neurons in the *Drosophila* brain. We use genome editing techniques to insert Protein Quantitation Reporters into endogenous genomic loci in three different genomes for quantitation of endogenous protein levels. The Protein Quantitation Ratioing technique allows for measurements of endogenous or exogenous protein amounts in

single living cells, to relate cellular phenotypes as a function of protein concentrations.

3-G-180 Efficient gene delivery into the mouse hindbrain using in utero electroporation

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Manipulation of gene expression via recombinant viral vectors and creating transgenic knock-out/in animals has revolutionized our understanding of genes that play critical roles during neuronal development and the pathophysiology of neurological disorders. Target-specific genetic manipulations are made possible in Cre-lines, albeit costly, labor-intensive and time consuming. Thus, alternative methods of gene manipulations have been adapted to address important biological questions. In this study, we utilized an in utero electroporation technique which involves efficient delivery of a hindbrain-specific enhancer/promoter construct into the 3rd ventricle of live mouse embryos to investigate GFP expression pattern in the mouse hindbrain. We created a GFP DNA construct containing a Krox20 B enhancer and β -globin promoter to drive GFP expression in the hindbrain and subsequently introduced it into the lateral ventricles of mouse E12 to E13.5 embryo allowing it to flow to the 3rd ventricle. Electrical currents were applied to allow DNA uptake into the cell and confocal images from fixed brain slices were analyzed for GFP expression. By using a cell-type specific enhancer as well as region specific injection and electroporation, robust expression of GFP was observed. Our long-term goal is to establish the utility of this technique for performing specific genetic manipulations to unravel critical molecular substrates underpinning functional and morphological remodeling of synapses as well as understanding the pathophysiology of certain disorders targeting hindbrain.

3-G-181 Excross: a tool multigene expression mapping in the mouse brain

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Most mental disorders are complex, caused by many genes possibly interacting with various environmental factors. Compounding this

etiological complexity is the heterogeneity of gene expression in the brain, with the majority of genes differentially expressed across brain regions or time. Excross helps understand these expression pattern by providing genome and brain wide views of gene expression. Excross combines data across user provided gene sets to show heatmaps of developmental expression and voxelwise 3D views of adult mouse brain expression (<http://excross.chibi.ubc.ca/>). We use large and expensive public gene expression atlases (Allen Brain atlases) which are underused for the study of polygenic brain diseases because the data is not easily accessible for study beyond the single gene scope. In contrast, we provide a fast tool that only requires a list of mouse genes. A similar tool for exploring human brain gene expression is under construction and available for demonstration.

3-G-182 The Virtual Reality Stroop: Impulsivity and Attention Assessment

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Impulsivity is a multifactorial construct (Evenden, 1999) and no single task can assess all its component. The Stroop is known to assess impulsivity and attention (Lezak, et al. 2012). The VR-Stroop assess impulsivity (both motor and cognitive) and elicit a "stroop-effect" (Henry, Joyal, & Nolin, 2012). For this study, performances on the virtual task were combined with eye-tracking data to determine if it would further improve sensitivity. Volunteers were recruited among the general population (39 females and 13 males, mean age: 26.92 ± 10.63, 20-63). The VR-Stroop consists of color words presented on the screen of a virtual apartment. Concomitantly, color words are spoken. Correct answers required participants to identify matching auditory and visual stimuli (i.e. when the participant hears blue and the color word is written with a blue ink). Distractors also appeared throughout the task. Eye-tracking data were collected via FaceLAB (Seeing Machines). Eye-tracking data was not associated with commission errors ($p > 0.05$) but with omissions errors. Omissions errors were inversely associated with the time spent on the TV ($r = -0.501$, $p < 0.0001$) and associated with the number of deviations ($r = 0.413$, $p = 0.004$). These results suggest that the VR-Stroop alone is sufficient. Eye-tracking data do not improve significantly the sensitivity of this task. It should be explore if eye movements differ throughout

the task or are affected by a certain type of distractor. Further applications are required, especially with clinical populations.

