The Canadian Association for Neuroscience presents

9th Annual Canadian Neuroscience Meeting 2015

ABSTRACT BOOKLET

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www.can-acn.org

INVITED SPEAKERS

Presidential Lecture:

Melvyn Goodale, University of Western Ontario

How We See and Hear Stuff: Visual and Auditory Routes to Understanding the Material Properties of Objects

Almost all studies of object recognition, particularly in brain imaging, have focused on the geometric structure of objects (i.e. 'things'). Until recently, little attention has been paid to the recognition of the materials from which objects are made (i.e. 'stuff'), information that is often signalled by surface-based visual cues (the sheen of polished metal) as well as auditory cues (the sound of water being poured into a glass). But knowledge about stuff (the material properties of objects) has profound implications, not only for understanding what an object is, but also for the planning of actions, such as the setting of initial grip and load forces during grasping. In recent years, our lab has made some headway in delineating the neural systems that mediate the recognition of stuff (as opposed to things), not only in sighted people but also in blind individuals who use echoes from tongue clicks to recognize the material properties of objects they encounter. I will discuss evidence from both neuropsychological and fMRI studies demonstrating that lateral occipital regions in the ventral stream play a critical role in processing the 3-D structure and geometry of objects, whereas more anteromedial regions (particularly areas in the parahippocampal gyrus and collateral sulcus) are engaged in processing visual and auditory cues that signal the material properties of objects.

Plenary Symposium:

Seeing and moving: how the brain controls vision and gaze

Brian Corneil, *Robarts Research Institute*

Through the looking glass: reflections of sensory and cognitive processing in the motor periphery

The oculomotor system, which rapidly moves or stabilizes the line of sight, is one of the best-understood motor systems in the human brain. While this system is often studied via discrete saccadic eye movements made with the head restrained, orienting of the line of sight can be brought about by coordinated movements of the eyes, head, and body, and may also incorporate subtle changes in pupil diameter. A key oculomotor area is the superior colliculus (SC), which coordinates an ancient orienting reflex via outputs that distribute widely within the brainstem and spinal cord to saccadic and other premotor and autonomic circuits. There are key differences in the response properties of such downstream circuits, with saccadic circuits in particular having the highest threshold for engagement. Because of such differences, non-saccadic circuits are, somewhat paradoxically, more responsive to subtle changes in upstream SC signaling. In my talk, I will illustrate how this framework provides a unifying explanation to a variety of curious findings, including short-latency neck and limb muscle recruitment time-locked to the onset of visual stimuli, the modulation of such responses with cognitive state, and the elaboration of non-saccadic responses following sub-threshold stimulation of the frontal cortex.

Christopher Pack, McGill University

A sensorimotor role for oscillations in the visual cortex

Brain activity is often observed to be oscillatory, meaning that it increases and decreases in strength at regular intervals. Oscillations at particular frequencies often vary in strength depending on a sensory stimulus or the cognitive state of the subject. As a result, oscillations have figured prominently into many models of brain function, particularly the hypothesis that oscillations provide a way to synchronize the timing of long-range communication across neural ensembles.
In this presentation I will focus on the spatial structure of oscillations measured via the local field potentials (LFP) in primate visual cortex. In particular I will discuss recent recordings from area V4 of monkeys implanted with chronic multi-electrode recording arrays. Our results show that there is a spatial pattern of oscillatory activity across retinotopic maps of visual space, and that this pattern is reorganized by the execution of saccadic eye movements. Saccade-related LFP patterns in turn seem to regulate the timing of single-neuron spiking activity, providing a possible basis for optimizing perisaccadic visual perception. I will suggest that well-known phenomena such as perisaccadic remapping, saccadic suppression, and

saccadic momentum are consistent with a role for oscillations in linking oculomotor commands with visual processing.

Featured Plenary Speaker:

Mayank Mehta, UCLA

From Virtual Reality to Reality: How Neurons Make Maps

All animals move through space. What are the sensory and biophysical mechanisms that generate mental maps of space? How do these maps contribute to behavior? Despite tremendous progress these questions have not been fully resolved, partly because it is difficult to precisely measure, let alone manipulate, the wide range of sensory and motor variables that change when subjects move in space. Hence, we have developed a noninvasive, immersive and multisensory virtual reality system where precisely controlled stimuli determine the surrounding virtual space, and nonspecific stimuli are spatially uninformative. We simultaneously measured rats' behavioral performance and the activities of thousands of neurons from the hippocampal circuit while rats performed complex tasks, including the Virtual Morris Water Maze task. We also developed computational techniques to decipher the emergent neural dynamics. This integrative, experiment-theory approach provided many surprising results. For example, when only the visual landmarks provide spatial information, more than half of hippocampal neurons shut down and the remaining active neurons are unable to form robust spatial maps, contrary to commonly held theories. Instead, additional multisensory cues are required to generate spatially selective activity. Indeed, inclusion of consistent locomotion cues generates spatial maps, but they encode relative distance traveled, not an allocentric representation of space. Theta rhythm too is significantly altered in virtual reality. We propose a "multisensory-pairing" hypothesis for hippocampal function where the entorhinal-hippocampal circuit forms rapid associations between multisensory stimuli using both cooperative and competitive mechanisms. This can explain the formation of diverse representations of space under different conditions.

Plenary Symposium:

Mike Salter, Sick Kids

From Receptors to Pain: The Molecular Dynamics of Pain

Neuron-microglial interactions are increasingly recognized as being key for physiological and pathological processes in the central nervous system. Microglia have been found to play a causal role in neuropathic pain behaviours resulting from peripheral nerve injury, and a core neuron-microglia-neuron signaling pathway has been elucidated. Within the dorsal horn, microglia suppress neuronal inhibition by a cascade involving activation of microglial P2X4 receptors causing the release of brain derived neurotrophic factor (BDNF). BDNF acts on trkB receptors which leads to a rise in intracellular chloride concentration in dorsal horn nociceptive output neurons, transforming the response properties of these neurons. In addition to suppressing inhibition, peripheral nerve injury causes activity-dependent facilitation at dorsal horn glutamatergic synapses which enhances nociceptive transmission. This enhancement is mediated by intracellular signaling networks involving serine/threonine and tyrosine kinases within nociceptive transmission neurons. Key for this enhancement is facilitation of NMDA receptor function by Src family tyrosine kinases. Recently we have discovered that microglia-to-neuron signaling is not only critical for pain hypersensitivity after peripheral nerve injury but also for the paradoxical hyperalgesic effect of morphine and other opioids. We anticipate that by targeting microglia-neuron signaling pathways new therapeutic strategies for chronic pain as well as its comorbid sequelae may be developed.

Lisa Topolnik, Universite Laval

Synaptic integration and plasticity gradients in dendrites of hippocampal inhibitory interneurons

Hippocampal interneurons play a critical role in the spatiotemporal organization of principal cell assemblies and formation of memory fields. The synaptic mechanisms responsible for recruitment of distinct subtypes of interneurons in governing network activity are a matter of intense investigation, as it is still largely unknown how do interneurons integrate multiple inputs during

specific brain states. In addition, understanding the mechanisms of multiple forms of synaptic plasticity experienced by interneurons and providing for functional segregation of GABAergic inputs converging onto principal cells remain an open question. Using two-photon microscopy and whole-cell patch-clamp recordings in combination with computational simulations, we have examined the mechanisms of synaptic integration and plasticity along a somatodendritic axis of hippocampal basket cells (BCs). Significant fluctuations in the summation of excitatory inputs through a variable contribution of GluA2-lacking AMPA vs NMDA receptors have been detected in dendritic branches of parvalbumin-positive BCs, with a direct impact on the synapse-specific integration and direction of long-term plasticity. In contrast, cholecystokinin-positive BCs have shown a variable gain function for excitatory inputs but also a somatodendritic gradient in the expression of Cav3.1 Ca2+ channels, which controlled LTP induction at inhibitory synapses. The lifetime of Ca2+ elevations in dendrites of BCs was critical in pacing down their activity through the cell type-specific induction of depression at excitatory or potentiation at inhibitory synapses. These data indicate that afferent inputs can differentially activate the two subtypes of BCs through the cell type- and input-specific dendritic mechanisms, providing for flexible recruitment of BCs during network activity.

Featured Plenary Speaker:

Karel Svoboda, HHMI Janelia Farm Research Campus

Illuminating the neural circuits underlying tactile decisions

Optical methods are revolutionizing our understanding of neural circuits. Cellular imaging allows measurements of cording of information in populations of defined cell types and subcellular structures. Optogenetic manipulations permit testing for causality of patterns of neural activity and behavior. We use these tools to dissect the circuit mechanisms underlying tactile decision making in behaving mice.

Keynote Lecture:

Clay Reid, Allen Institute for Brain Science

Functional Connectomics at the Allen Institute

The current decade is emerging as golden age of neuroanatomy. Connectomics began was defined a decade ago, mostly as an aspiration for the future, but is likely to emerge as a mature field in this decade. In the first published use of the term (Sporns, Tononi, and Kotter, 2005 PLos Comp Biol), it was recognized that connectomics should be considered on multiple scales, from the macroscale of entire brains to the microscale of individual synaptic connections between neurons. At the Allen Institute, we have begun a ten-year program to study the cerebral cortex of mice and humans. The mouse program, called MindScope, concentrates on the cortico-thalamic visual system and seeks to examine the computations that lead from visual input to behavioral responses. In this program, there is a strong emphasis on neuroanatomy, or connectomics at a macro- and microscale. Already a large-scale study of mesoscale connectivity in the mouse brain has been completed (Oh et al., 2014 Nature). Future work will include further mesoscale connectivity atlases that concentrate on the mouse visual system, as well as microscale connectivity of local cortical circuits. At a microscale, we have demonstrated that the relationship between structure and synaptic connectivity can be studied in local cortical circuits by combining in vivo physiology with subsequent network anatomy with electron microscopy (Bock et al., Nature, 2011; and subsequent studies), leading towards a functional connectome (Reid, 2012, Neuron). I will examine the near-term and long-term prospects for microscale connectomics and argue that connectomics at all scales must be combined with functional studies to fully exploit its great promise.

Plenary Symposium:

Mei Zen, University of Toronto

The Development and Operation of the C. elegans Motor System

Animals sense environments and respond with changes in motility. These sensorimotor behaviors are fundamental to life, and governed by nervous systems. All nervous systems undergo postnatal development, predicting changes in circuit connections. There is however surprisingly little understanding of how synaptic wiring changes, and how they affect behaviors during development. In C. elegans, the 302 neuron adult nervous system was reconstructed by serial section electron microscopy

(EM), yielding the first connectome for an entire animal. Yet the adult nervous system starts from 220 neurons at birth; the animal also grows fourfold in size, adding new glia, muscles and epidermis. Throughout postnatal development, the nervous system remains functional, even as new neurons, glia and non-neuronal partners integrate into existing circuits. Combining EM, calcium imaging, optogenetics and molecular genetics, we are addressing how the growing nervous system remodels and regulates motor behaviors across development. Applying acquired insights on motor circuit function, we have further established relevant C. elegans models for human disorders, and applying them to address underlying physiological defects.

Douglas Altshuler, University of British Columbia

Visual motion perception in avian flight

Flying birds use diverse sensory information to guide their flight, and the available evidence suggests that visual information plays a prominent role in flight control. For example, the avian midbrain in general, and the optic tectum in particular, are relatively much larger than homologous regions in other vertebrates. Although there is considerable information available about the neural circuitry for sensing and processing visual motion in birds, there is a major gap in our understanding of how motion perception is used during flight. Our research aims to unify electrophysiological and immunohistochemical investigations of neural circuitry with behavioural studies of visual guidance. We work with hummingbirds and zebra finches, which differ in flight behaviour and neuroanatomy but have fast wingbeat frequencies and similar muscle physiology. Our most recent work has demonstrated that hummingbirds are extraordinarily sensitive to global visual motion during hovering flight, even when the motion is small and displayed in the presence of prominent stationary features. Current research is focused on how visual motion processing guides other flight modes and flight mode transitions, and how cellular responses to visual motion differ across flight behaviours and species.

Featured Plenary Speaker:

Kristin Scott, UC Berkeley

Taste processing in Drosophila

The ability to identify food that is nutrient-rich and avoid toxic substances is essential for an animal's survival. Although olfaction and vision contribute to food detection, the gustatory system acts as a final checkpoint control for food acceptance or rejection. The fruit fly Drosophila melanogaster tastes many of the same stimuli as mammals and provides an excellent model system for comparative studies of taste detection. The relative simplicity of the fly brain and behaviors, along with the molecular genetic and functional approaches available in Drosophila, allow the examination of gustatory neural circuits from sensory input to motor output. We have utilized a combination of molecular, behavioral, and calcium imaging studies to determine the taste ligands that different gustatory neurons detect and how taste information is processed in the higher brain. More recently, we have begun to examine how hunger, satiety and learning influence activity in taste circuits and regulate feeding decisions. These studies provide insight into how taste compounds are detected and processed by the brain.

Parallel Symposia:

Symposium 1: ***Glial handling of neuronal functions: from synapses to blood flow***

Overview:
Glia are very dynamic cells that regulate a large variety of neuronal functions. This workshop will highlight some recent aspect of the dynamic and pluripotent roles that glial cells play in the regulation of various neuronal functions. This will span from the fine regulation of molecular organisation at synapses to the whole in vivo brain activity and functions. Stéphane Oliet will discuss the dynamic regulation of glutamate transporters in processes of astrocytes while Keith Murai will discuss the role of glial interactions in shaping dendritic spines. Marie-Eve Tremblay will discuss the interplay between astrocytes and microglial cells in the regulation of synapse elimination and Hélène Girouard will then discuss the regulation of blood flow by astrocytes as a function of neuronal activity. This would represent an opportunity to have a workshop regrouping young Canadian investigators (Girouard and Tremblay) with more senior researchers (Oliet and Murai).

Speakers:
Stéphane H. R. Oliet | Université de Bordeaux

Surface dynamics of the astrocytic glutamate transporter GLT-1

Marie-Ève Tremblay | Université Laval
Microglial remodeling of neuronal circuits in the healthy brain

Keith Muray | McGill University
Neurons actively sustain the unique molecular and physiological properties of astrocytes in the adult brain through morphogen signaling pathways

Hélène Girouard | Université de Montréal
The astrocytic contribution to neurovascular coupling in health and disease

Symposium 2: *Development and Processing of Vocal and Social Communication*

Overview:
There is tremendous information carried in all vocalizations. For instance, we are very sensitive to human voices and can readily recognize others by listening to their voice. Our vocal inflections also have emotional content which relay how we feel, and what we hope to elicit in others. This means that our communication system is fundamentally a social one, and many other social animals share the basic properties of human vocal communication. This conceptual link allows us to study the communication systems in humans and other species. Recently, much progress has been made in understanding the neural mechanisms underlying various components of communication in both humans and non-human animals. These studies reveal how the brain extracts, represents, and encodes not only the physical features of a communication signal but also their perceptual representations and other abstract quantities (eg, semantic meaning). In this symposium, we will discuss new and exciting data that identify these important relationships across a variety of animals (songbirds, cats, monkeys, and humans) using an integrative approach of psychophysical, electrophysiological, and functional imaging techniques.

Speakers:
Yale E. Cohen | University of Pennsylvania
Mechanisms Underlying Auditory Decision-Making

Stephen G. Lomber | University of Western Ontario
Vocalization Processing Along a "What" Processing Pathway in Auditory Cortex

Sarah M.N. Woolley | Columbia University
Neural Basis and Behavior of Social Communication

Susan A. Graham | University of Calgary, Hotchkiss Brain Institute
Preschoolers' Real-Time Processing of Vocal Emotional Information

Symposium 3: *Shaping inhibition: new insights into the development and function of GABAergic inhibitory interneurons in the cortex*

Overview:
In the mammalian cortex, GABAergic inhibitory interneurons are remarkably diverse in terms of morphology, connectivity, and physiological properties. In addition to their recognized roles in maintaining the E/I balances, recent studies have suggested that different subtypes of inhibitory interneurons can be involved in sensory processing, learning and memory, and cognitive behavior. In this symposium, speakers will present data using a variety of approaches, including genetic manipulations, electrophysiology, optogenetics, and in vivo two-photon imaging to provide new insights into the development and function of GABAergic inhibitory interneurons. Dr. Graziella Di Cristo will discuss how neural activity regulates the innervations of cortical basket cells. Dr. Melanie Woodin will address the molecular mechanisms of synaptic plasticity of inhibitory synapses in the hippocampus. Dr. Mingshan Xue will move into in vivo system and show how different subtypes of interneurons equalize E/I ratios during visual processing. Dr. Simon Chen will focus on the role of subtype-specific reorganization of inhibitory circuits during motor learning in awake and behaving mice.

Speakers:
Graziella Di Cristo | Université de Montréal
Mechanisms regulating GABAergic cell innervation fields in the adolescent brain

Melanie Woodin | University of Toronto
Inhibitory Synaptic Plasticity and Chloride Regulation in the Hippocampus

Mingshan Xue | Baylor College of Medicine
Inhibitory synapses equalize excitation-inhibition ratios across cortical neurons

Simon Chen | University of California, San Diego
Cell-type specific reorganization of inhibitory circuits during motor learning

Symposium 4: ***Neural stem cells in cognitive repair and aging***

Overview:
An evolving body of work indicates that resident stem cells function to maintain and in some cases repair tissues. These findings have led to the idea that if we could recruit these stem cells, then we could enhance repair or regeneration. For example in rodents, exercise, learning, and enriched environment enhance, and stress and aging suppress neurogenesis or oligodendrogenesis. Here, Liisa Galea will present data on how estrogens affect hippocampus-dependent neuroplasticity and cognition and how reproductive experience moderates those effects in aging. Cindi Morshead will present on how metformin repairs stroke damage in postnatal mice by enhancing neurogenesis. Don Mabbott will talk about his recent findings (in review in New Eng J Med) on how exercise greatly improves cognition and increases hippocampal volume and white matter in children with brain injuries. David Kaplan will provide a short overview and short talk on maternal influences on adult neurogenesis in progeny.

Speakers:
David Kaplan | The Hospital for Sick Children
Introduction, and Long-term effects of maternal infection and diabetes on neural stem cell pools

Lisa Galea | University of British Columbia
Estrogens, memory, neuroplasticity and aging: the good, the bad and the ugly

Cindi Morshead | University of Toronto
Activating endogenous stem cells to promote brain repair and cognitive recovery

Donald Mabbott | The Hospital for Sick Children
Training the brain to repair itself

Symposium 5: ***Imaging brain complexity***

Overview:
The brain is a complex organ, containing billions of neurons, each connected through synapses to several thousand other neurons. Therefore an appreciation of how the brain works necessarily involves understanding how information is integrated both in individual neurons, as well as across brain regions. This symposium brings together 4 Canadian researchers tackling this complexity at both the micro- and macroscales. Using new tools to image dendritic activity at high speed in vivo, Podgorski will show how sensory inputs are integrated in a single neuron in the developing Xenopus. Frankland has developed whole brain activity dependent mapping approaches in mice, and used graph theoretical approaches to define functional networks engaged by fear memory. Mohajerani has developed voltage-sensitive dye wide-field imaging approaches to investigate hippocampal-cortical interactions during sleep. Ko uses FDG-PET in patients with movement disorders, and will present graph theoretical analyses to understand the underlying cause of aberrant network activity.

Speakers:
Kasper Podgorski | Howard Hughes Medical Institute
Comprehensive 3D imaging of synaptic activity in the awake brain

Majid Mohajerani | University of Lethbridge
In vivo optical imaging assessment of mouse cortical-hippocampal dialogue during sleep

Paul Frankland | The Hospital for Sick Children
Pharmacogenetic interrogation of a fear memory network

Ji Hyun Ko | University of Manitoba
Network analysis approach with metabolic PET imaging in neurodegenerative movement disorders

Symposium 6: ***Are you what you eat? Impact of diet on mesocorticolimbic circuit***

Overview:
Obesity can be viewed as a disorder of decision-making. Feeding is not only governed by homeostatic energy signals, but also by stress,

variety and availability of low cost calorically dense foods, habitual factors and even previous diet that govern our decisions of what and when to consume. This symposium will explore the neurobiological mechanisms of how diet can influence our feeding behavior. Dr. Fulton will demonstrate that lipid type can influence signaling and behavior within the mesolimbic system. Dr. Winstanley will demonstrate that impulse control is affected in rats consuming a high fat diet. Dr. Borgland will demonstrate that a cafeteria diet promotes compulsive eating and dysfunction in the orbitofrontal cortex. Finally, Dr. Dagher will show functional imaging data on decision-making, food valuation, and appetite control as they relate to weight gain in humans. Together, this symposium will implicate diet-induced alterations in the mesocorticolimbic system resulting in changes in ingestive behaviour.

Speakers:
Thierry Alquier | University of Montreal
Regulation of mesolimbic function, reward and feeding by lipids

Catharine Winstanley | University of British Columbia
Steady-state consumption of a high-fat diet can decrease impulse control even in the absence of excessive weight gain

Stephanie Borgland | Hotchkiss Brain Institute
Compulsive eating reduces inhibitory control of pyramidal neurons of the lateral OFC

Alain Dagher | McGill University
Brain Endophenotypes of Obesity

Symposium 7: *Establishment and maintenance of cell diversity in sensory system function*

Overview:
The degeneration of neurons in sensory systems and their associated pathologies, such as loss of vision and olfaction, represent a growing problem in our aging population. The development of stem cell based regenerative therapies in sensory systems requires a fundamental understanding of the molecular mechanisms underlying the generation and function of these neurons. This symposium will describe how both intrinsic and environmental factors impinge on the generation and function of neurons in the visual, olfactory, and somatosensory systems. Dr. Cayouette will present a novel transcriptional cascade controlling temporal identity progression in retinal progenitor cells and how it might improve strategies for cell replacement therapy. Dr. Wallace will discuss the importance of maintaining morphogen responsiveness for the production of neurons in the retina. Dr. Deppmann will discuss the function of TNF receptor family members in the formation of somatosensory neurons and their importance for proprioception and touch sensation. Dr. Cloutier will describe how cell-cell interactions influence both the generation and survival of sensory neurons in the olfactory epithelium.

Speakers:
Valerie Wallace | Toronto Western
Notch and Hedgehog cross talk in neural progenitors converges on Gli2 activity

Michel Cayouette | Institut de recherches cliniques de Montréal
A Conserved Regulatory Logic Controls Temporal Identity in Mouse Neural Progenitors

Christopher Deppmann | University of Virginia
Molecular Rheostats Governing Sensory Perception

Jean-François Cloutier | McGill University
Cellular interactions in the control of neural progenitor cell differentiation

Symposium 8: *Homeostatic plasticity: molecular mechanisms and physiological function*

Overview:
Synapses have the capacity to alter their strength in a process called synaptic plasticity. Plasticity can occur at individual synapses in the form of LTP and LTD, forming the cellular basis of learning and memory, or more globally during homeostatic plasticity in order to regulate neuronal firing rates and network activity. Homeostatic plasticity was originally described as a scaling of excitatory synapses. Recent investigations have shown that homeostatic plasticity involves different synapse types, occurs over different spatial scales and in multiple brain regions. Despite advances in understanding the molecular mechanisms underlying homeostatic plasticity, its impact on information processing or on learning and memory through interaction with LTP/LTD remain largely unknown.

This symposium will highlight the various forms and functions of homeostatic plasticity, including plasticity of excitatory and inhibitory synapses, the involvement of glia, and the role of this plasticity type in different physiological states such as inflammation, stress and sleep.

Speakers:
David Stellwagen | McGill University
TNF-mediated suppression of striatal reward dysfunction

Jaideep S. Bains | Hotchkiss Brain Institute
State-dependent plasticity in stress circuits

Salvatore Carbonetto | McGill University
Dystroglycan Mediates Homeostatic Plasticity at GABAergic Synapses

Graham Diering | John Hopkins University
Homeostatic scaling-down of excitatory synapses during sleep

Symposium 9: *Regulatory mechanisms in cortical neurogenesis*

Overview:
The regulation of neurogenesis in the developing cerebral cortex is highly dynamic and complex, being influenced by gene regulatory programs interacting with epigenetic mechanisms to establish cell fates. The symposium with open with a lecture from Dr. Carol Schuurmans (University of Calgary) on her recent findings concerning the role of the proneural factors Neurog2 and Ascl1 in priming cell lineage selection in the neocortex. This will be followed by a lecture by Dr. Stefano Stifani (McGill University) on mechanisms that antagonize the functions of proneural factors during cortical neurogenesis and regulate the transition from neurogenesis to gliogenesis. The third lecture by Dr. Ruth Slack (University of Ottawa) will highlight recent findings that mitochondrial function impinges on cortical cell fate decisions. The final talk of the session by Dr. David Picketts (Ottawa Hospital Research Institute) will discuss the crucial role of chromatin remodelling in balancing cortical progenitor proliferation with differentiation.

Speakers:
Carol Schuurmans | University of Calgary
Cortical lineages are primed by the competing lineage determinants Neurog2 and Ascl1

Stefano Stifani | McGill University
Regulation of neurogenic and anti-neurogenic transcription factors during murine cortical neurogenesis

Ruth Slack | University of Ottawa
Mitochondrial -mediated regulation of stem cell maintenance and cell fate decisions

David Picketts | Ottawa Hospital Research Institute
Defining the role of chromatin remodeling proteins in balancing progenitor expansion with differentiation during cortical neurogenesis

Symposium 10: *New insights into classical memory issues.*

Overview:
This symposium brings together a group of internationally renowned researchers who study cutting edge issues in memory processing. The talks will discuss issues involved from simple to complex systems. Specifically, using wild-type and mutant strains of c. elegans, Prof. Rankin explores nuanced issues on the molecular neurobiology of memory consolidation mechanisms. Using a technique that enables single cell resolution over brain areas, Prof Sauvage will discuss the specific areas implicated within the medial temporal lobe mediating memory tasks. Prof Bolshakov will discuss a completely new finding in neuroplasticity. All previous work has shown that the molecular and cellular correlates induced by learning are reversed when amnesia is induced. Prof Bolshakov found that learning induced changes in pre-synaptic efficacy. Surprisingly, he found that reconsolidation blockade led to a reduction in the post-synaptic mechanisms but spared the pre- synaptic changes induced by learning. The pioneering work in forgetting byProf Kida shows that forgetting is a neurobiologically conserved phenomenon and not brain dysfunction as commonly believed.

Speakers:
Catharine Rankin | University of British Columbia
Rethinking habituation: New Insights into the Complexity of the Simplest Form of Learning

Magdalena Sauvage | Ruhr University Bochum
New evidence for a segregation of spatial and non-spatial memory subnetworks along the proximodistal axis of the hippocampus

Vadim Bolshakov | McLean Hospital, Harvard Medical School
Diminishing fear by disrupting retrieval-induced synaptic restabilization

Satoshi Kida | Tokyo University of Agriculture
Erasure of recent and remote fear memory by enhancing forgetting through increase in adult hippocampal neurogenesis

Symposium 11: *Linking nervous system development with function*

Overview:
Nervous system developmental mechanisms have been studied for many years now, but their precise link to specific neural circuit functions remains unclear. Considering the high number of neurological disorders that arise because of developmental defects, this symposium seeks to link specific developmental events and aspects of neural circuit function. We will present evidence tying the molecular specification of spinal interneuron subclasses to locomotor circuits. We will discuss the impact of developmental sensory experience on the connectivity and function of the vertebrate visual system. We will discuss the dimorphic cellular and genetic mechanisms that direct the formation of a sex-specific neuronal circuit required in females for reproduction. Finally, we will explore how molecular mechanisms controlling neuronal stem cell development contribute to the function of cognitive neural circuits. The emergent theme of this symposium is that linking developmental processes with circuit function is essential to understanding neurodevelopmental disorders.

Speakers:
Freda Miller | Hospital for Sick Children
Understanding cognitive disorders: from neural stem cells to neurons

Ying Zhang | Dalhousie University
Distinctive developmental pathways of functional subpopulations of V3 interneurons in the mouse spinal cord

Douglas Allan | University of British Columbia
Genetic mechanisms underlying sexually dimorphic development of female-specific neural populations in Drosophila

Edward Ruthazer | McGill University
How sensory experience controls circuit wiring in the developing visual system

Symposium 12: *Dysregulated synaptic plasticity in models of brain disorders*

Overview:
Synaptic plasticity is a fundamental neural process critical for brain development and function. Dysregulations in synaptic plasticity are closely associated with many neurological and mental disorders, including Alzheimer's disease (AD), Huntington's disease (HD), Neuropathic pain and drug abuse, which represent a major burden to our society. Therefore, investigating synaptic plasticity and how it is altered in these brain disorders is a key to the understanding and treatment of these diseases. In this symposium, we will bring together leading researchers working on synaptic plasticity in various animal models of brain disorders; Dr. Dumont will present studies on compulsive behaviors in rats including compulsive drug intake, Dr. Min on neuropathic pain, Dr. Sepers on transgenic models of HD, and Professor Collingridge on current state of synaptic plasticity and mechanisms underlying synaptic degeneration that is a core feature of AD. Therefore, the proposed symposium will be timely and of interest to a wide range of audience.

Speakers:
Éric C. Dumont | Queen's University
Altered plasticity at glutamate and GABA synapses in compulsive behaviours in rats

Min Zhuo | University of Toronto
Aberrant synaptic plasticity and treatment in animal models of neuropathic pain and anxiety

Marja D. Sepers | University of British Columbia
Endocannabinoid-mediated synaptic plasticity at cortico-striatal synapses in the YAC128 model of Huntington's disease

Graham L Collingridge | University of Bristol
Dysregulated synaptic plasticity in models of Alzheimer's disease

POSTER SESSION 1

A – Development

1-A-1 Lipid mediator prostaglandin E2 alters calcium homeostasis during neuronal differentiation in neuroectodermal stem cells

Jennilee Davidson[1], Hongyan Li[1], Dorota Crawford[1]

[1]York University

Lipid mediator prostaglandin E2 (PGE2) is an endogenous molecule that plays an important role during early development of the nervous system (NS). Abnormal PGE2 signaling due to environmental impacts can result in the pathology of the NS and has been implicated in neurodevelopmental disorders like Autism Spectrum. We previously showed PGE2 influences gene expression, migration, proliferation and differentiation of neuroectodermal stem cells (NE-4C). In this study, we aimed to elucidate if PGE2 can alter calcium homeostasis in neuronal differentiation of NE-4C cells. Using ratiometric fura-2AM calcium imaging we assessed cytosolic and growth cone calcium dynamics of differentiated NE-4C cells. We have found PGE2 alters calcium homeostasis in a time- and dose-dependent response. We found 3 and 24 hours of 0.1, 1 and 10 µM PGE2 exposure on day 12 significantly increased both cytosolic and growth cone mean intracellular calcium level ([Ca2+]i) as well as the amplitude and minimum (min)/maximum (max) levels of fluctuation in the growth cones. When PGE2 was continuously present during differentiation, cytosolic [Ca2+]i significantly changed depending on the concentration, whereas growth cone mean, amplitude and min [Ca2+]i did not change, but max [Ca2+]i increased significantly. Our results show that PGE2 can disrupt calcium homeostasis during neuronal differentiation in a time- and dose-dependent manner. Our data suggests that prenatal increase in PGE2 may adversely impact calcium signaling in differentiating neurons and result in pathologies of the NS.

1-A-2 Spatio-Temporal Heterogeneity of the Spinal Cord Central Canal

Kathryn Douglas[1], Dongho Lee[1], Jane Roskams[1]

[1]University of British Columbia

We used data from the Allen Spinal Cord Atlas (ASCA) to mine for cell-specific gene expression to enhance our understanding of the identity of spinal cord progenitors. The ependymal cell layer (ECL) of the central canal (CC) of the spinal cord is considered to be a neural stem cell niche, as cells isolated from it possess the potential to differentiate into astrocytes, oligodendrocytes, and neurons in vitro and when transplanted into brain neurogenic zones. Using in-situ hybridization (ISH) data from the ASCA, we discovered temporal and spatial segmentation of CC cells expressing distinct groups of genes. We categorized each gene as exhibiting expression at either postnatal day 4 (P4), postnatal day 56 (P56) or both. These ages are relevant to understanding the mechanisms of neurogenesis and gliogenesis, as the P4 spinal cord is capable of regenerating functionality following spinal cord injury, whereas the P56 spinal cord is not. Gene ontology analysis suggests that these different regions of the CC may contribute in different ways to spinal cord regeneration and repair. We show here that many of the gene expression patterns seen in ISH from the ASCA are maintained by the corresponding proteins in the ECL: this differential protein expression suggests the existence of subpopulations of ependymal cells. Furthermore, we find that the CC ependymal cell niche experiences altered protein expression after different types of injury or insult, such as spinal cord injury or demyelinating lesion. These results have potential applications for spinal cord regeneration and repair.

1-A-3 The Role of Activator E2Fs in Stem Cell Maintenance during Neurogenesis

Raghda Gemae[1], Mireille Khacho[1], Alysen Clark[1], Kristen Stevens[1], David Park[1], Ruth Slack[1]

[1]University of Ottawa

The recent discovery of adult neural precursor cells (NPCs) holds much hope for the potential regeneration of damaged brain tissue. However, their use has been limited by their low numbers and relatively quiescent state, particularly in the aging brain. Previous studies from our laboratory have demonstrated a crucial role for the Rb/E2F pathway in NPCs regulation and proliferation, in addition to the importance of E2F3 in the regulation of the pluripotency factor, Sox2, in NPCs. As activating E2Fs (1-3) are regulators of gene transcription, it is important to understand how they function to

maintain the NPCs population during embryogenesis and in the adult brain. Knowing that E2F2 expression has not been previously demonstrated in the brain, we have investigated the role of E2F1 and E2F3 in neurogenesis embryonically by knocking both these genes out using an Emx1Cre and a NestinCreERT2 to specifically delete E2F3 in the cerebral cortex. To eliminate possibilities that E2F2 may be playing a role in the E2F1/3 double knockout, these results will be compared to an E2F1/2/3 triple knockout Emx1Cre model. Our results show a decrease in NPCs proliferation, suggesting that E2F1 and E2F3 are essential for the maintenance of stem cells and neurogenesis from embryogenesis to adulthood, and that they regulate multiple genes required for stem cell self renewal. This will be beneficial in the development of therapeutics to enhance neurogenesis in the context of brain damage or neurodegenerative disease.

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1-A-4 Hoxb8 intersection defines a role for Lmx1b in excitatory dorsal horn neuron development, spinofugal connectivity and nociception

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Spinal cord neurons respond to peripheral noxious stimuli and relay this information to higher brain centers but the molecules controlling the assembly of such pathways are poorly known. In this study, we use the intersection of Lmx1b and Hoxb8::Cre expression in the spinal cord to genetically define nociceptive circuits. Specifically, we show that Lmx1b, previously shown to be expressed in glutamatergic dorsal horn neurons and critical for dorsal horn development, is expressed in nociceptive dorsal horn neurons and that its deletion results in the specific loss of excitatory dorsal horn neurons by apoptosis, without any effect on inhibitory neuron numbers. To assess the behavioral consequences of Lmx1b deletion in the spinal cord, we used the brain-sparing driver Hoxb8::Cre. We show that such a deletion of Lmxb1 leads to a robust reduction in sensitivity to mechanical and thermal noxious stimulation. Furthermore, such conditional mutant mice show a loss of a subpopulation of glutamatergic dorsal horn neurons, abnormal sensory afferent innervation and reduced spinofugal innervation of the parabrachial nucleus and the periaqueductal gray, important nociceptive structures. Altogether, our results demonstrate an important role for the intersection of Lmx1b and Hoxb8::cre expression in the development of nociceptive dorsal horn circuits critical for mechanical and thermal pain processing.