3-G-183 Effective Long-term Upper-Limb Tremor Treatment in Parkinson Disease Patients

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Tremor is the most visible symptom of Parkinson Disease (PD), which significantly impacts the patient's quality of life. Two major aspects that prevent physicians from treating tremor are inadequate objective assessments for tremor and lack of tremor-specific therapy. 23 PD patients underwent kinematic assessment over 64 weeks, receiving Botulinum neurotoxin type A (BoNT-A) injections every 4 months. Recordings were taken with patients in rest, posture, and kinetic states. Degrees of freedom (DOF) at each joint were: flexion-extension, pronation-supination, radial-ulnar at wrist, flexion-extension at elbow, and flexion-extension, abduction-adduction at shoulder. Based on kinematic data and the physician's clinical experience, BoNT-A injection sites and dose parameters were determined. An average decrease of 75% in tremor amplitude at the wrist, 65% in the elbow and 50% in the shoulder was seen following the first treatment cycle. Maximal grip strength was reduced by 20% but did not impact function. The UPDRS rest tremor score (item 20) decreased from 2.75 to 1.81 and action tremor (item 21) decreased from 1.67 to 0.77 by week 16. The Fahn-Tolosa-Marin tremor rating scale showed significant improvement over 8 months. Tremor did not improve for 6 of the 27 patients who had noticeable advances in other PD symptoms. Tremor treatment in PD patients using kinematic guided BoNT-A injections improved tremor scores. The use of objective kinematic assessment allows physicians to pinpoint tremor sources in the arm, increasing efficacy of BoNT-A treatment.

3-G-184 Functional optical imaging of the retina through intrinsic signals

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Optical imaging of retinal intrinsic signals (RIS) is a method that measures changes in light reflectance of the retina that occur following visual stimulation. The aim of this study is to examine the characteristics of RIS as a function of stimulation conditions and to determine the cellular origin of these signals. Experiments were performed on anesthetized rabbits in scotopic condition. RIS imaging was performed using a fundus camera, illuminated by near infrared light. Retinal stimulation consisted of a diffuse flash of green light. Electroretinograms were concurrently recorded. The activity-dependency of RIS was evaluated by observing the effects of stimulus intensity on RIS amplitude and by comparing the RIS with the ERG waves. The cellular origin of RIS was studied by blocking different retinal cell types by injections of various pharmacological agents. Strong correlations were found between RIS amplitude and stimulus intensity as well as with the ERG b-wave. No RIS were obtained when the photoreceptors' activity was isolated with aspartate, suggesting that photoreceptors activity is not at the source of RIS. A small but significant decrease in RIS amplitude was observed using TTX, suggesting a partial role for ganglion cells activity in RIS. The administration of bipolar cells blockers (PDA and APB) affected the RIS amplitude in a dose-dependent manner. Our results indicate that RIS are dynamic signals that can reflect the various levels of retinal activity. RIS originate from the inner retina cellular activity with a strong contribution of bipolar cells.

3-G-185 A Computer model of neuron swelling and shrinkage under synaptic activity. How much can we expect from holographic microscopy?

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Water fluxes through neuron membranes are essential in regulating homeostasis while osmolarity is challenged by heavy synaptic activity or high frequency spiking. The membrane displacement caused by the volume change in response to such activity may be only of a few tens of nanometres. The technique of holographic microscopy, a novel measurement method, enables us to assess these small volume changes using interferometry. However, indirect computations are necessary to infer magnitude and duration of synaptic current from

the phase shift measured by holographic microscopy. In order to achieve such computations, we build a computer model to describe a neuron response to synaptic and spiking activity in terms of changes in volume and refractory index. The reverse use of our model allows computation of magnitude of synaptic events from phase shifts and refractory index. Water fluxes in our model are controlled by passive and active mechanisms of water transport such as cation-chloride-cotransporters and aquaporines. Our model pinpoints some inconsistencies in our current state of knowledge that could be due to unknown volume regulating mechanisms or to poorly described biomechanical properties of membrane, suggesting further experimental investigation. Moreover, we show that a train of synaptic events should be well measured while only large individual synaptic events could be detected but not precisely quantified.