1-A-5 Docosahexaenoic acid status and neurodevelopment at birth are comparable in controls and neonates born to well-controlled gestational diabetes mellitus

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Background/aims: Docosahexaenoic acid (DHA) is a key fatty acid for neurodevelopment. Non-esterified DHA seems to be the preferred form for building-up brain membranes. Lower DHA levels are reported in blood of fetus born to gestational diabetes mellitus (GDM). The aims of this study were to evaluate at birth whether non-esterified DHA in cord blood is 1) modified by GDM and, 2) associated with neurodevelopment of the offspring.

Methods: Offspring of 46 pregnant women (21 controls, 25 with diet- or insulin-controlled GDM) were recruited. Cord blood was collected for measuring DHA in the non-esterified fatty acids (NEFA), the phospholipids (PL), the cholesteryl esters (CE) and the triacylglycerols (TAG). An EEG was performed to the newborns 48 hours post-delivery. EEG data were recorded using a 15-channel bi-polar montage. Data were sampled at 200 Hz and acquired for 10 minutes. We developed a new method allowing identification of artifact-free periods and then computing the mean spectral power.

Results: In GDM vs. controls, maternal A1C was 5.3 ± 0.5% vs 5.4 ± 0.3% (P = 0.803), respectively; in cord plasma, DHA levels in the NEFA, the PL, the CE and the TAG were similar in both groups. The mean signal of the 15 EEG electrodes was -0.986 ± 0.005 dB in GDM vs. -0.985 ± 0.005 dB in controls (P = ns).

Conclusion: Our novel electrophysiological method allows for reliable recording of brain activity in newborns. Neurodevelopment was similar in neonates with comparable cord esterified and non-esterified DHA, under tight-controlled GDM or physiologic conditions.

1-A-7 The Effect of Microglia on Progenitor Cells During Tuberal Hypothalamic Development

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Microglia influence during central nervous system (CNS) inflammation and injury is an active area of research. However, new research has shown microglia begin to invade the embryo brain around embryonic (e) day 10.5 in mouse, suggesting a unique role microglia in CNS development. My overall research is focused on asking three questions: (1) what are the microglia populations present during development; (2) what mechanisms direct and attract microglia towards the developing fetal brain; (3) determine what functional role microglia plays in the developing hypothalamus.

I have systematically identified the timing of microglia invasion into the developing hypothalamus and microglia activation state using fluorescent imaging approaches. I have defined the spatiotemporal timing of microglia invasion in the developing embryonic tuberal hypothalamus and am now determining the influence of microglia on developing cell populations by using pharmacological knock down models. Specifically, pregnant CD1 mice were sacrificed and embryonic brain tissue was harvested on e11.5, e13.5, e15.5 and e17.5. Cryosectioned brains were labeled with microglia and hypothalamic markers for fluorescent imaging followed by stereological analyses employed to quantify the hypothalamic microglia population. My data suggest a possible role for microglia in maintaining hypothalamic progenitor populations given their pivotal position during development, where they can interact with progenitors.

1-A-8 In Situ Imaging of Intracellular Axon Guidance Signaling

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Despite important advances in our understanding of the mechanisms of axon guidance, the location and timing of the intracellular events leading to growth cone behavior in relation to guidance cue detection remain obscure. One emerging approach is the imaging of intracellular signaling in situ, which has the powerful advantage of linking biochemical-level events with specific cellular structures. We are using this approach to investigate the molecular cascade downstream of ephrin:Eph signaling in the context of spinal motor axons, where it plays a significant role in their guidance. Specifically, we characterized antibodies raised against the phosphorylated forms of candidate effector proteins in cultured motor neuron growth cones. Using immunohistochemistry, we show robust, localized, and ephrin dose-dependent phosphorylation of known Eph effectors, the Src family kinases. The guanine nucleotide exchange factor and Eph effector ephexin, is similarly phosphorylated in growth cones after exposure to ligand. These phosphorylation events occur in dense puncta that co-localize with EphB receptors and ephrin ligands, suggesting that signaling occurs in small, contained environments within the growth cone. By imaging signaling events downstream of axon guidance signals, we may begin to link the known biochemistry with the emergent behavior of growth cones. The multiple overlapping signals presented to growth cones during development suggest that molecular convergence events mediating their integration may be localized in time and in space.

1-A-9 Neuronal activity of surrounding axons instructs retinal ganglion cell axon growth in the Xenopus laevis visual system

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Competing presynaptic inputs use neuronal activity to select their appropriate postsynaptic partners during the development of neuronal circuits. We have previously shown that in Xenopus tadpoles, an occasional stray retinal ganglion cell (RGC) axon is misdirected to the ipsilateral tectum where the other retinal inputs originate from the contralateral eye. Two-photon imaging of ipsilateral RGC axons revealed that asynchronously stimulating the two eyes upregulated branch additions in the ipsilateral axon. In contrast, synchronous stimulation stabilized the arbor. Synaptic transmission is required for the

axon to be stabilized by synchronous stimulation, as both tetanus toxin light chain (TeNT-LC) expression to prevent synaptic transmission and MK801 treatment to block NMDA receptors prevented the stabilization. The enhancement of branch dynamics under asynchronous stimulation conditions was not prevented by these manipulations. Thus, we sought to determine whether it was primarily mediated by unpaired firing activity in the lone axon or by activity in its neighbors. We found that stimulation of the ipsilateral eye alone is not sufficient to upregulate axonal branch motility, but stimulation of the contralateral eye alone can enhance axonal branch motility by the ipsilateral axon. Thus, firing of surrounding contralateral axons non-cell-autonomously promotes branch motility in the non-coactive ipsilateral axon.

1-A-10 Imaging the longitudinal development of structural sex differences in the mouse brain using in vivo manganese enhanced magnetic resonance imaging

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Previous research has demonstrated that the development of the brain differs in males and females, and some areas show sexual dimorphisms beginning in neonatal life, a critical period where many sex differences are thought to emerge. However, a comprehensive, whole brain assessment of where and when sex differences emerge in the brain is not yet available. This project aims to characterize the development of male-female differences in the whole mouse brain and establish the trajectory of these sex differences in the brain. Ten male and ten female C57Bl/6 mice were longitudinally imaged with manganese enhanced magnetic resonance imaging (MEMRI) at 90 micron resolution (imaging protocol from Szulc et al, 2015, in press). Up to seven mice were imaged simultaneously, in vivo, at postnatal days 3, 5, 7, 10, 17, 23, 29, 36 and 65 (fig1a). Image registration techniques were used for analysis. Preliminary results show that all sex differences in the brain are present by postnatal day 23, while different sexual dimorphisms in the brain emerge at different times between birth and day 23. For example, the medial amygdala, which is known to be larger in males, does not show any difference until postnatal day 10 when the sex difference emerges, and then remains stable until the last time point at postnatal day 65 (fig1b). Longitudinal in vivo MEMRI effectively captures developmental changes that occur in the male and female brain, and will allow us to further explore more detailed development of sex differences in the brain longitudinally.

1-A-11 AF1Q Interacts with TCF7 to Facilitate Neural Stem Cell Proliferation

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ALL1-fused from chromosome 1q (AF1q), originally considered as an oncogenic factor, has been implicated in the pathogenesis of neurodegeneration. AF1q is highly expressed during neurodevelopment, but its specific functions and molecular mechanisms in neural system remained elusive. Our study here demonstrated that AF1q facilitated neural stem cell proliferation. Since previous studies have shown WNT signaling being involved in neural stem cell proliferation, we examined whether AF1q could induce cell proliferation by activating Wnt signaling pathway. Reporter assay showed AF1Q could activate WNT signaling. And coimmunoprecipitation (CO-IP) analysis demonstrated that AF1q bound specifically to T-cell factor/lymphoid enhancer binding factor-7 (TCF/LEF7), which stabilized TCF7 and facilitated TCF7 translocation into nucleus. Additionally, we identified the amino acid 11-20 on AF1Q is sufficient for the binding of TCF7. Furthermore, the phosphorylation of Serine 11 on AF1Q is required for the binding of TCF7. The AF1Q-S11F mutant decreased the activation of WNT signaling and was unable to induce cell proliferation. Our study here identified AF1Q as an important factor in neurodevelopment by interacting with TCF7 and regulating WNT signaling pathway.

1-A-12 Identification of a novel interaction between Pannexin 1 and Collapsin response mediator protein 2 that regulates neuronal development

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Pannexin 1 (Panx1) is highly expressed in the brain and forms channels permeable to ions and metabolites of up to 1 kDa, including ATP. Panx1 was originally detected in mature neurons. We discovered that Panx1 is also expressed in developing neurons of the ventricular zone and positively regulate their proliferation and migration, while negatively regulating neurite outgrowth. To understand the specific molecular players involved in Panx1 signaling in developing neurons, we performed the first unbiased proteomics screen for Panx1 protein interactions partners. Here we present data for a direct interaction with collapsin response mediator protein 2 (Crmp2), a protein that plays a key role in neuronal maturation by positively regulating neurite outgrowth. We demonstrate that Panx1 and Crmp2 associate both in vitro and in vivo, in developing and mature neurons, and identify the specific region of Panx1 interacting with Crmp2. Next we investigate the hypothesis that Panx1 negatively regulates neurite outgrowth by sequestering Crmp2 using a cell-permeable (TAT) peptide to disrupt the Panx1/Crmp2 interaction. Consistent with this hypothesis, neural precursor cells treated with this interfering peptide show increased neurite outgrowth. Overall, we present several novel findings that expand our understanding of the cellular role of Panx1 in the brain, and the molecular mechanisms underlying its function in neural cells.

1-A-13 Autocrine/paracrine control of neural precursors: an integrated proteomic and transcriptomic approach for defining the cortical precursor niche

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The development of the mammalian brain is a finely tuned process that integrates a precise genetic program with information from the cell environment to generate the three cell lineages required to build the brain: neurons, astrocytes and oligodendrocytes. Radial glial precursor cells are thought to be responsible for the production of these cell types in the cerebral cortex. While a host of different secreted factors (for example the neurotrophins) have been shown to play roles in the proliferation or differentiation process of radial glial cells, very few have been shown to be made by the radial glia themselves (or their progeny) and influence these processes in an autocrine/paracrine manner. In a recent example, we have shown that interleukin-6 (IL-6) can act in an autocrine/paracrine manner to influence radial glial cell proliferation. Spurred by this result, we have devised an integrated proteomic and transcriptomic approach to identify the diversity of secreted factors which influence radial glial cell proliferation and differentiation. Here, we report the use of this approach to define the cortical precursor growth factor milieu, and demonstrate the general importance of autocrine/paracrine mechanisms on radial glial cell proliferation and differentiation.

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

1-B-14 Acute Actions of Gabapentinoids on Neuropathic Spinal Cord Slices: Preferential Actions on Excitatory Neurons

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The α2δ ligands, gabapentin and pregabalin, are treatments for neuropathic pain. However, their mechanism of action is poorly understood. We propose an acute mechanism of action based on data showing that clinically relevant doses of gabapentin significantly decrease overall dorsal horn excitability in approximately 30 min as measured using confocal Ca2+ imaging and patch-clamp electrophysiology of ex vivo spinal cord slice preparations. In addition, we have shown that the acute actions of the gabapentinoids in ex vivo slices correlates with their ability to supress chronic neuropathic pain in animal models using rats subject to sciatic chronic constriction injury (CCI). We characterized excitatory delay firing neurons and inhibitory tonic firing neurons based on firing pattern. Our results indicate that gabapentin decreases excitatory drive to excitatory neurons and increases excitatory drive to inhibitory neurons. Furthermore, by examining postsynaptic firing frequency in response to current ramps, we have established that the effect of gabapentin depends on modification of synaptic transmission as opposed to changes in postsynaptic ion channels. Previous studies of the gabapentinoids in in vitro models have failed to identify any major acute effect since these studies have been mainly carried out on naïve animals or with no reference to effects on specific

cell types. Mechanisms of these acute, cell-type specific actions of gabapentinoids remain to be elucidated.

1-B-15 Projection-target dependent effects of orexin and dynorphin on VTA dopamine neurons

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Ventral tegmental area (VTA) dopamine neurons are a critical part of the neural circuits that underlie reward and motivation. Dopamine neurons send dense projections to many brain areas and recent observations suggest that the properties and functional output of dopamine neurons can be segregated on the basis of projection target. The output of dopamine neurons is regulated by lateral hypothalamic (LH) orexin (also known as hypocretin) neuronal input. These neurons are critically involved in mediating both arousal and reward and can release both excitatory orexin peptides and the inhibitory kappa opioid agonist dynorphin. We have previously demonstrated that orexin and dynorphin exert balanced but opposing effects on VTA DA neurons, but circuit specific effects of these peptides has been largely overlooked. Here we are investigating the effect of orexin and dynorphin on projection-target specific VTA dopamine neurons. To do this, we inject retrograde beads into either the nucleus accumbens (NAc) or the basolateral amygdala (BLA) of adult Pitx3-enhanced green fluorescent protein (Pitx3-eGFP) mice. We then use whole-cell patch clamp electrophysiology to determine the effects of bath application of orexin and dynorphin on the firing rate of fluorescently labeled dopamine neurons. The probability of nucleus accumbens shell-projecting dopamine neurons responding to both orexin and dynorphin is greater than of the VTA-BLA projection neurons. These preliminary results suggest that there may be projection-dependent effects of the LH orexin/dynorphin input to the VTA.

1-B-16 Two-photon optogenetics for controlling PDE activity in living neurons

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Phosphodiesterases (PDEs) degrade cAMP and cGMP, which are essential for synaptic plasticity and memory, providing important regulatory control. However the exact function of PDEs in synaptic plasticity is unclear due to the lack of techniques to locally monitor and control their activity. Here we report the development of two-photon optogenetic tools to manipulate PDEs in a spatiotemporally precise manner, to study their synaptic function. Mammalian PDEs are divided into 3 types: cAMP specific (PDE4,7,8), cGMP specific (PDE5,6,9) and PDEs that hydrolyze both (PDE1-3,10,11). To activate PDE by two-photon illumination, we utilized the recently described light sensitive domain PAS-GAF-PHY from Deinococcus radiodurans linked to the conserved catalytic domain of Homo sapiens PDE2A forming light activated phosphodiesterase (LAPD) (Moglich et al, 2014). To validate LAPD, we first examined the LED light-dependent PDE activity in vitro by measuring cGMP/cAMP degradation with an ELISA. The level of applied cGMP/cAMP decreased within seconds of illumination, indicating strong photoactivation. To achieve necessary precision to target PDEs at a single synapse we used two-photon laser excitation in vitro. At 1000nm, cGMP/cAMP concentrations decreased rapidly within minutes, indicating LAPD photoactivation. The photoactivation for cGMP was less than half compared to cAMP, reflecting the natural affinities of PDE2A. We will discuss other photoactivatable PDEs as two-photon optogenetic tools, for use in in vivo studies of second messengers in synaptic plasticity during learning and memory.

1-B-17 Microglia rapidly adopt a filopodia-rich phenotype upon oxygen depletion by sensing tissue acidosis

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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 long-term stroke recovery, however the acute responses of microglia to the metabolic stresses of ischemia remain unclear. Here, we used two-photon imaging in vivo and in acute brain slices to monitor the initial effect of anoxia on the morphological phenotype and dynamic properties of microglia. The highly

ramified morphology of resting microglia is rapidly transformed during oxygen depletion with extension of fine actin-dependent filopodia followed by retraction of microtubule-dependent ramifications. This rapidly reversible switch in morphology drives significant changes in microglial sensing behavior, affecting microglial cells capacity to respond to tissue damage. Several observations indicate that increased intracellular cyclic AMP is a key trigger of both filopodia extension and retraction of ramifications. Our data suggests that during short anoxia insults, this phenotypic switch is induced by the accompanying acidic shift in the extracellular environment. This pH drop causes microglia to adopt a filopodia-rich phenotype through an increase in cyclic AMP induced by activation of the Gs-coupled proton-sensing receptor TDAG8. Characterizing the highly specialized sensing structures of microglia and defining the molecular cues responsible for the functional switch of microglial behaviour observed upon oxygen depletion will likely provide promising targets for stroke treatment.

1-B-19 Activity-Regulated Trafficking of the Palmitoyl-Acyl Transferase DHHC5

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Neuronal activity can regulate the palmitoylation of proteins, which in turn controls the dynamic localization of proteins to and from synaptic compartments. Here we report that DHHC5's ability to palmitoylate substrates in an activity-dependent manner is dependent on changes in its subcellular localization. Under basal conditions DHHC5 is bound to PSD-95 and Fyn kinase and is stabilized at the synaptic membrane through Fyn-mediated phosphorylation of a tyrosine residue within the endocytic motif of DHHC5. In contrast, DHHC5's substrate, δ-catenin, is highly localized to dendritic shafts, resulting in the segregation of the enzyme/substrate pair. Neuronal activity disrupts the DHHC5/PSD-95/Fyn kinase complex, resulting in DHHC5 endocytosis, translocation to dendritic shafts, and association with δ-catenin. Following DHHC5-mediated palmitoylation of δ-catenin, DHHC5 and δ-catenin are trafficked together back into spines where δ-catenin enhances cadherin stabilization and AMPA receptor surface insertion. This study demonstrates how palmitoyl acyl-transferases regulate synapse plasticity in response to activity.

1-B-20 Mitochondria regulation of neuronal structural and functional plasticity

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The classical view of mitochondria as housekeeping organelles acting in the background to simply maintain cellular energy demands has been shaken by mounting evidence of their direct and active participation in synaptic plasticity in neurons. Time-lapse imaging has revealed that mitochondria are motile in dendrites, with their localization and fusion and fission events regulated by synaptic activity. Recent studies have also shown that mitochondrial protein cascades classically associated with apoptosis are involved in neural plasticity and metaplasticity in healthy cells. To investigate the role of mitochondria on plasticity modulation we performed in vivo imaging of neuronal morphology and activity, and real-time mitochondria tracking in the awake visual system of Xenopus laevis tadpoles. The tri-dimensional analysis of each individual mitochondrion in the entire neuronal dendritic tree show that mitochondria are faster, smaller, and translocate towards motile and stable filopodia 30 min after LTD induction, but no mitochondrial changes were observed in LTP neurons. Our data supports evidence of mitochondria as central organelles controlling the spatiotemporal expression of LTD. A complex model is emerging of an intimate and dynamic relationship between mitochondrial function and neuronal plasticity. More refined measures of the spatiotemporal dynamics of mitochondrial proteins, and their correlation to local functional and structural plasticity are required to decipher precise pathways regulating plasticity and metaplasticity.

1-B-21 Scavenging reactive oxygen species initiates GABA A receptor-mediated electrical suppression in anoxia-tolerant turtle neurons

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Anoxia induces hyper-excitability and cell death in mammalian brain but in the anoxia-tolerant western painted turtle (Chrysemys picta bellii) neuronal

electrical activity is suppressed (i.e., spike arrest), adenosine triphosphate (ATP) consumption is reduced, and cell death does not occur. Electrical suppression is primarily the result of enhanced γ-aminobutyric acid (GABA) transmission; however, the underlying mechanism responsible for initiating oxygen-sensitive GABAergic spike arrest is unknown. In turtle pyramidal neurons there are three types of GABAA receptor-mediated currents: spontaneous inhibitory postsynaptic currents (sIPSCs), giant IPSCs and tonic currents. The aim of this study was to assess the effects of reactive oxygen species (ROS) scavenging on these three currents since ROS levels naturally decrease with anoxia and may serve as a redox signal to initiate spike arrest. We found that anoxia, pharmacological ROS scavenging, or inhibition of mitochondrial ROS generation enhanced all three types of GABA currents, with tonic currents comprising ~ 50% of the total current. Application of hydrogen peroxide inhibited all three GABA currents, demonstrating a reversible redox-sensitive signaling mechanism. We conclude that anoxia-mediated decreases in mitochondrial ROS production are sufficient to initiate a redox-sensitive inhibitory GABA signaling cascade that suppresses electrical activity when oxygen is limited. This unique strategy for reducing neuronal ATP consumption during anoxia represents a natural mechanism in which to explore therapies to protect mammal

1-B-22 Regulation of Chloride Homeostasis by NMDA Receptors

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Within the family of electroneutral cation-chloride cotransporters (CCCs), there are two members primarily responsible for setting the reversal potential for GABA (EGABA): NKCC1 and KCC2. In immature neurons, NKCC1 predominates and mediates the influx of chloride, resulting in high intracellular chloride ([Cl-]i) and depolarizing GABA currents. In mature neurons, KCC2 is robustly upregulated and maintains low [Cl-]i, which is required for hyperpolarizing GABAergic inhibition. Recently, kainate receptors (KARs) have been observed to post-translationally regulate KCC2 expression and function. In this study, we use biochemical and electrophysiological techniques to investigate the regulation of KCC2 and NKCC1 by another class of ionotropic glutamate receptors: N-methyl-D-aspartate receptors (NMDARs). In vivo and in heterologous expression systems, co-immunoprecipitation assays revealed that NMDAR subunits GluN2A and GluN2B interact with KCC2 and NKCC1. Previously, the Ca2+-dependent protease calpain was found to cleave KCC2 upon Ca2+ influx due to NMDAR activation, thereby disrupting KCC2 function and EGABA. Interestingly, GluN2A and GluN2B were found to interact with the non-functional KCC2 cleavage product as well as with full length KCC2 monomers and oligomers. These unexpected interactions between excitatory ionotropic glutamate receptors and inhibitory cation-chloride cotransporters suggest the existence of a functional glutamate-dependent complex capable of regulating neuronal chloride homeostasis.

1-B-23 Heterogeneous populations of neural stem progenitor cells and astrocytes express brain lipid binding protein in aged human neurogenic niches

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New neurons are continuously generated in two main regions of the adult human brain: the subventricular zone (SVZ) of the lateral ventricle and the dentate gyrus (DG) of the hippocampus. Data on the dynamics of adult neurogenesis in humans have demonstrated moderate decline during aging. However, the cellular organization of the neural stem progenitor cells (NSPCs) and the niche-maintaining cells as well as their proliferation activities in aged humans have yet to be fully explored. In this study, we reveal the cellular heterogeneity of the neurogenic niches and show brain lipid binding protein (BLBP) expression in subpopulations of NSPCs using triple immunofluorescence on formalin-fixed brain samples from aged humans. We find spatial heterogeneity along the lateral ventricles, as evidenced by variable thickness of the progenitor-rich astrocytic ribbon and cell-scarce hypocellular gap, which appear to correlate positively with proliferative activity. The integrity of the ependymal cells is also highly variable between different regions of the SVZ. In addition, we demonstrate BLBP expression in the proliferative Sox2+/Nestin+ NSPCs of the astrocytic ribbon, and in GFAP+ astrocytes of the hypocellular gap. In the aged hippocampus, we

identify multiple subtypes of BLBP+ cells: the radial glia and putative NSPCs in the DG and the protoplasmic astrocytes in the molecular layer, both of which exhibit mitotic activities. Thus, our data provide a comprehensive spatial map of the aged human subventricular zone and the expression pattern of BLBP in both neurogenic niches

1-B-24 Nicotinic receptor signaling in principal neurons of the mouse hippocampal formation during postnatal development

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The normal development and function of the hippocampal formation depends on afferent acetylcholine (ACh) neurotransmission that is mediated by nicotinic acetylcholine receptors (nAChRs). Although the α4β2* isoform constitutes a major class of nAChR in the hippocampus, the ability of these receptors to mediate nicotinic signaling in glutamatergic pyramidal neurons of the hippocampus is not well understood. We first sought to determine whether functional α4β2* nAChRs are present on pyramidal neurons located within the cornu ammonus (CA) area 1 of the hippocampus in male CD1 strain mice during postnatal development. Whole-cell electrophysiological responses to ACh in CA1 pyramidal neurons were measured within acute brain slices in the presence of antagonists to both α7 nAChRs and muscarinic receptors. We found that ACh elicited postsynaptic inward currents and facilitated neuronal excitation, and that the magnitude for both of these nicotinic responses was greatest in CA1 pyramidal neurons during the first and second postnatal weeks of life compared with later developmental ages examined. Since the hippocampal formation also comprises the additional CA areas, dentate gyrus, subiculum and entorhinal cortex, we are now continuing to characterize α4β2* nAChR function within the principal neurons of these regions. Our findings demonstrate that α4β2* nAChRs mediate nicotinic signaling in principal neurons of the developing hippocampus, and suggest a role for these receptors in the formation and maturation of hippocampal learning and memory networks.

1-B-25 Inhibitory Synaptic Transmission and KCC2 Function in the Brain of Huntington's Disease

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Proper GABAA-mediated synaptic inhibition requires low levels of neuronal Cl- that is mainly achieved by the K+-Cl- cotransporter, KCC2. When there is a decrease in KCC2 expression or when KCC2's ability to efficiently extrude Cl- decreases, it results in reduced synaptic inhibition contributing toward the pathophysiology of numerous neurological disorders. Huntington's disease (HD) is a progressive neurodegenerative disorder that is caused by mutations in the Huntington protein (Htt). Previous proteomic analysis using mass spectrometry has revealed that the KCC2 encoding gene, Slc12a5, is highly enriched in the Htt proteome, and this interaction decreases when Htt is mutated (mHtt). Based on the critical role of KCC2 in neurophysiological function, and the reported decease in KCC2 interaction with Htt in the Huntington brain, we propose that KCC2 function is compromised in neurons with mHtt. Using co-immunoprecipitation from COS7 cells co-transfected with full length KCC2 and wild type Htt, we found that KCC2 is capable of precipitating Htt. We also performed the experiment in reverse direction and observed that Htt also precipitates KCC2. We are currently determining whether the KCC2: mHtt interaction is reduced or absent compared with the KCC2: Htt interaction. We are also evaluating the physiological and pathological relevance of the interaction. The identification of the KCC2: Htt interaction and its fundamental molecular pathways could represent novel insights into the pathogenesis of HD.

1-B-26 Homeostatic scaling of excitatory synapses during sleep

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Homeostatic scaling is believed to regulate neuronal firing through global, non-Hebbian, adjustments to synaptic weights while maintaining the information stored through Hebbian plasticity mechanisms. While scaling has clearly been demonstrated in neurons in culture, true physiological functions of

homeostatic scaling in vivo are not known. Sleep plays an essential role in normal cognitive functions. Evidence suggests that the benefits of sleep may occur via synaptic mechanisms, including a global weakening of synapses to restore synaptic homeostasis which is perturbed by synaptic strengthening during waking life. Using sub-cellular fractionation, biochemistry and quantitative proteomics, we characterized the changes that occur in forebrain excitatory post-synaptic densities (PSD) during sleep. We observed reduced levels of synaptic AMPA receptors and reduced AMPA receptor phosphorylation consistent with global synaptic weakening during sleep. Further, our data suggest that multiple signaling pathways become down-regulated during sleep suggesting possible mechanisms to drive sleep-dependent synaptic weakening. Sleep-dependent removal of AMPA receptors requires the immediate early gene Homer1a, a protein known to drive homeostatic down-scaling and to regulate sleep. These findings reveal part of the molecular mechanisms at play during sleep and suggest that the physiological function of homeostatic scaling-down may be to renormalize synaptic strength during sleep.

1-B-27 Identification of Na+/H+ exchanger as a possible second target for Bactridine 2

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Bactridine 2 (Bact-2) is an antibacterial toxin from Tityus discrepans venom which modifies the function of isoforms 1.2, 1.4 and 1.6 voltage dependent sodium (Nav) channels. In previous works, we had determined that Bact-2 induced Na+ outward rectifying currents in DRG neurons. We found that these currents, were amiloride sensitives and could correspond to the Na+/H+ exchanger (NHE) coupled to an H+ conductance. To corroborate the effect of Bact-2 as NHE modulator, we characterized the effect of Bact-2 in the regulation of pHi in DRG neurons, using the NH4+ prepulse technique and the pH indicator BCECF-AM. Bact-2 inhibited the pH recovery mediated by NHE, after an acid load of cells, but to a lesser extent than amiloride, a known NHE inhibitor. On the other hand, unexpectedly, cariporide a specific inhibitor for NHE isoform 1 (NHE1) truncated the acid load compared to the control pre-pulse, maintaining pHi values more basic than controls. Since NHE-1 participates in the regulation of early neurite morphogenesis, we also studied the effect of Bact-2 on axon extension, using embryonic hippocampal neurons. We observed that cariporide increased the total axon length. Unfortunately, we were unable to detect significant differences with Bact-2 at the concentration used. These results, suggest that Bact-2 affect the regulation of cellular pH, probably by modulating some NHE isoforms. Finally, in the case of NHE-1, it is possible that we need to test higher concentrations of Bact-2, or that Bact-2 acts on isoforms different from NHE-1.

1-B-28 Altered TORC1-dependent protein synthesis dysregulates the excitatory-inhibitory balance and dendritic branching in vivo

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During early development, neurons undergo extensive growth and rearrangement of their connections, which ultimately leads to the formation of functional circuits. While TORC1-dependent protein synthesis has been implicated in dendritic growth and branching, its role in synapse formation is less clear. Using electrophysiological recordings and two-photon imaging, we therefore investigated the role of TORC1-dependent translation in synapse stabilization and maturation in vivo in the retinotectal system of Xenopus laevis tadpoles. We show that tectal neurons that were electroporated with Rheb, an upstream activator of TORC1, exhibited enhanced AMPA mEPSC amplitudes and frequencies, as well as larger AMPA/NMDA ratios. Interestingly, GABA mIPSC amplitudes or frequencies were not affected, resulting in a significant imbalance in the excitatory-inhibitory (E/I) ratio. To further investigate how a TORC1-mediated E/I imbalance could affect the cell's integration into the circuit, we performed receptive field mapping of excitatory and inhibitory inputs onto Rheb-expressing neurons and observed that TORC1 activation selectively results in larger excitatory receptive fields, suggesting that the developmental refinement of retinotopic inputs to tectal neurons may be dysregulated. Moreover, neurons expressing Rheb have significantly larger and more complex dendritic arbors. These experiments therefore

suggest that TORC1 activity is critical for regulating the number and maturity of excitatory synapses and also contributes to setting the E/I balance in the developing brain.

1-B-29 Calcium Responses to Single Action Potentials in Spinal Cord Lamina I Neurons are Mediated by T-Type VGCCs

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Lamina I neurons of the spinal cord are critical for the integration of nociceptive information from the periphery and relay this information to the brain. Lamina I neurons exhibit hyperexcitability and decreased inhibition in chronic pain models. Voltage-gated calcium channels (VGCCs) have been implicated in the development of chronic pain symptoms, however their functionality in lamina I neurons is poorly understood. Here, we develop an approach to measure calcium responses evoked by individual action potentials (APs) in lamina I neurons. We made current-clamp recordings from the soma of lamina I neurons, loaded via the patch pipette with the calcium indicator Oregon Green Bapta-1 (OGB1). APs were induced by current injection. Simultaneous two-photon imaging of OGB1 fluorescence in the somata, dendrites, and dendritic spines of lamina I neurons allowed for analysis of calcium responses. Single APs induced robust ΔG/R increases in the soma (peak ΔG/R = 0.06, n= 20 cells), nucleus (peak ΔG/R = 0.03, n= 6 nuclei), dendrites (peak ΔG/R = 0.2, n=20 dendrites) and dendritic spines (peak ΔG/R = 0.07, n=5 spines). We found that calcium responses were prevented by the addition of tetrodotoxin. The effects of cadmium, nickel, and TTA-P2 demonstrated that calcium responses were mediated by T-Type VGCCs. These findings suggest that single APs are capable of opening T-Type VGCCs, and eliciting calcium entry into the entire dendritic arbour and nucleus. Calcium influx caused by AP firing could aid integration of inputs and alter gene transcription to upregulate excitability.

1-B-30 Anoxic regulation of mitochondrial membrane potential and ROS production leads to electrical suppression in turtle cerebral cortex

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The anoxia-tolerant western painted turtle (Chrysemys picta bellii) has uniquely adapted to surviving extended periods of low O2 by reducing the activity of energy-consuming pathways. The largest such pathway is the re-establishment of ionic gradients following excitatory transmission. We hypothesize that the mitochondria serve as the nexuses of glutamatergic receptor down-regulation and GABAergic receptor up-regulation with the goal of reducing energetic demand. Here, we show that a mild depolarization of the mitochondrial membrane potential (MMP) during anoxia results in the release of Ca2+, which reduces glutamate receptor activity in pyramidal neurons. MPP collapse is prevented by action of the F1F0-ATPase, which maintains the MPP by pumping H+ out of the matrix. This activity functions in a pH-sensitive manner, as analysis of matrix pH by confocal microscopy using the pH-sensitive dye SNARF-1 indicates a mild acidification of the mitochondrial matrix during anoxia. The reduction in mitochondrial O2 consumption during anoxia limits the production of ROS, which we have found to increase both tonic and phasic GABAergic signaling. We have observed inter-neurons in the turtle cortex identified as stellate neurons that burst fire (0.09 ± 0.01 Hz) at the same frequency as GABAergic phasic currents and number of action potentials per burst doubles during anoxia, suggesting that mitochondrial signaling in these neurons controls widespread GABAergic signaling in the turtle cortex.

1-B-31 Serine 863 regulates surface expression of GluA1 and is Phosphorylated by PAK3

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PAK kinases are highly expressed in the brain, and are known to play a critical role in actin polymerization and dendritic spine morphogenesis. Disruption of PAK kinases activity or expression has been linked to abnormal synapse formation, dysfunctional plasticity, and diseases affecting cognition. Several studies demonstrate that aberrant AMPAR trafficking impairs memory and cognition. Dynamic control of AMPAR recycling to and from synapses is regulated by posttranslational modifications and interactions with accessory proteins. We identified a novel site in AMPAR subunit GluA1 (serine 863) that is rapidly

phosphorylated following brief TTX-mediated disruption of neuronal activity, and can regulate surface expression of AMPARs. Activation of PAK3 through the small GTPases Cdc42 and Rac1 enhances phosphorylation of S863. In contrast, targeted loss of PAK3 expression disrupts activity-dependent phosphorylation of S863 in neurons. The receptor tyrosine kinase EphB2 has been shown to regulate Cdc42 and Rac GTPase activity, in addition to modulating synaptic recruitment of AMPAR. In this study we demonstrate that activation of EphB2 enhances GluA1 phosphorylation at S863. Furthermore, we identify EphB2 as a novel interactor of the guanine-nucleotide exchange factor Zizimin1. In vivo activation of either Cdc42 or Rac1 by Zizimin1 is sufficient to stimulate GluA1 phosphorylation at S863. Collectively, our findings outline a novel signaling cascade that includes EphB2, Zizimin1 and PAK3 in the regulation of S863 phosphorylation and AMPAR trafficking in neurons.

1-B-32 Interactions between excitatory synapse proteins and KCC2 influence KCC2 function and GABAergic inhibition

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A balance between synaptic excitation and inhibition is essential for normal brain function. When this delicate balance is disrupted, it can lead to neuronal hyperexcitability, resulting in alterations in neuronal network activity and the onset of various neurological disorders. KCC2 is the neuron-specific K+-Cl- co-transporter that extrudes Cl- from neurons and thus maintains intracellular Cl- levels low, which is critical for GABAergic inhibition in the mature nervous system. In addition to its role in regulating inhibitory transmission, KCC2 also plays an important role at the excitatory synapse. Here, KCC2 functions as structural support essential for dendritic spine formation and maintenance, and contributes to the structural organization of AMPA receptors at the excitatory synapse. We sought to investigate the interaction between proteins expressed at excitatory synapses and KCC2. We hypothesize that these interactions may form the basis of a protein complex important for the excitation-inhibition balance. To investigate these interactions, we used co-immunoprecipitation assays in heterologous systems and in vivo. Our work sheds light on the effects that excitatory synapse proteins have on KCC2, suggesting a potential role for this interaction in the regulation of excitation-inhibition balance in the CNS.