3-G-186 Gene Delivery to the Spinal Cord using MRI-guided Focused Ultrasound

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Gene therapy shows promise in preclinical animal models for the treatment of spinal cord injury (SCI) and spinal muscular atrophy (SMA). For SCI, intraparenchymal injections allow for targeted gene transfer, but this delivery method is invasive and impractical to cover large areas of the cord. Intrathecal injections are also invasive, and although they allow for delivery to a larger area, gene transfer throughout the entire spinal cord and brain may result in detrimental side effects. Here, we demonstrate that MRI-guided focused ultrasound (MRgFUS) can be used to transiently permeabilize the blood-spinal cord barrier and allow for targeted, non-invasive delivery of an adeno-associated virus (AAV) expressing a reporter gene, from the blood to the rat spinal cord. Immunohistochemistry, histology, and confocal microscopy were used to determine the location, efficacy and cell-specificity of gene delivery 13

days post treatment. The results show successful transduction of spinal cord neurons and oligodendrocytes. Overall, this demonstrates that MRgFUS-mediated delivery of AAV is an effective method of non-invasive, targeted gene delivery to the spinal cord.

IBRO – International Brain Research Association

3-IBRO-187 Absence of endosomal SNAREs vti1a and vti1b led to significant neuronal degeneration in central as well as peripheral nervous system.

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SNARE (Soluble NSF attachment protein receptor) proteins play vital role in membrane trafficking events. SNAREs vti1a and vti1b share 30% similarity in their amino acid sequences and have a distinct but overlapping subcellular localization. Vti1a has role in trans-Golgi network/early endosomal fusion whereas vti1b is linked with late endosomal fusion/lysosomal degradation events. Mice deficient of both endosomal SNARE proteins, vti1a and vti1b, die during intrauterine life just before birth, whereas single knockouts and triallelic mice survive and reach normal age without difficulty. These KO mice have various deficits in central (CNS) and peripheral nervous system (PNS). In CNS, they show wide ventricles and lack several fibre tracts including anterior commissure. Corpus callosum thickness is greatly reduced and thalamocortical axons cannot cross pallio-subpallial border. On the other hand, only a few corticothalamic fibres can reach to thalamus. In case of PNS, KO mice show various degrees of neurodegeneration in number of ganglia. Trigeminal (TG), dorsal root (DRG) and nodose-petrosal ganglia show severe neurodegeneration (98%) whereas vestibular and cochlear ganglia show only 15-25% degeneration. This neurodegeneration was due to the lack of delivering efficient plasma membrane required during axonal growth cone formation. Disparity in neurodegeneration among these ganglia could be due to the distance between the ganglia and their target. Overall phenotype suggests that neuronal cell require adequate endosomal traffic events to produce their normal axonal growth.

3-IBRO-188 NMDA-R Affects Cellular Process Formation in Tilapia Melanocytes; a Model for Pigmented Adrenergic Neurons in Process Formation and Retraction

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NMDA-R is an important glutamate receptor implicated in neurogenesis, neuronal migration, maturation and cell death, thus we investigated the role of NMDA-R potentiation by glutamate/KCN and its inhibition by ketamine in the behavior of fish scale melanocytes in vitro. This is aimed at establishing the regulatory role of NMDA-R in this cell type (melanocytes isolated from Tilapia) in a similar manner to what is observable in the mammalian neurons. Glutamate treatment caused formation of short cellular processes localized directly on the cell body while ketamine treatment (inhibition of NMDA-R) facilitated elongation of secondary cellular processes (highly branched) from primary major processes (Less branched); co-incubation of glutamate and ketamine induced short and highly branched process formation. Cyanide toxicity induced degeneration and reduction of cell size while co-treatment of cyanide and ketamine gave changes similar to that observed in glutamate-ketamine co-incubation. NMDA-R is present in the melanocytes. Activation of the receptor reduced elongation process, while inhibition of the receptor facilitated cell process elongation and branching. This confirms that like pigmented adrenergic cells of the nervous system, this cell contains NMDA-R and this receptor also regulates cell process elongation. The study also showed that inhibition of NMDA-R in melanocytes gave opposite outcomes to the role of the receptor in developing neurons; a function that is protective in adult neurons.

3-IBRO-189 Microbats appear to have adult hippocampal neurogenesis, but post-capture stress causes a rapid decline in the number of neurons expressing doublecortin

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A previous study investigating potential adult hippocampal neurogenesis in microchiropteran bats failed to reveal a strong presence of this neural trait. As microchiropterans have a high field metabolic rate and a small body mass, it is

possible that capture/handling stress may lead to a decrease in the detectable presence of adult hippocampal neurogenesis. Here we looked for evidence of adult hippocampal neurogenesis using immunohistochemical techniques for the endogenous marker doublecortin in 10 species of microchiropterans euthanized and perfusion fixed at specific time points following capture. Our results reveal that when euthanized and perfused within 15 minutes of capture, abundant putative adult hippocampal neurogenesis could be detected using doublecortin immunohistochemistry. Between 15 and 30 minutes post-capture, the detectable levels of doublecortin dropped dramatically and after 30 minutes post-capture, immunohistochemistry for doublecortin could not reveal any significant evidence of putative adult hippocampal neurogenesis. Thus, as with all other mammals studied to date, bats, including both microchiropterans and megachiropterans, appear to exhibit substantial levels of adult hippocampal neurogenesis. The present study underscores the concept that, as with laboratory experiments, studies conducted on wild-caught animals need to be cognizant of the fact that stress (capture/handling) may induce major changes in the appearance of specific neural traits.

3-IBRO-190 Histamine impairs midbrain dopaminergic development in vivo by activating histamine type 1 receptors

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Histamine (HA) regulates sleep-wake cycle, synaptic plasticity and memory in adult organisms. Dopaminergic specification in the embryonic ventral midbrain (VM) coincides with increased HA brain levels. To study the effect of HA receptor stimulation on dopamine (DA) neuron generation, we administered HA to DA progenitors, both in vitro and in vivo. Embryonic day 12 (E12) VM expressed HA receptors H1R, H2R and H3R. These undifferentiated progenitors increased intracellular calcium upon HA addition. In HA-treated cultures, DA neuron numbers significantly decreased. We performed intrauterine injections in the developing VM to investigate HA effects in vivo. HA administration

to E12 rat embryos notably reduced VM Tyrosine Hydroxylase (TH) staining 2 days later, without affecting GABA or serotonin neurons. qRT-PCR and Western blot analyses confirmed that several markers important for the generation and maintenance of DA lineage were significantly diminished. We injected H1R or H2R antagonists to identify the receptor responsible for the detrimental effect of HA on DA lineage and found that activation of H1R was required. To identify the cell type susceptible to HA action, we injected embryos of different developmental stages, and found that neural progenitors (E10 and E12) were responsive, whereas differentiated DA neurons (E14 and E16) were not susceptible to HA actions. Accordingly, the expression of H1R is co-localized with the neural precursor marker Nestin at E12, but not at E14. These results reveal a novel action of HA affecting dopaminergic lineage during VM development.