1-B-33 Modulation of PTEN/mTOR pathway through Ndfip1 over-expression promotes neuronal survival and regeneration following injury

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Spinal cord injury involves a mechanical injury to the axons which results in progressive degeneration of the axons and subsequent neuronal cell death. PTEN plays as a negative regulator of axon outgrowth and regeneration. Pharmacological inhibition or genetic ablation of PTEN increases neurite outgrowth and axon regeneration after injury. However, the nuclear form of PTEN is crucial for neuronal survival. Ndfip1 is an adaptor protein which regulates PTEN trafficking to the nucleus causing its reduction in the cytosol and increasing its pro-survival nuclear pool. This mechanism has not been previously shown in neurons, making Ndfip1 a potential factor for down-regulating PTEN in the neuronal cytosol, while maintaining its role in their survival.

Here we have shown that Ndfip1 expression in cultured neurons resulted in the reduction of PTEN level in the cytosol leading to the activation of mTOR pathway. Moreover, nuclear trafficking of PTEN was stimulated by overexpression of Ndfip1 and overexpression of Ndfip1 increased neuronal survival after mechanical injury. Stretching of axon in an in vitro model for mechanical injury resulted in an increase in apoptotic death of cultured cells. However, neurons overexpressing Ndfip1 showed an increased survival compared to control neurons expressing GFP. Furthermore, we have shown that Ndfip1 expression could reduce cleavage of caspase-3 and could also inhibit the degradation of dephosphorylated NF200. Additionally our results indicate that Ndfip1 can promote axonal outgrowth formation of longer axons in injured neurons.

1-B-34 Activity-dependent and bi-directional plasticity of glutamate synapses on striatal projection neurons in cortico-striatal co-cultures.

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Striatal synaptic plasticity and dendritic spine morphology are implicated in numerous psychiatric and neurodegenerative disorders, including Parkinson's Disease (PD). However, few studies have explored the molecular mechanisms underlying this plasticity, especially within the context of PD-linked gene mutations. We recently reported that mutations in the protein leucine rich repeat kinase 2 (LRRK2; the most common genetic risk factor for PD) alter synaptic activity of cortical neurons in cultures from LRRK2 G2019S knock-in mice: glutamatergic transmission was increased, and the phosphorylation status of presynaptic proteins was reduced. Here, we explored the effects of synaptic silencing, NMDAR-dependent long-term potentiation (LTP) and mGluR1/5 dependent long-term depression (LTD) in cortico-striatal co-cultures. We quantified striatal spiny projection neuron (SPN) spine morphology and density by confocal microscopy to examine the activity-dependence of structural plasticity, and measured changes in synaptic proteins, including surface expression of glutamate receptors. Manipulating cortical glutamatergic activity significantly altered spine structure and synaptic proteins, particularly after chronic treatment. LTP increased spine density and AMPA-type glutamate receptor subunit density/intensity; the reverse was observed for LTD. This study provides a useful assay to explore the mechanisms regulating SPN synaptic transmission and plasticity, and is now being extended to investigate PD-linked mutations and how altered synaptic activity may contribute to PD pathology.

1-B-35 Spatiotemporal transformations of local calcium dynamics during clustered synapse development

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Pyramidal neurons establish thousands of synaptic connections during early postnatal brain development, marking a key period in neural circuit assembly. Despite the eminent role of calcium in synapse regulation, remarkably little is known about intracellular calcium dynamics during synaptogenesis. Using whole-cell electrophysiology, two-photon calcium imaging and glutamate uncaging in acute hippocampal slices, we found that synaptic NMDA receptor activation during a narrow developmental epoch triggered ryanodine receptor-dependent calcium-induced calcium release (CICR) at CA1 pyramidal cell dendrites. This NMDAR-mediated CICR was a major determinant of spine calcium kinetics, and drove local calcium signal propagation to nearby spines. Moreover, NMDAR-CICR coupling enabled dendrites to biochemically encode a range of distinct spatiotemporal features of synaptic input. We hypothesized that this mechanism may spatially regulate the development of synaptic ensembles in vivo. To test this prediction, we mapped synapse maturation by probing the functional AMPAR and NMDAR content of neighbouring spines and found unequivocal evidence for clustered synapse development along individual dendritic segments. Our results reveal novel developmental features of NMDAR-dependent calcium dynamics suited to instruct the assembly of synaptic microcircuit motifs apt to exploit the nonlinear regimes of dendritic information processing. Dysregulation of these mechanisms may be involved the genesis of subtle microcircuit disturbances underlying neurodevelopmental disorders.

1-B-36 Short-term consumption of high fat food increases long lasting excitatory synaptic transmission onto VTA dopamine neurons

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Dopamine neurons of the ventral tegmental area (VTA) are part of a critical circuit for reward seeking and hedonic feeding. Consumption of energy dense, palatable food activates the mesolimbic dopaminergic circuit to reinforce food intake. It is well known that the sensory properties, such as the perceived palatability, can drive food intake. However, it is unknown if consumption of palatable food can have lasting effects on future food intake or food seeking behaviours. Here, we test the hypothesis that short-term consumption of palatable food can drive future feeding behavior and consumption. Mice given 24 h access to a sweetened high fat diet (SHF) had increased anticipatory and food approach behaviors as well as consumption 2 days later compared to mice given only 1 h access or chow. Immediately after 24 h SHF intake, increased endocannabinoid tone onto excitatory inputs to

dopamine neurons was offset by increased glutamatergic synaptic density onto dopamine, but not non-dopamine neurons. This was followed days later by an increase in glutamatergic synaptic strength that lasted at least a week. Finally, increased food approach behaviour and consumption two days after short-term access to SHF was reversed with intra-VTA administration of insulin, known to selectively induce a synaptic depression at glutamatergic inputs to the VTA. In conclusion, the short-term consumption of SHF induces a long lasting strengthening of excitatory synaptic transmission onto VTA dopamine neurons, indicating a potential mechanism underlying increased food approach behaviors and consumption.

1-B-37 The ciliary gene, EFHC1, implicated in human epilepsy, modulates dopamine signalling in C. elegans

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Aberrant dopamine signalling is associated with various forms of epilepsy. An important question is whether alterations in dopamine signalling contribute to epileptogenesis or are merely a consequence of increased neuronal excitability. In this work, we provide evidence for a direct link between the ciliary protein, EFHC1, commonly mutated in juvenile myoclonic epilepsy, and dopamine signalling. C. elegans EFHC1 localizes to a subset of ciliated dopaminergic neurons responsible for modulating specific behaviors based on food availability. In the presence of food, cilia are mechanically activated, leading to synaptic release of dopamine that is then able to modulate neuronal activity. Interestingly, while EFHC1 is enriched at cilia, it also localizes to synapses. Mutations in EFHC1 cause phenotypes consistent with increased dopamine signalling due to spontaneous dopamine release. Overactivity of a pre-synaptic voltage-gated calcium channel in overlapping cells has the same effect, suggesting that EFHC1 may negatively regulate this voltage-gated calcium channel at the synapse to prevent spontaneous dopamine release. This represents the first evidence that a gene implicated in epilepsy directly influences dopamine signalling and suggests a possible involvement of ciliary proteins in epileptogenesis.

1-B-38 Optogenetic control of second messenger dynamics in dendritic spines during synaptic plasticity using a two-photon approach

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Cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger involved in synaptic plasticity, such as LTP (long-term potentiation). Here we study the role of postsynaptic cAMP in the structural plasticity of dendritic spines by developing two-photon optogenetics and live imaging techniques in cultured hippocampal slices. Dendritic spines are thought to change their shape and properties during LTP. To understand cAMP's role in this process, we prepared a genetically-encoded optical cAMP sensor and used two-photon FRET (Fӧrster resonance energy transfer) microscopy to image neurons during LTP. Time-lapse imaging of two-photon FRET in living neurons allowed us to detect a transient increase in cAMP after inducing LTP via strong tetanus, but we did not detect a change after caged-glutamate uncaging (early LTP induction), suggesting a role of cAMP in late LTP. We have also established a two-photon optogenetic technique to activate PAC (photoactivatable adenylyl cyclase) at targeted dendritic spines to locally produce cAMP. We found that postsynaptic cAMP enhanced dendritic spine enlargement during the plasticity, and it was required within the first minute after induction of spine structural plasticity, suggesting the intracellular signaling critical for the structural plasticity last for less than one minute. The rapid effect of cAMP is requires PKA, but not HCN (hyperpolarization-activated cyclic nucleotide) channels or protein synthesis. We will discuss a role for postsynaptic cAMP in the dendritic spine structure reorganization during synaptic plasticity.

1-B-39 Sex differences in the involvement of spinal P2X4 receptors and BDNF in pain hypersensitivity induced by peripheral nerve injury

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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 essential role for P2X4 receptor-driven release of brain-derived neurotrophic factor (BDNF) from spinal microglia in the maintenance of pain hypersensitivity. However, we recently demonstrated that microglia do not mediate pain hypersensitivity in female mice. The role of BDNF in mediating pain hypersensitivity in females remains unknown. Consequently, we investigated the role of spinal BDNF in neuropathic pain in female and male mice. Neuropathic pain was modeled in mice via spared nerve injury. Mechanical sensitivity was measured using von Frey fibers. First, we found that intrathecal application of BDNF (0.5µg/mouse) in naïve mice induces pain hypersensitivity in either sex. Second, we determined that pain hypersensitivity after spared nerve injury was reversed in males but not females through inhibition of TrkB functioning via spinal application of TrkB-Fc (0.5µg x3 days) and y1036 (5.0µg). Furthermore, we found that inhibiting P2X4 receptors with TNP-ATP (5.0µg) reversed pain hypersensitivity in males but not in females. Our experiments provide evidence indicating that female mice do not use P2X4 receptors or BDNF to mediate neuropathic pain hypersensitivity. Taking into consideration sex differences in the spinal mediation of chronic pain may greatly improve future treatment development.

1-B-40 Enhanced thalamic GABAAR-mediated spill-over inhibition elicits anesthetic-like changes in electrocortical activity that do not require T-type Ca2+ channel activation

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Increased thalamic GABAA receptor (GABAAR)-mediated inhibition can trigger changes in electrocortical activity indicative of sedation. GABAAR-mediated inhibition can be either tonic (i.e. extrasynaptic), phasic (i.e. synaptic), or spill-over (i.e. requiring both synaptic and extrasynaptic GABAARs). Importantly, the alterations in thalamic activity elicited by the general anesthetic etomidate require both synaptic and extrasynaptic GABAARs in vitro. Here we test 2 hypotheses in vivo: 1) enhanced thalamic spill-over inhibition elicits changes in electrocortical activity that resemble those elicited by etomidate, such as increased 8-12Hz and 12-30Hz activity, decreased 1-4Hz activity, and increased spindle-like oscillations; and 2) thalamic T-type Ca2+ channels, which promote 1-4Hz activity, do not mediate these changes. Microperfusion of the extrasynaptic δGABAAR positive allosteric modulator DS2 (100μM), which promotes spill-over inhibition, into the thalamus effected electrocortical activity in wild-type mice, but not in mice lacking δGABAARs. Specifically, during non-REM sleep DS2: (i) increased 8-12Hz and 12-30Hz power (both p<0.032), (ii) decreased 1-4Hz power (p<0.001), and (iii) increased spindle-like oscillations (p=0.034). The electrocortical effects of DS2 were unaffected by blocking T-type Ca2+ channels with TTAP2 (300μM). These results indicate that enhanced thalamic spill-over inhibition elicits changes in electrocortical activity that resemble those elicited by etomidate at the thalamus and do not require activation of T-type Ca2+ channels.

1-B-41 cAMP-dependent protein kinase inhibits a7 nicotinic receptor activity in layer 1 cortical interneurons through activation of D1/D5 dopamine receptors

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Phosphorylation of ion channels, including nicotinic acetylcholine receptors (nAChRs), by protein kinases plays a key role in the modification of synaptic transmission and neuronal excitability. α7 nAChRs are a major subtype of nAChRs in the CNS. Serine 365 in the M3-M4 loop of α7 is a phosphorylation site for protein kinase A (PKA). We used pharmacological and molecular approaches to target PKA and monitor alterations in α7 nAChR activity. In layer 1 interneurons of mouse prefrontal cortex, α7 nAChR currents were decreased upon stimulation with 8-Br-cAMP, a PKA activator. In HEK293T cells, dominant negative PKA abolished 8-Br-cAMP's effect of diminishing α7 nAChR currents, while a constitutively active PKA catalytic subunit decreased α7 currents. In brain slices, the PKA inhibitor KT-5720 nullified 8-Br-cAMP's effect of attenuating α7 nAChR responses, while applying a purified PKA catalytic subunit in the pipette significantly decreased

α7 currents. There was no effect of 8-Br-cAMP on the function of mutant α7 receptors, in which serine 365 was mutated to alanine. The reduced α7 current was due to a decrease in surface expression of α7 nAChRs. The D1/D5 dopamine receptor agonist SKF83822 attenuated α7 nAChR currents from interneurons and this attenuation of nAChR current was prevented with KT-5720. These results demonstrate that dopamine receptor mediated activation of PKA attenuates nicotinic neurotransmission in prefrontal cortical interneurons, which may be a contributing mechanism of dopamine modulation of cognitive behaviors such as attention or working memory.

1-B-42 Norepinephrine Protects Synapses from Depotentiation by Priming Translation-Dependent LTP.

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Norepinephrine (NE) is a key neuromodulator that controls the longevity of distinct forms of synaptic plasticity such as LTP. Activation of beta-adrenergic receptors (b-ARs) is known to boost persistence of LTP in area CA1 of mouse hippocampal slices. We tested the hypotheses that: 1) NE primes synapses for subsequent long-lasting LTP (an effect known as "metaplasticity"); 2) NE-induced metaplasticity requires activation of specific subtypes of b-ARs; 3) This metaplasticity serves to protect potentiated synapses from activity-induced weakening (depotentiation, DPT). Using population EPSP recordings from CA1 of mouse hippocampal slices, we show that NE (10uM) applied well before weak tetanic stimulation (1 x 100-Hz, 1s) induces metaplasticity of LTP by engaging beta-1 adrenergic receptors. LTP persistence was enhanced by prior NE application. Interestingly, 5-Hz stimulation induced DPT in control potentiated neurons, but not in neurons pre-treated with NE before LTP induction. The NE-induced metaplasticity of LTP required protein synthesis, as evidenced by a significant reduction of LTP persistence when slices were acutely treated with a translational inhibitor, cycloheximide, during NE application. Thus, NE primes synapses to undergo long-lasting potentiation when a stimulus that is otherwise sub-threshold for induction of persistent LTP is later applied. One function of NE may be to prime intracellular mechanisms that protect, or immunize, potentiated synapses from activity-induced weakening. (Funded by CIHR)

1-B-43 IKCa channels are a critical determinant of the slow AHP in hippocampus

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Of the many classes of potassium channels recognized in central neurons, few have as key a role in regulating the frequency and pattern of spike discharge as calcium-gated potassium channels. However, an unidentified class of calcium-gated potassium channel that generates a slow AHP (sAHP) that can last seconds exerts a far greater influence on the excitability of almost all neurons. We recently found that a sAHP in cerebellar Purkinje cells is generated by a third class of "intermediate conductance calcium-gated potassium channel" (IKCa) that was not believed to be expressed in central neurons. We used patch recordings in rat or mouse in vitro tissue slices to test the hypothesis that CA1 pyramidal cells express IKCa channels. Under conditions of BK, SK and Kv7 channel block we recorded a potassium current derived from calcium-activated channels of intermediate conductance that is sensitive to IKCa channel blockers (ie TRAM-34) and enhanced by IKCa agonists. The sAHP is known to exert significant control over pyramidal cell excitability by restricting EPSP summation and mediating spike accommodation. Internal infusion of 1 µM TRAM-34 blocked the sAHP evoked by repetitive stratum radiatum stimulation (5-30 pulses, 50 Hz). TRAM-34 further blocked spike accommodation during synaptic trains or direct current injection in wild type but not IKCa knockout animals. These data reveal the expression of IKCa channels in CA1 hippocampal pyramidal cells that exert a significant contribution to the calcium-dependent component of the sAHP. *Shared first authorship. Funded through CIHR.

1-B-44 Effects of wild-type and mutant huntingtin on the in vitro corticostriatal synapse

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Huntington disease (HD) is caused by a CAG expansion in huntingtin (HTT), leading to early corticostriatal dysfunction, progressive striatal death and motor, psychiatric and cognitive disturbances. Huntingtin-interacting protein 14 (HIP14) is a palmitoyl acyltransferase responsible for palmitoylation and localization of HTT and synaptic proteins. HIP14 function is potentiated by wild-type HTT (wtHTT) and impaired by mutant HTT (mHTT). Thus, a hypothesis has emerged whereby HIP14 dysfunction in the presence of mHTT results in deficient palmitoylation and mislocalization of its substrates, contributing to early aberrant neuronal transmission in HD. A corticostriatal co-culture model was utilized to study the effects of wtHTT and mHTT on the distribution and palmitoylation of synaptic HIP14 substrates. Immunocytochemical analyses of YAC128 co-cultures expressing mHTT indicate altered localization of the presynaptic HIP14 substrates cysteine string protein and synaptotagmin-1 compared to wild-type, HTT heterozygous, or wtHTT-overexpressing YAC18 co-cultures. These changes correspond with a 15-20% reduction in substrate palmitoylation. Electrophysiological recordings reveal a dramatic reduction in spontaneous excitatory events in YAC128 co-cultures as well as impaired transmitter replenishment following synaptic release. Postsynaptic AMPA receptor distribution and glutamate response are largely unaffected by HTT expression level or mutation. Altogether, these findings suggest that early corticostriatal dysfunction in HD may be mediated primarily through presynaptic mechanisms.

1-B-45 Homeostatic influence on Hebbian plasticity rules at central synapses

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Changes in fine circuit architecture of the brain are believed to participate in neural computation and information storage. This malleability of neural circuits is dependent on the capacity of individual synapses to express rapid structural and functional modifications in response to incoming activity, through mechanisms collectively referred to as Hebbian synaptic plasticity. In the face of perpetual changes in network excitability, slower-acting homeostatic synaptic plasticity (HSP) mechanisms operate bidirectionally to regulate the strength of synapses in order to tune cellular excitability within a defined range. Interestingly, homeostatic and Hebbian synaptic plasticity operate through partially overlapping mechanisms to modify synaptic strength, including through postsynaptic regulation of excitatory glutamate receptors of the AMPA and NMDA subtypes. As such, homeostatic adaptions are well poised to exert strong control over Hebbian synaptic plasticity rules, although this possibility has received little direct attention. Using an established model of HSP in organotypic hippocampal slices, we investigated whether and how homeostatic mechanisms influence Hebbian plasticity rules at CA1 synapses. We show that HSP mechanisms powerfully influence the ability of CA1 neurons to express subsequent synaptic plasticity through Hebbian induction mechanisms. A complete elucidation of the mechanistic interplays between homeostatic and Hebbian synaptic plasticity will contribute to a more comprehensive understanding of how information is processed and stored in the brain.

1-B-46 Control of trauma-induced epileptogenesis in mice and its age dependency

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Severe brain damage leads to epilepsy several months to years after the trauma. The mechanisms leading to epileptogenesis are unknown. We used a model of cortical trauma epilepsy (TIE) in mice cutting the white matter underneath a cortical region. We hypothesized that the reduction in afferent excitatory inputs in the undercut region leads to the upregulation of excitability and, this leads to epilepsy. This homeostatic process might be age-dependent. We performed undercut in the somatosensory area in young and adult C57/BL6 mice and implanted LFP and EMG electrodes for continuous electrographic recordings for at least two months. In the following weeks, we found only isolated interictal spikes in young animals, but all old mice revealed recurrent seizure activities. We used the DREADD technology to test the hypothesis that controlling the level of excitability will control the development of epilepsy. Target cortical regions were injected with AAV-hM3D(Gq) or AAV-hM4D(Gi) and infected neurons were continuously activated via an osmotic pump. We found that activation of

hM3D(Gq), which leads to depolarization and increased firing in infected neurons, reduced the occurrence of seizures in chronic stages, while the activation of hM4D(Gi), which induces a hyperpolarization of neurons, strongly increased the occurrence and severity of spontaneous seizures. We conclude that TIE is age-dependent and that recovering the normal level of activity in the undercut region could lead to the prevention of epileptogenesis.

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1-B-47 Identifying sources of study-to-study variability in neuronal electrophysiology data

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Recently, there has been a major effort by neuroscientists to systematically organize and integrate vast quantities of brain data (Allen Brain Atlas, NeuroLex, NeuroMorpho, NeuroElectro). For example, meta-analysis of electrophysiological data using NeuroElectro.org has shown that various experimental conditions, or metadata (animal age, recording temperature, electrode type) can in part explain study-to-study variance in neuronal electrophysiology values (resting membrane potential, input resistance, spike half-width). Here, we employ a large scale text-mining approach, supplemented with manual curation, that provides us access to quantitative data from hundreds of thousands of published neuroscience articles. Specifically, our goal is to identify additional metadata that may explain more of the electrophysiological variance and further reconcile experiment-to-experiment differences. We are exploring how external (recording) and internal (pipette) chemical compositions affect variance of intrinsic and synaptic measurements (i.e. long term potentiation). Our literature-based approach serves to cover this gap in evidence by extracting metadata from a large corpus of neuroscience articles. Ultimately, normalizing electrophysiology values based on experimental conditions within each neuron type will allow us to link electrophysiological diversity to corresponding differences in gene expression levels and disease phenotypes.

1-B-48 A brain-wide analysis of neuronal transcriptomic and electrophysiological diversity

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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 statistical 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; R2 = .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.

1-B-49 Mild traumatic brain injury produces more immediate and prolonged LTP deficits in the juvenile female brain

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Traumatic brain injury (TBI) is the leading cause of disability in individuals under 45 years of age, with mild TBI (mTBI) accounting for the majority of cases.

The juvenile brain is in a period of robust synaptic reorganization and myelination, making adolescence a particularly vulnerable time to incur a TBI. Learning and memory deficits that involve the hippocampal formation are often observed following mTBI in adults. To examine this issue we examined changes in electrophysiology following closed-head mTBI in male and female Long-Evans rats (25-28 days of age). Synaptic plasticity of field excitatory post-synaptic potentials (fEPSPs) was assessed at either one hour, one day, seven days, or 28 days following mTBI in either the dentate gyrus (DG) or cornu ammonis area 1 (CA1) regions of the hippocampus. In female rats, the CA1 region ipsilateral to the impact showed a significant reduction in long-term synaptic plasticity (LTP) as early as one hour following mTBI. Similar LTP deficits were apparent at one day in the DG, and persisted to 28 days following injury. In male rats, a deficit in both DG- and CA1-LTP was apparent in the ipsilateral hemisphere by seven days following injury, but these deficits did not persist until 28 days post-injury. These data suggest that the juvenile brain is susceptible to mTBI-induced impairments in plasticity, and sex and regional differences are apparent in the expression and recovery of synaptic plasticity following mTBI.

1-B-50 APP facilitates the RCAN1-mediated apoptosis

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Individuals with Down syndrome (DS), caused by trisomy of chromosome 21, inevitably develop characteristic Alzheimer's disease (AD) neuropathology including neuritic plaques, neurofibrillary tangles and neuronal loss after middle age. Amyloid β protein (Aβ), the major component of neuritic plaques, is the proteolytic product of amyloid β precursor protein (APP). APP and regulator of calcineurin 1 (RCAN1) gene on chromosome 21 play a pivotal role in promoting plaque formation and neuronal apoptosis. However, the mechanism underlying AD pathogenesis in DS is not well defined. In this study, we demonstrated that APP significantly increased RCAN1 level in cells and transgenic mice. Overexpression of APP significantly reduced the expression of two proteasome subunits, proteasome α subunit 5 (PSMA5) and proteasome β subunit 7 (PSMB7), leading to inhibition of proteasomal degradation of RCAN1. Furthermore, knockdown of RCAN1 expression attenuated APP-induced neuronal apoptosis. Taken together, the results clearly showed that APP has a previously unknown function in regulating RCAN1-mediated neuronal apoptosis through proteasome pathway. Our study demonstrates a novel mechanism by which overexpression of APP and RCAN1 causes neurodegeneration and AD pathogenesis in DS and it provides new insights into the potential of targeting APP-induced proteasomal impairments and RCAN1 accumulation for AD and DS treatment.

1-B-51 Cannabinoid receptor 1 and Somatostatin receptor subtypes colocalization in rat brain

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Somatostatin (SST) and cannabinoids exert many overlapping functions in central and peripheral tissues via binding to their cognate receptors which belong to GPCR superfamily. Whether these receptor subtypes are expressed in same cell specifically in central nervous system is not well understood. Recently, we described that somatostatin (SST) is colocalized with cannabinoid receptor 1 (CB1) in rat brain hypothalamus and hippocampus. In the present study, by using double-labeled immunofluorescence, we determined the distribution and colocalization of SSTR1-5 and CB1 in rat brain regions including cortex, striatum and hippocampus. Results presented revealed a receptor subtype-and brain region-specific colocalization between CB1 and SSTRs. In cortical brain regions, CB1 and SSTRs colocalize in both cell bodies and apical dendrites in different layers with various intensities. In comparison, colocalization in striatum was less frequently seen among all SSTRs with distinct patterns in projection neurons and interneurons. In hippocampus, colocalization in both neuronal cell bodies and dendrites was observed in CA1-3 and dentate gyrus. In addition to the neuronal population showing colocalization, neurons displaying either CB1 or SSTRs were also seen in different brain regions in receptor specific manner. Our results provide first comprehensive study on colocalization between CB1 and SSTR subtypes and establish a close relationship between these two receptor families which can be explored as possible

therapeutic intervention in certain pathological conditions.

C - Disorders of the Nervous System

1-C-52 Dopamine terminals mediate vesicular release of L-DOPA-evoked enhancement of dopamine in the 6-OHDA lesioned striatum.

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Administration of L-DOPA (≥25 mg/kg) to rats with extensive nigrostriatal degeneration (≥90%) resulted in enhanced dopamine (DA) efflux that was impulse-independent and/or unaffected by DA transporter inhibition (Abercrombie & Zigmond, 1990). However, L-DOPA-derived DA is indeed sequestered into vesicles in striatal DA terminals (Omiatek et al, 2013). The present study utilized microdialysis to determine whether increased DA efflux evoked by L-DOPA in the 6-OHDA-lesioned striatum is mediated by vesicular release. Reverse-dialysis L-DOPA [1 uM] resulted in a significant increase in DA efflux. Changes in DA in response to further experimental manipulations were dependent on the severity of the lesion (moderate [0.4-1.5 nM] or severe [<0.3 nM]) indicated by extracellular basal concentration of DA. In moderately lesioned rats, L-DOPA-evoked DA response was attenuated significantly by inhibition of vesicular transport or omission of calcium from the perfusate. Further, the DA response was eliminated by the D2 agonist quinpirole, and restored by the addition of the D2 antagonist eticlopride, suggesting a role for D2 autoreceptor regulation of DA release. In contrast, in severely lesioned rats, L-DOPA-evoked DA appeared to be from a cytosolic pool as it was unaltered by the same treatments. The present findings suggest that with moderate nigrostriatal degeneration, synaptic release of newly synthesized DA involves sequestration into vesicles and calcium-dependent exocytosis from surviving DA terminals.

1-C-53 In ALS, misfolded wtSOD1 induced by pathological FUS or TDP-43 transmits intercellularly and is propagated misfolding-competent

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Clinically indistinguishable cases of amyotrophic lateral sclerosis (ALS) can be caused by either inheritable mutation in the genes encoding SOD1, TDP-43, FUS, among others, or can occur sporadically. Misfolded SOD1 has been detected in both familial and sporadic ALS patients, despite SOD1 mutations accounting for only ~2% of cases. We previously reported that pathological FUS or TDP-43 kindles misfolding of human wtSOD1 in living cells. Here, we used human cell cultures and mouse primary neural cultures expressing human wtSOD1, to establish that FUS or TDP-43-induced misfolded SOD1 can traverse between cells through incubation of untransfected cells with conditioned media, triggering conversion of endogenous SOD1. This spread is arrested by pre-incubating the conditioned media with SOD1 misfolding-specific antibodies, demonstrating their therapeutic potential. We find that recipient cells pre-treated with SOD1-siRNA do not contain misfolded SOD1, implying that endogenous SOD1 is required as substrate for active conversion. Furthermore, transfection of TDP-43 into cells triggers its cleavage, mislocalization and hyperphosphorylation; these properties are not observed in untransfected cells incubated with conditioned media from TDP-43 transfected cells, further confirming that the transmission of SOD1 misfolding occurs independently of TDP-43.

1-C-54 Rauwolfia vomitoria root extract improves behaviour and provides region-specific reduction in Aβ plaque coverage in the 5xFAD mouse model of Alzheimer's Disease.

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Rauwolfia vomitoria (RV) root extract contains medicinal alkaloids and is used to treat pain, anxiety, psychosis, hypertension and cancer in Africa (Fapojuwomi & Asinwa, 2013. Gr J Med Sci. 3, 37-41). We have shown that RV reduces anxiety-like behaviour without altering motor activity of CD-1 mice (Bisong, et al., 2011. J Ethnopharm. 132, 334-339). The present study investigated the effects of RV on learning, memory and amyloid beta (Aβ) plaque formation in the brains of 6 month old 5xFAD mice. Nine female wild type (B6SJLF2) and nine female 5xFAD mice were dosed with RV (0, 4, or 8 mg/kg, i.p) for 51 days and given behavioural tests for locomotion, anxiety, learning and memory. They

were then sacrificed and their brains removed, cut in 40μm coronal sections and analyzed by immunohistochemistry using polyclonal anti-Aβ antibodies with diaminobenzidine as the chromogen for brightfield microscopy. These sections were imaged at 2.5x magnification and the percent of different brain areas covered by Aβ plaques measured. Behavioural results for locomotion, anxiety, novel object recognition and spatial learning will be presented. There was a significant decrease in percent area covered by Aβ plaques in hippocampus as well as motor and somatosensory cortex in RV treated mice. There was no effect of RV on Aβ plaque load in the auditory cortex, visual cortex, or basolateral amygdala. These results indicate that RV affects behaviour and reduces Aβ plaque load in certain brain areas in 5xFAD mice and should be investigated further as a potential therapy for Alzheimer's disease.

1-C-55 Neuroanatomical and microstructural differences in the brain of a Mecp2 mouse model of Rett syndrome

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder associated with mutations in Mecp2 which leads to an early-life regression in cognitive function and quality of life. These behavioural deficits have been attributed to a loss of dendritic complexity of neurons within the brain. Although magnetic resonance imaging (MRI) has demonstrated alterations in the RTT neuroanatomy, a direct association between the mesoscopic neuroanatomy and the microscopic cellular environment has not been established. Thus, the objecitve of this study was to determine whether the reduced neuronal complexity can be detected with diffusion weighted MRI; reduced dendritic complex in RTT brain leads to increased measures of water diffusion. Hemizygous and wild type Mecp2-tm.1Hzo brains were: a) imaged ex vivo with a diffusion-weighted MRI sequence, b) aligned with an image registration algorithm and c) compared based on a whole-brain, voxel wise analysis of fractional anisotropy (FA), a measure of water diffusion used to infer the order of the cellular environment. Differences in FA were found in many regions of the brain, particularly the cortex, hippocampus, corpus callosum and cerebellum (5% FDR). We are in the process of performing a localized Golgi stain to the regions that show FA changes. This study will be used to determine whether FA can be used as a biomarker of the organization of cellular environment in the RTT brain. These tools can then be used to evaluate treatment success in future studies that aim to reverse the structural impairments of neurons in the RTT brain.

1-C-56 Age-related changes in frailty in the 3xTgAD and 5xFAD mouse models of Alzheimer?s disease

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Frailty is a measure of the accumulation of age-related physical deficits [Howlett SE, Rockwood K. 2013. Age Ageing. 42, 416-423]. Although the concept of frailty was developed for humans, the Frailty Index (FI) can also be used to assess age-related disabilities in mice. The mouse FI consists of 31 health-related variables (integument, digestive, ocular, auditory, urogenital, respiratory systems, signs of sickness, abnormalities in body temperature and weight) [Whitehead J et al., 2014. J Gerontol A. 69, 621-632], with higher FI scores indicating increased frailty. We found significant differences in the lifespan of 3xTgAD and 5xFAD mouse models of AD compared to their wildtype controls. In order to have a measure of aging that was independent of chronological age, we calculated the FI for all of the mice from 3-28 months of age in our animal colonies. FI score was positively correlated with age in both the 5xFAD and 3xTgAD mice and, in both genotypes, males had higher FI scores than females. The 5xFAD mice had higher FI scores than their wildtype (C57BL6xSJL) controls, but there was no difference in their rate of deficit accumulation. The 3xTgAD mice had a higher rate of deficit accumulation than their wildtype (B6129S/F2) controls, indicating that they have a faster rate of age-related frailty than their wildtype controls. These results indicate that both 3xTgAD and 5xFAD mice show more age-related indices of frailty than their wildtype controls, which may explain the shortened lifespan of these mice.

1-C-57 Effects of uncoupling 2B-NMDA receptors from PSD-95 by Tat-NR2B9c in Huntington?s disease corticostriatal co-culture

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Huntington's disease (HD) is a neurodegenerative disorder caused by expansion in the CAG repeat region of the Huntingtin (Htt) gene. Numerous studies suggest that mutant Htt (mHtt) expression causes deficiency in the major astroglial glutamate transporter and elevation in extrasynaptic NMDA receptor (NMDAR) expression, especially those containing GluN2B subunit, in striatum. These changes may contribute to NMDAR overactivation and cell death. Uncoupling of PSD-95 from GluN2B with a disrupting peptide, NR2B9c, can rescue the increased vulnerability of HD striatal neurons to NMDA-induced apoptosis, suggesting the peptide has therapeutic potential in HD. However, little is known about its effects on synaptic function. Here, we investigated whether TatNR2B9c treatment to weaken the PSD-95/GluN2B interaction can ameliorate synaptic signaling changes in striatal neurons in corticostriatal co-culture from the YAC128 HD mouse, a model that expresses human Htt of 128 repeats. Surprisingly, the peptide reduced rather than improved pro-survival signaling. Moreover, 1 hour treatment with TatNR2B9c decreased synaptic GluN2B-NMDAR, which may result in diminished synaptic NMDAR activity and contribute to impaired survival signaling. Currently, we are testing whether NR2B9c peptide impairs NMDAR synaptic function permanently. In addition, we will assess whether this peptide rescues other changes, such as enhanced extrasynaptic NMDAR and impaired synaptic vesicle release in co-culture. Together, these studies will lay the foundation for a preclinical trial of Tat-NR2B9c peptide in HD mice.