3-IBRO-191 A novel role for medial prefrontal cortex in taste aversion memory

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The role of the medial prefrontal cortex (mPFC) in taste aversion memory processing has been scarcely investigated. Few works have studied its participation in conditioned taste aversion (CTA) extinction memory and only one work described a prefrontal b-adrenergic receptors requirement in CTA memory formation. Nevertheless, it has recently been described that mPFC neurons can encode aspects of gustatory stimuli suggesting that this cortex could be part of the encoding taste network. In this work, we studied the involvement of the mPFC in taste memory processing using the CTA task in rats. We microinfused the protein synthesis inhibitor emetine, the GABA_A receptor agonist muscimol or the N-methyl-D-aspartate (NMDA) receptors antagonist AP-5 into the mPFC before CTA training. We found that all these treatments impaired the formation of CTA long-term memory, but not its acquisition. Furthermore, we infused muscimol before a 72h-retention test session and observed impairment in CTA long-term-memory retention. These results indicate that neural activity, protein synthesis and NMDA receptors in the mPFC are necessary during training or early after for CTA memory consolidation and that this

cortex is required for normal CTA memory retrieval. Altogether, our findings suggest that the mPFC is an essential structure taking part in the CTA memory processing network.

3-IBRO-192 Enriched environment and neuronal plasticity in the hippocampus of adolescent and adult mice

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Enriched environment (EE) on rodents positively regulate the remodeling of neural circuits, promoting memory consolidation, hippocampal long-term changes in the strength of synaptic weight and neurogenesis. However, the fine mechanisms by which environment shapes the brain at different development stages and the duration required to induce such changes are still a matter of debate. In this study, weaned mice were housed in EE for 4, 6 or 8 weeks and compared with matched control mice raised in standard environment (SE). To investigate the differential effects of EE on immature developmental and mature brains, we also housed adult mice (8 weeks old) for 4 weeks in EE. We studied the influence of onset and duration of EE housing upon the structure and function of hippocampal neurons. We found that 1) EE enhances neurogenesis in the hippocampus at youth but not at adulthood stage, 2) EE increases the number of synaptic contacts at every stage, 3) EE modifies differentially long-term potentiation as well as spontaneous and miniature activity at the glutamatergic synapses depending on the onset and duration of EE. Our study emphasizes the importance of environment of life, particularly cognitive, sensory and motor stimulations, on brain plasticity during postnatal maturation.

3-IBRO-193 Supernumerary formation of olfactory glomeruli and morphological recovery following continuous exposure to ligands of specific olfactory receptors

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Olfactory glomeruli are formed by the convergence of axons of the same type of sensory neurons onto the olfactory bulb. Although the anatomical organization of

glomeruli is conserved across species, their particular role in olfactory processing is not fully understood. Also, it remains unclear whether sensory experience actively participate in the formation of glomeruli as in the further process of refinement and remodelling. We studied the composition, formation and maintenance of glomeruli in knock-in mice whose I7 and M72 primary afferents express GFP and beta-gal, correspondingly. Animals were continuously exposed to heptaldehyde and acetophenone, cognate ligands of these olfactory circuits during different stages of postnatal development. Our results revealed that exposure from postnatal day (PD) 0 to 20 led to the formation of permanent supernumerary I7 and M72 glomeruli in a dose and time dependent manner. Glomeruli in exposed mice were formed within the same regions of olfactory bulb and occupy small space volumes compared to non-exposed mice. When exposed from PD 5 to 10 glomeruli were high in number, had an irregular shape, and were interconnected, nonetheless if odorant exposure is interrupted until PD 20, glomeruli morphology is recovered and their number slightly decreased. We suggest that local reorganization of the primary afferents caused by odorant exposure could participate in the process of formation of supernumerary glomeruli and that the developing olfactory system through sensory experience actively maintains glomerular morphology and number.