1-C-58 The Small RNA Molecule miR-16-5p is an Early Biomarker of Neurodegeneration and a Potential Target for Therapy in Prion Disease

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Introduction: Prion disease is a uniquely infectious, fatal neurodegenerative disease. Interestingly, the clinical stage of this disease can take years to present which may offer a window for therapeutic intervention. Studies on a mouse model of prion disease in our lab uncovered an alteration in expression of microRNA (miRNA) in the hippocampus during this period. MiRNA are small, non-coding RNA molecules that bind to complementary regions in the 3'untranslated region of target mRNA, inhibiting protein synthesis. We hypothesize that one specific miRNA, miR-16-5p, targets mRNA involved in neurodegeneration, and thereby acts as a neuroprotective miRNA. Methods: Primary hippocampal neurons are dissected from mice at embryonic day 18 and treated with a lentiviral vector at maturity. This vector either encodes miR-16 or miR-ZIP, causing overexpression or knockdown of miR-16, respectively. Evaluation of cellular morphology is performed via confocal microscopy. In order to determine which mRNA are targets of miR-16 in hippocampal cells, Argonaute immunoprecipitation (Ago-IP) coupled to microarray analysis is utilized. Validation of targets is carried out by real-time PCR and western blot.
Results/Conclusion: MiR-16 has been successfully overexpressed and knocked down in hippocampal cell cultures. Ago-IP is underway, and targets were predicted using a variety of software programs. Many predicted target genes are involved in hippocampal neuron excitotoxicity such as NOS1, GRIN1, GRIN2B and AKT3. This supports the hypothesis that miR-16 is involved in preventing neurodegeneration.

1-C-59 Two unique activities in the brain of Parkinson's disease model rats: High-Voltage-Spindles and Beta-Oscillation

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Exaggerated beta oscillations in the cortical-basal ganglia-thalamic loop are currently believed was related to the motor symptoms of patients with Parkinson's disease (PD), as well as to the movement abnormalities observed in PD-model animals. In our studies, we have found exaggerated beta oscillations and High-Voltage- Spindle (HVSs) episodes increases in the M1 of PD rats. Interestingly, exaggerated beta oscillations was only occurred in 6-OHDA injection side but HVS appear bilaterally. Reports have indicated that are related to an awake, idling state of animals and disappear right after the latter start to move. Similarly, excessive beta oscillations are

attenuated during voluntary movements. Our studies further reveal that both HVSs and beta oscillations are suppressed by stimulating the subthalamic nucleus (STN) with electric shocks similar to those used in clinical applications of DBS. Beta-frequency oscillations could be suppressed and other oscillations at higher frequencies (40-50 Hz and 85 Hz) were formed in ipsilateral M1-LFP by administration of apomorphine but couldn't be influenced by treatment with amphetamine. Additionally, we couldn't find significant coherence between M1-upper layer (layer I, II/III of primary motor cortex) and M1-deepper layer (layer V) in intact side of hemi-PD model and in normal rats from the results. However, LFP from ipsi. M1-upper layerand ispi. M1-deeper layer coherence emerged in the 30-40 Hz with a strong anti-phase relationship hemi-PD model rats.

1-C-60 Neurophysiologic response to bilateral vs. unilateral therapy for chronic stroke patients with varying degrees of motor impairment

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For patients with chronic stroke, it is believed excitation of the primary cortex of the non-lesioned hemisphere (NLH) exacerbates motor deficits by exaggerating transcallosal inhibition (TCI) upon the lesioned hemisphere (LH). However, recent evidence suggests the NLH may play a compensatory role in recovery for patients with greater motor impairment. If true, then therapies recruiting the NLH would elicit a more adaptive role of the NLH. Therefore, we tested the hypothesis that therapy involving the NLH (bilateral) would lower TCI exerted upon the LH compared to therapy only involving the LH (unilateral); an effect that would become more pronounced with increasing impairment. In a crossover repeated-measures design, six patients with varying degrees of motor impairment (Fugl-Meyer: 15 to 59) underwent a single session each of unilateral and bilateral therapy. We measured excitation of the NLH and TCI it exerts upon the LH using transcranial magnetic stimulation. Overall, bilateral therapy resulted in a greater reduction of TCI (A), where the effect was more pronounced in the more impaired patients (B). However, greater reduction in TCI was associated with less of an increase in excitability of the NLH (C). Our preliminary results suggest that bilateral therapy may invoke an adaptive rather than inhibitory influence of the NLH with greater motor impairment, where excitation of the NLH may influence the degree TCI is reduced. Future work will test whether behavioral outcomes following bilateral therapy is superior to unilateral for patients with greater motor impairment.

1-C-61 Parkinson's disease targets intrinsic brain networks

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Network propagation hypothesis of neurodegenerative diseases (e.g. Alzheimer's disease) proposes that degeneration spreads through brain via intrinsic brain networks. Here we test this hypothesis in Parkinson's Disease (PD). We used Parkinson's Progression Markers Initiative data including T1 MRI, Unified Parkinson's Disease Rating Scale motor score (UPDRS3) and striatal binding ratio (SBR) of 232 PD patients and 117 controls. Brain atrophy was calculated using deformation based morphometry (DBM) and decomposed into spatially independent maps using independent component analysis (ICA). ICA maps were compared to seed based functional connectivity map with Substantia Nigra (SN) as seed. We also investigated the relation between regional functional connectivity and structural deformation. ICA estimated 30 deformation maps. PD patients had higher deformation values in only one map (p=.003) including the entire basal ganglia, amygdala, hippocampus, insula, anterior cingulate cortex, and premotor areas. PD-ICA deformations were correlated with SBR (r=-.22,p<.001) and UPDRS3 (r=-.26,p<.0001). PD-ICA network was spatially correlated with SN functional network (r=-.3,p<.0001). Regional functional connectivity was significantly correlated with regional structural deformation (r=-.41,p<.10-6). Regions demonstrating disease-related atrophy in PD correspond to an intrinsic network in healthy brain. Also, the deformation in a given region was inversely proportional to functional distance from the

presumed disease epicenter in SN. This supports network propagation hypothesis in PD.

1-C-62 Striking differences in the neuroanatomical phenotype of the Neuroligin3 R451C knock-in and the Neurexin1α knock-out.

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Background - Neurexin and neuroligins are synaptic cell adhesion genes that have been associated with autism (Jamain et al., 2003; Kim et al., 2008). Neurexins are on the pre-synaptic side and bind to neuroligin on the post synaptic side. Alterations in either neuroligins or neurexins could alter the excitatory/inhibitory balance (Etherton et al., 2009, 2011). Objectives - To compare the volumetric differences of the neuroligin3 R451C knock-in (NL3 KI) with the neurexin1α (NRXN1α) knock-out mouse. Methods - In total, 48 fixed mouse brains were examined (8 NL3 KI with 8 WT and 10 WT (B6/SV129), 13 NRXN1α (%2B/-), and 9 NRXN1α (-/-). A multi-channel 7T MRI using a T2-weighted, 3-D fast spin-echo sequence was used to acquire images at 56 μm isotropic resolution (Lerch et al. 2011). To compare the groups the images were registered together. Volume differences were calculated voxelwise and in 62 regions. Results - 14 regional differences were found in both the NL3 KI and the NRXN1α (-/-), but the differences were in opposing directions, smaller in NL3 and larger in NRXN1α. Several white matter structures were affected indicative of structural connectivity differences. One of the largest differences was the 12% decrease in the hippocampus in NL3. The hippocampus was not affected in the NRXN1α model (%2B3%, FDR of 16%). In addition to the neuroanatomical differences in the hippocampus, the electrophysiology and behaviour are quite different (Etherton et al., 2009, 2011). Therefore, care must be taken in assessing genetic manipulations involving similar genes or genetic pathway

1-C-63 VIP interneuron re-modelling during stroke recovery.

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During stroke recovery, pyramidal neurons in the peri-infarct cortex undergo structural re-arrangements, at both the level of dendritic branches and spines. In contrast, very little is known about how stroke affects the morphology of cortical interneurons. This knowledge gap should be addressed because many of these interneurons release GABA, acetylcholine and regulate cortical excitability, which is profoundly disrupted after stroke. Of note, interneurons expressing vasoactive intestinal peptide (VIP) specialize in inhibiting other classes of inhibitory cortical neurons, such as those expressing parvalbumin (PV) and somatostatin (SOM). Further, VIP neurons have been shown to regulate cerebral blood flow. Using longitudinal in vivo imaging and Cre-dependent transgenic mouse lines, we are currently investigating how stroke alters the growth and stability of dendritic arbors, spines and axons in VIP interneurons. Preliminary data suggest that, like their pyramidal neuron counterparts, VIP neurons lose a significant number of dendritic spines in the first few weeks after stroke. The dendritic arbors of these neurons are generally stable, although some branch tips clearly retracted from week to week. Based on these preliminary findings, we believe that peri-infarct VIP neurons lose afferent excitatory input which may permit excessive inhibitory activity (in downstream PV and SOM neurons) in the recovering cortex. The functional consequences of stroke related changes in VIP interneuron structure and excitability will be explored in future studies.

1-C-64 Novel motor cortical output pathways following spinal cord injury despite extensive corticospinal loss revealed by optogenetic mapping

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Regeneration of lesioned fibers is limited in the adult mammalian CNS, but many individuals that sustain spinal cord injuries (SCI) undergo some spontaneous functional recovery. Cortical plasticity is one mechanism thought to underlie this recovery, but how the motor cortex responds to spinal cord injury (SCI) longitudinally is largely unresolved. We applied optogenetic motor mapping in Channelrhodopsin-2 expressing mice before and at multiple time points after a C3/C4 dorsal column SCI to map changes in motor cortical topography and output within the

same animals following bilateral ablation of the dorsal corticospinal tract and dorsal column sensory afferents. We find that cortical motor maps of the limbs are greatly diminished in area and magnitude in the early stages of injury. However, by 2-3 weeks post-injury, pre-injury forelimb and hindlimb output, map area, and short latency to movement are re-established. Thus, the adult mammalian motor cortex has the capacity to spontaneously re-route motor output following SCI. Optogenetic motor mapping provides a quantitative assessment of motor cortical output, can be performed repeatedly in individual animals from acute to chronic stages of injury, and represents a novel longitudinal quantitative assay to assess treatment following SCI.

1-C-65 Behavioural comorbidities related to psychiatric disorders in a rat model of absence epilepsy: effects of the T-type calcium channel blocker Z944

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The Genetic Absence Epilepsy Rats from Strasbourg (GAERS) strain of Wistar rats are commonly used as a rodent model of childhood absence epilepsy. In addition to the occurrence of absence seizures in 100% of GAERS, research suggests that the animals also display characteristics of psychiatric disorders including heightened anxiety and increased sensitivity to dopamine agonists. However, a detailed behavioural assessment of the strain has yet to be completed. Therefore, we tested GAERS and Non-Epileptic Control (NEC) rats on a battery of cognitive tests relevant to psychiatric disorders. We found that GAERS rats performed similarly to NEC rats on a test of tactile recognition memory. However, their performance was impaired on a visual recognition test at a one hour, but not five minute, delay. The GAERS strain also showed a profound disruption of crossmodal memory (tactile-to-visual memory) when compared to the NECs. In a classical fear conditioning paradigm, GAERS rats showed normal learning and memory to a conditioned stimulus but impaired extinction and reinstatement. Latent inhibition was also dramatically reduced in GAERS rats. Acute administration of the T-type calcium channel blocker Z944 (10 mg/kg; i.p.) reversed the crossmodal memory deficit in the GAERS rats. These results suggest that the GAERS strain is useful for studying the comorbidities in absence epilepsy as well as the cognitive symptoms of some psychiatric disorders. Experiments with Z944 reveal that altered T-type calcium channel activity may underlie certain cognitive deficits in the GAERS strain.

1-C-66 Thioredoxin system modulates neural stem cell proliferation and differentiation: Implication on Neurotrauma treatment

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Thioredoxin(Trx) system is a major controller of cellular redox status which regulates oxidation/reduction of thiol groups in signaling proteins involved in cell survival & proliferation. Increased availability of Trx1 has been shown to enhance survival & proliferation in neural precursor cells(NPCs). Their contribution towards repair after CNS trauma remains limited. In this study, we hypothesized that; availability of Trx will optimize NPC's contribution to repair after neurotrauma. To increase Trx tissue deposition we employed TAT peptide mediated delivery method to ensure intracellular delivery of Trx(I-Trx). A noTAT protein was used to compare the advantage of intracellular delivery, identified as Extracellular Trx(E-Trx). The effect of I-Trx and E-Trx on NPCs proliferation was quantified in cultures after local delivery. Our in vitro data indicates an increased cell proliferation in brain and spinal cord-derived NPCs in response to Trx treatment. This effect was more pronounced when cells were treated with I-Trx. Additionally, I-Trx increased oligodendrocytes in NPC differentiation. To test efficacy of Trx transduction in preclinical neurotrauma models, we used focal permanent cortical devascularization and clip compression spinal cord injury in rats. Preliminary results from focal devascularization showed an increased number of proliferating NPCs in subventricular zone. We are currently investigating effect of Trx transduction after spinal cord injury. This study represents the first application of intracellular Trx delivery for potential treatment of neurotrauma.

1-C-67 The role of eukaryotic elongation factor-2 kinase (eEF2K) activity in Alzheimer?s disease pathogenesis and relevance for novel therapies.

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Defects in neuronal energy metabolism and synaptic plasticity are thought to underlie cognitive dysfunction and neuronal loss in Alzheimer's disease (AD), and potentially other neurological diseases. The AMP-responsive protein kinase (AMPK), and its downstream target the eukaryotic elongation factor-2 kinase (eEF2K) couple neuronal energy metabolism to neural activity. This mechanism allows for tight regulation of dendritic protein translation in register with synaptic activity, and plays a pivotal role in modulating synaptic strength and plasticity. Previously, we have shown that tumor cells exploit the AMPK-eEF2K axis in developing resistance to nutrient deprivation (Leprivier, G. et al, Cell, 2013), and targeting this pathway represents a potential treatment strategy in cancers. Our recent findings suggest that aberrant activation of the AMPK-eEF2K pathway may also be relevant to neurodegeneration in AD. Previous studies show that eEF2K activity, measured by the phosphorylation of its substrate eEF2, is enhanced in AD brains. We have also carried out detailed analysis in two AD transgenic mouse models (APPPS1 and 3x-Tg AD), and utilized pharmacological and gene silencing approaches in primary neuronal cultures to understand the relevance of AMPK-eEF2K pathway activity on amyloid-β induced deficits in dendritogenesis and oxidative stress. Our results suggest that the AMPK-eEF2K axis may provide a link between aberrant synaptic activity and neurodegeneration in AD, and represents a novel target for developing mechanism based therapies in AD and related dementias.

1-C-68 Characterization of AMPAR surface recycling and synaptic transmission in a novel D620N knock-in mouse model of Parkinson?s disease.

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The pathogenic D620N (DN) mutation in vacuolar protein sorting 35 (VPS35) is linked to late-onset, autosomal-dominant Parkinson's disease (PD). VPS35 is a core component of the retromer complex, involved in endosomal recycling and intracellular trafficking. Data from overexpression models suggests that the mutation confers a loss of function in AMPA-type glutamate receptor (AMPAR) recycling, resulting in aberrant synaptic connectivity. Here we explore early synaptic dysfunction in a novel D620N knock-in mouse model of PD. Western blot and co-immunoprecipitation were performed in tissue from wild-type and mutant mice to explore differences in retromer subunit expression, and binding of neurotransmitter receptors. Fluorescence recovery after photobleaching was used to assay AMPAR surface recycling. Whole-cell patch clamp and immunocytochemistry were used to explore differences in synapse number and response amplitude in cultured cortical cells. We found alterations in VPS35 binding to multiple neurotransmitter receptors, including AMPARs. Cultured cortical cells showed alterations in surface recycling of AMPARs, spontaneous glutamate release, and synaptic strength. Here we conclude that the D620N mutation alters glutamatergic synapse maintenance, and that VPS35 may be involved in the recycling/maintenance of surface populations of other neurotransmitter receptors. Many genes linked to PD appear are involved in synaptic transmission; thus, understanding the role of VPS35 is important for uncovering how disruptions of neurotransmission lead to neurodegeneration in PD.

1-C-69 Alcohol tolerance & histone modifications: histone methylation plays a role in altered response to second alcohol exposure

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Chronic alcohol consumption produces gene expression changes in the brain. A microarray study found altered regulation of histone methylation might underlie altered gene expression in alcoholic brains. To investigate whether histone methylation also plays a role in behavioral changes associated with previous exposure to alcohol we developed a

model using C. elegans to measure a key behavioral indicator of previous experience of alcohol exposure: tolerance, defined in DSM-IV, as a "markedly diminished effect with continued use of the same amount of alcohol". We pre-exposed C. elegans to 200mM ethanol for 24 hrs, and tested for behavioral changes on 200mM ethanol after 2 hrs recovery. Wildtype animals pre-exposed to ethanol showed diminished responses to ethanol compared to those without pre-exposure, suggesting that pre-exposure increased wildtype's tolerance to ethanol. To see whether histone methylation plays a role in our model, we tested animals defective in regulating histone methyltransferases (HMTs), an enzyme required for methylation of lysine residues on histones. We tested two mutants: one carrying a mutation in set-2, a HMT transcriptional activator, and another carrying a mutation in set-11, a HMT transcriptional repressor. Neither mutant showed a diminished response to ethanol following pre-exposure, suggesting that both activator and depressor HMTs were involved in tolerance. Further studies using this model could reveal whether HMTs involve in altering the gene expression profile and behavioral responses following previous exposure to alcohol.

1-C-70 Assessment of attention behaviour and cholinergic signaling in male mice following developmental ethanol exposure.