3-IBRO-194 Memory reactivation and gene expression in striatum, hippocampus and amygdala

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The course of a memory continues after consolidation as this can be modified by retrieval. Extinction or reconsolidation can take place after a memory is retrieved; those processes can attenuate or strengthen that memory, respectively. Pharmacological studies have found elements needed to retrieve a memory; nevertheless different approaches might be used to identify new ones that could play a role after retrieval. In this study we investigated the temporal and regional expression of genes that code for proteins in the MAPK pathway following memory retrieval. We evaluated mRNA levels of *chrm1*, *erk1*, *arc* and

zif268 that code for the cholinergic muscarinic receptor M1, a MAPK, a cytoskeleton protein and a transcription factor, respectively. Gene expression was analyzed in striatum, amygdala, and dorsal hippocampus using Real-Time PCR. At 30 min after memory retrieval of an inhibitory avoidance task, an increase in arc associated to retrieval was observed only in striatum. On the other hand, zif268 increased at 30 min in striatum and hippocampus. The increase of zif268 was maintained in striatum and hippocampus up to 90 and 180 minutes, respectively. We show for the first time that arc and zif268 mRNAs were increased in striatum after memory retrieval and we propose that these changes could be related to processes taking place following memory reactivation. These findings provide insights for manipulating zif268 to change the fate of a memory after retrieval.

3-IBRO-195 Na⁺-dependent glutamate/aspartate transporter (GLAST/EAAT-1) signalosome in Bergmann glia

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Glutamate, the main excitatory amino acid transmitter in the vertebrate brain triggers a wide variety of signal transduction cascades that regulate protein synthesis at the transcriptional and translational levels. Activity-dependent differential gene expression has been attributed to the activation of ionotropic and metabotropic glutamate receptors, however recent findings had shown that the electrogenic Na⁺-dependent glutamate transporters, responsible of its removal from the synaptic cleft participate in glutamate-induced signaling. Although these transporter proteins are present in neurons and glia cells, the vast majority of the transport takes place in glial cells. Within the cerebellum, Bergmann glia cells are responsible for most of glutamate uptake activity through the Na⁺-dependent glutamate/aspartate transporter (GLAST/EAAT-1). With this in mind we decide to investigate GLAST/EAAT1 signaling partners, using cultured Bergmann glial cells, immunoprecipitation assays coupled to Western blot and we measured GLAST activity through radioligand transport assays. Though this approach we were able to demonstrate that the

GLAST signalosome includes among other proteins, the Na⁺/K⁺ ATPase, the Na⁺/Ca²⁺ exchanger (NCX), mTOR, p60Src and Glutamine Synthetase (GS). Interestingly, the use of specific inhibitors for the proteins mentioned reduces GLAST activity, suggesting that signalosome integrity is needed for the proper Glu removal. Our results add new mediators of Glu effects and strengthen the critical role of Bergmann glia in cerebellar glutamatergic neurotransmission.

3-IBRO-196 Regulation of the glutathione and reactive oxygen species during cerebellar granule neurons development

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Reactive oxygen species (ROS) are highly reactive molecules derived from molecular oxygen, these molecules could act as signaling molecules, regulating numerous physiological processes. In this study we aim to determine the relevance of ROS during cerebellar granule neurons (CGN) development. For this purpose we evaluated the levels of ROS and glutathione content in CGN cultures from 0 to 8 days in vitro (DIV). We found that during the first 2 DIV, CGN gradually increase the levels of ROS measured by dihydroethidium oxidation. The highest levels were founded at 2 DIV, this increment was sustained until 3 DIV. After 3 DIV, the levels of ROS return to the basal levels founded at 1 DIV. In addition, the levels of reduced glutathione (GSH) increased two fold from 0 to 1 DIV, preceding the peak of ROS founded at 2 and 3 DIV. The high levels of GSH were sustained until 5 DIV, and at 8 DIV the levels of GSH returned until the basal levels founded at 0 DIV. To determine the relevance of this increase in GSH levels during CGN development, we inhibited the synthesis of GSH with BSO during different periods. When GSH synthesis was inhibited with 48 h treatments from 0 to 2 DIV, the totality of CGN died, which was completely rescued by the antioxidant Euk-134. However, these treatments did not alter the cell viability from 5 DIV onwards. These results suggest that the balance between the antioxidant and pro-oxidant systems are essential for normal CGN development.



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