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Chronic prenatal exposure to ethanol can produce a spectrum of adverse effects known collectively in humans as Fetal Alcohol Spectrum Disorder (FASD). Although deficits in attention rank among the most common and persistent behavioural components of FASD, the mechanisms underlying this outcome are not known. The objective of this study was to determine effects of developmental ethanol exposure on attention behaviour and on acetylcholine signaling in adult male C57BL/6 mice. We measured attention performance in adult male mice that were exposed via oral gavage to either ethanol or sucrose during prenatal and postnatal development using the five-choice serial reaction time test for sustained visual attention. In these experiments, mice in the ethanol treatment group performed with lower accuracy during initial training sessions and then exhibited a greater rate of omissions under challenging training conditions when the light stimulus was presented for short time periods. Mice in the ethanol treatment group also performed with greater impulsivity during the most challenging test conditions, and this effect was corrected by acute treatment with the acetylcholinesterase inhibitor galantamine. We are now continuing to assess neuronal physiology and responses to cholinergic stimulation in the prefrontal cortex of these same mice. This work confirms the presence of attention deficits in this mouse model of FASD, and suggests that dysregulation of the cholinergic system underlies certain aspects of this behavioural outcome

1-C-71 Investigation of --alpha-synuclein phenotype in primary cortical cultures from LRRK2 knockout mice

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Parkinson's disease (PD) is the second most common neurodegenerative disorder with a prevalence of between 2-5% in people over age 65 . Clinically, PD is characterized by rigidity, tremor, bradykinesia, and postural instability , but is often accompanied by non-motor symptoms including autonomic dysfunction, anosmia, REM sleep behaviour disorder, mood disorders, and dementia . PD is pathologically defined by the death of dopaminergic nigrostriatal neurons with Lewy bodies composed primarily of misfolded α-synuclein (α-syn) in surviving neurons . The most common genetic cause of PD is mutation of the gene that encodes leucine-rich repeat kinase 2 (LRRK2), a large, multi-domain protein with functions in vesicular recycling and neurotransmitter release. We are currently investigating α-syn pathology in primary cortical neurons and whether LRRK2 modulates this phenotype. Addition of synthetic α-syn pre-formed fibrils (PFFs) to primary neuronal cultures has been previously shown to induce aggregation of α-syn, neuronal network dysfunction, and eventual neuronal death . Examining differences in the response of wild type and LRRK2 knockout primary cortical neurons to PFFs will elucidate if LRRK2 is involved in the harmful effects of PFFs. This information could be crucial in the characterization of early molecular correlates of the disease and in

the design of therapeutic and neuroprotective strategies for PD.

1-C-72 Assembly of the mammalian palmitoylome indicates a pivotal role for palmitoylation in diseases and disorders of the nervous system

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Palmitoylation involves the reversible addition of the fatty acid palmitate to cysteine residues. The fatty acid moiety promotes membrane binding as well as protein-protein interactions and regulates protein function and activity, through direct palmitoylation of catalytically active cysteines. Like phosphorylation, palmitoylation is regulated by many palmitoylating and depalmitoylating enzymes. Alterations in palmitoylation of various proteins have been implicated in many diseases including obesity, cancer and neurodegenerative diseases, such as Alzheimer disease and Huntington disease. We have compiled a non-redundant list of palmitoylated proteins from 15 palmitoylation proteomic studies from various mouse, rat and human tissues. This list was subjected to an unbiased systems biology approach for biomarker-based disease enrichment and, for the first time, demonstrates that palmitoylation plays a crucial role in diseases and disorders of the nervous system. In particular, Schizophrenia, Heredodegenerative disorders, Chorea and HD were among the top 5 diseases. Indeed, not only was palmitoylation found to be enriched in 'synaptic vesicle fusion and recycling in nerve terminals,' but a significant amount of the synaptic proteome was found to be palmitoylated (~41%). Ultimately, this suggests that aberrant palmitoylation may play a key role in many nervous system diseases. This may provide a myriad of novel targets for the treatment of HD and diseases of the nervous system.

1-C-73 Isolated and combined effects of early-enriched environment and treadmill walking in a model of cerebral palsy in rats: motor behavior aspects

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Cerebral palsy (CP) is a disorder attributed to non-progressive lesions in the immature brain. The treatment of CP is focused on enhance general abilities, physical strength, prevent complications and improve quality of life. However, scientific evidence of the treatments are scarce and the biological mechanisms have not been elucidated. The aim of this research was to evaluate the effects of the early-enriched environment (EE), the treadmill walking (TW) and the association of both (EETW), in order to simulate the reality of clinical rehabilitation. CP model was induced by maternal exposure of low doses of bacterial endotoxin (lipopolysaccharide), perinatal anoxia and hindlimb sensorimotor restriction of male offspring. Pups were divided in 8 groups: (CP), (CP+EE), (CP+TW), (CP+EETW) and its respectively control groups (Figure 1). At 31 and 52 days of life (P31 and P52), the ladder walking test (LWT) and barrow narrow test (BNT) were performed. Data were analyzed by ANOVA for repeated measures followed by Tukey test. At P31 the CPEE decreased the error score, evaluated in the LWT, and the error steps, in BNT when compared with the CP (p<0.001) and CPTW groups (p<0.01). The groups CPTW and CPEETW were able to reverse the error score and error step when compared the day P31 with P52 (p<0.01). Factors such as novelty, motivation and social integration found in EE seem to be crucial in helping to motor recovery. This research joins others in order to elucidate the effects of the treatments used in clinical rehabilitation of CP.

1-C-74 Restoring ability to form new, and recover old "lost", memories in mice that model Alzheimer's disease

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The clinical hallmark of Alzheimer's disease (AD) is a progressive decline in cognitive function. Increasing evidence indicates that b-amyloid (Ab) disrupts neurotransmission and synaptic function, possibly by promoting excessive internalization of postsynaptic

AMPA-type glutamate receptors (AMPARs). These findings suggest that the memory deficits described both in patients affected by early AD and mouse models of AD may be due to high levels of Ab promoting the loss of postsynaptic AMPAR. Here we tested the hypothesis that excessive AMPAR internalization could account for the memory deficits seen in AD mouse models. We found that increasing Ab either acutely or chronically in mouse model of AD (TgCRND8, 5xFAD, HSV viral injection of mutated APP) disrupts both consolidation and reconsolidation of memory measured in hippocampal dependent tasks (Context fear conditioning and Water Maze). Preventing AMPAR endocytosis during memory encoding restored the ability of both acute and chronic mouse models to form new memories (consolidation) and to make these memories more persistent. Remarkably, preventing AMPAR endocytosis during a memory reminder enabled the recovery of an otherwise inaccessible old memory (reconsolidation) in AD mouse models. These findings elucidate the disruptive role of Ab in synaptic function and raise the possibility that restoring plasticity during memory encoding and/or retrieval by targeting the loss of postsynaptic AMPAR may help support the ability to form new memories as well as enable recovery of lost past memories in AD patients.

1-C-75 -NMDA R/+VDR Pharmacological Phenotype as a Novel Therapeutic target in Relieving Motor-Cognitive Impairments in Parkinsonism

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This study sets to investigate the effect of targeting calcium controlling receptors, specifically activation of Vitamin D3 receptor (VDR) and inhibition of N-Methyl-D-Aspartate Receptor (NMDAR) in the motor cortex of mice model of drug induced Parkinsonism. Also we demonstrated how these interventions improved neural activity, cytoskeleton, glia/neuron count and motor-cognitive functions in vivo. Methods: Adult mice were separated into six groups of n=5 animals each. 5mg/Kg body weight of haloperidol was administered Intraperitoneally for 7 days to block dopaminergic D2 receptors and induce degeneration in the motor cortex following which an intervention of VDR agonist (VDRA), and (or) NMDAR inhibitor was administered for 7days. A set of control animals received normal saline while a separate group of control animals received the combined intervention of VDRA agonist and NMDAR inhibitor without prior treatment with haloperidol. Behavioural tests for motor and cognitive functions were carried out at the end of the treatment and intervention periods. Subsequently, neural activity in the motor cortex was recorded in vivo using unilateral wire electrodes. We also, employed immunohistochemistry to demonstrate neuron, glia, neurofilament and proliferation in the motor cortex after haloperidol treatment and the intervention. Conclusion: Our findings suggests that calcium mediated toxicity is primary to the cause and progression of Parkinsonism and targeting receptors that primarily modulates calcium reduces the morphological and behavioural deficits in drug induced Parkins.

1-C-76 Patterns of APP fragments suggest that Alzheimer disease is the end-point of distinct processes in men and women

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Alzheimer's disease (AD) is identified by overt symptomatology, at a stage when there is evidence of extensive aggregation (as plaques) of the â-amyloid (Aâ) peptide. However, this late-stage in disease progression precludes effective disease management and underscores the crucial need to identify AD in earlier stages. Risk of AD is influenced by factors such as advancing age and genetics, including APOE å4 status. Several studies have reported that a single e4 allele increases the risk four-fold in women, but exerts little risk in men. Even so, clinical AD research continues to view male and female APOE å4 carriers as having equal risk. We examined autopsied control and AD brain samples for fragments of the Amyloid Protein Precursor (APP), including the Aâ peptide, and observed changes that clearly align with APOE å4 status in a sex-dependent manner. The increased detection of Aâ42 and Aâ40 in the guanidine extracted (insoluble/plaque) fraction are matched by decreases in the TBS-extracted (soluble) fraction. These changes were not consistent between cortical and hippocampal samples from the same donor. Furthermore, changes in expression of the receptor for advanced glycation end-products (RAGE) and for

the low density lipoprotein receptor-related protein (LRP) suggest that the changes in Aâ levels are due to disruption in clearance from the brain. This knowledge provides an understanding of how relative risk factors, e.g. sex and APOE å4 status, can interact to increase a given individual's absolute risk of developing AD.

1-C-77 Distribution of Somatostatin and Somatostatin Receptors in Human brain microvascular endothelial cell in β-amyloid induced Toxicity

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We recently described the role of somatostatin (SST) in lipopolysacchride (LPS) and cytokines induced inflammation using human brain microvascular endothelial cell line (hCMEC/D3) as model of blood brain barriers. Previous studies have indicated the pro-inflammatory role of amyloid in onset and progression of Alzheimer's disease (AD) via induction of IL-1β. Since the biological effect of SST is mediated by five different receptor subtypes, in the present study we determined the role of SSTR subtypes in BBB cells using β-amyloid. hCMEC/D3, grown in DMEM, were treated with 5μM of Aβ25-35 for 12-36 hr at 37ºC and processed for MTT assay for cell viability. In addition, SST and SSTR mRNA expression was determined using quantitative real time PCR and subcellular receptor expression using immunofluorescence staining. We also determined the expression of IL-1β mRNA upon treatment of A. hCMEC/D3 cells treated with Aβ25-35 exhibit time dependent changes in mRNA expression for SST and SSTR subtypes. We also found significant changes in subcellular expression of SSTR subtypes in hCMEC/D3. Furthermore, the expression of IL-1β mRNA in response to A was increased at 12 and 24 hr and decreases at 36 hr upon treatment. Collectively, our results indicate positive correlation between Aβ mediated changes in SST and SSTR subtypes in a receptor specific manner. These findings might shed new insight for the role of SSTR subtypes as potential therapeutic intervention in AD. This work was supported by grant from CIHR and NSERC and Seungil Paik is a recipient of CIHR fellowship.

1-C-78 Role of Thioredoxin Reductase in regulation of autophagic cell death in Neurons.

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Autophagy is an intracellular degradation process under the stress conditions. Lack of energy supply triggers the self-digestion process which delivers cytoplasmic constituents to the lysosome to maintain cellular homeostasis. Reactive oxygen species (ROS) regulate autophagy in several ways and are implicated in cell survival, development and death. ROS mediate their effects by oxidation of key proteins and a sophisticated set of thiols must constantly maintain the balance between the reduced and oxidized proteins. Thioredoxin (Trx) and its reducing enzyme TR is a major player in cell growth and survival but is dysregulated after trauma/neural diseases. While role of Trx1 in regulation of autophagy has been shown, we aimed to identify the impact of TR1 remains unidentified. Manipulation of TR1 by using Auranofin, a specific inhibitor of TR1, caused dose dependent cell death in SH-SY5Y neuronal cell lines. This was blocked by 3-methyladenine, an inhibitor of autophagy, but not Rapamycin, an inducer of autophagy. Inhibition of TR1 caused the cells to follow classical Type II (autophagic) cell death pathway evidenced by accumulation of LC3-II and activation of caspases (3 and 9) and PARP-1. Our data indicate an association between autophagy and endoplasmic reticulum (ER) stress markers IRE1 and ATF6. These results suggest that the availability/level of TR1 may be a regulatory control in regulation of endoplasmic reticulum stress. Using molecular tools such as gene/protein transfection, we are currently investigating the lack /gain of function studies.

1-C-79 Eye movements reveal sexually dimorphic deficits in children with fetal alcohol spectrum disorder

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We examined the accuracy and characteristics of saccadic eye movements in children with fetal alcohol spectrum disorder (FASD) compared with typically developing control children. Previous studies have found that children with FASD produce

saccades that are quantifiably different. Additionally, animal studies have found sex-based differences for behavioral effects after prenatal alcohol exposure. Therefore, we hypothesized that eye movement measures will show sexually dimorphic results. Children (5-18 yrs) with FASD (n=71) and typically developing controls (n=113) performed a visually-guided saccade task. Saccade metrics and behavior were analyzed for sex and group differences. Accuracy was significantly poorer in the FASD group, especially in males, which introduced significantly greater variability. Therefore, we conducted additional analyses including only those trials in which the first saccade successfully reached the target within a ±1˚ window. In this restricted amplitude dataset, the females with FASD made saccades with significantly lower velocity and longer duration, whereas the males with FASD did not differ from the control group. Additionally, the mean and peak deceleration were decreased in the females with FASD. These data support the hypothesis that children with FASD exhibit specific deficits in eye movement control and sensory-motor integration associated with cerebellar and/or brain stem circuits. Moreover, prenatal alcohol exposure may have a sexually dimorphic impact on eye movements, with males and females exhibiting differential patterns of deficit

1-C-80 Expression of a novel delta-opioid receptor isoform in human brain and a neuroblastoma cell line

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Delta-opioid receptor (DOR) is one of four opioid receptors. It has a role in pain, regulation of mood and neuroprotection, but there are no clinically used selective drugs acting on this receptor. There is only one known gene transcript encoding for the classic 7-transmembrane (7TM) domain G-protein coupled DOR. This simplicity is striking as the mu-opioid receptor (MOR) is diverse with dozens of transcripts encoding for truncated 6-transmembrane (6TM) domain receptors and single-TM domain chaperones in addition to the major 7TM isoform. 6TM MORs are an intriguing target, because they induce excitatory cellular responses in contrast to the inhibitory effects of 7TM isoforms. Additionally, a 6TM MOR selective ligand is a potent analgesic in animals, but lacks some of the problematic adverse effects of classic opioids, like morphine. The discovery of additional DORs could reveal receptor isoform specific effects and help in harnessing the receptor as a therapeutic drug target. By combining rapid amplification of 5' ends PCR with deep sequencing of long transcripts, we identified novel OPRD1 transcripts, all including previously unannotated exons. We are currently characterizing a transcript that produces a truncated 6TM DOR. We have determined its expression levels by quantitative PCR from a library of human central nervous system RNAs and BE(2)-C neuroblastoma cell line. The transcript was originally found from BE(2)-C cells, but we confirmed the expression in RNA derived from human brain. Studies are ongoing to reveal the mouse analogues of the novel human receptors.

1-C-81 The effect of ASD-associated mutations on neuronal development

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Over the last decade, the rate of children diagnosed with autism spectrum disorder (ASD) has risen steadily. Given the early onset of the disease, one hypothesis is that impairment of normal neuron growth and neural circuit connectivity during development contributes significantly to ASD pathogenesis. However, the mechanisms of these dysfunctions have yet to be elucidated. Although numerous genes have been identified as mutated in cases of ASD, with a model emerging of a combination of mutations whereby each contributes incremental susceptibility for disease expression, a few gene mutations appear to have strong and possibly monogenic action. To investigate the role of these mutations on neuronal morphological development and connectivity we employed in vivo imaging of the retinotectal system of the awake Xenopus laevis tadpole. Single-cell electroporation was used to transfect individual growing neurons for expression of ASD-associated mutations of neuroligin 3 (NLGN3) followed by two-photon rapid time-lapse imaging to capture full 3D dendritic arbor growth calcium transients in response to experience-driven plasticity training. Results elucidate the relationship between the environment and gene expression on functional and structural brain circuit development.

1-C-82 Differential expression of connexins in a mouse model of fetal alcohol syndrome

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Maternal alcohol exposure during gestation can cause serious injury to the fetus, resulting in the fetal alcohol syndrome (FAS) or the fetal alcohol spectrum disorder (FASD). FASD is associated with a range of physiological and neurobehavioral impairments, including increased seizure susceptibility. We developed a mouse model of FAS to study the expression pattern of connexins in the FAS brain. Prenatal alcohol exposure for the first trimester resulted in significant upregulation of Cx30 mRNA and Cx30 total protein in the hippocampus of FAS animals compared to age matched controls. Furthermore, surface level expression of both dimeric and monomeric Cx30 was found to be significantly upregulated in both hippocampus and cortex of the animals that were prenatally exposed to alcohol compared to age matched controls. On the surface levels, the fast migrating form and the C tail fragment of Cx43 were found to be upregulated in the hippocampus of mice that were prenatally exposed to alcohol. These results indicate that the expression of astrocytic connexins (Cx30, Cx43) is altered in the FAS model, and these changes could play a role in the alterations observed cerebral excitability of these animals. Supported by CIHR and CFFAR.

1-C-83 Delayed inhibition of VEGF signaling after stroke attenuates blood brain barrier breakdown and improves functional recovery in a co-morbidity dependent manner

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Diabetes is a common comorbidity in stroke patients and a strong predictor of poor functional outcome. To provide a more mechanistic understanding of this clinically relevant problem, we focused on how diabetes affects blood brain barrier (BBB) function after stroke. Since the BBB can be compromised for days after stroke and thus further exacerbate ischemic injury, manipulating its function presents a unique opportunity for enhancing stroke recovery long after the window for thrombolytics has passed. Using a mouse model of type 1 diabetes, we discovered that ischemic stroke leads to an abnormal and persistent increase in vascular endothelial growth factor receptor 2 (VEGF-R2) expression in peri-infarct vascular networks. Correlating with this, BBB permeability was markedly increased in diabetic mice which could not be prevented with insulin treatment after stroke. Imaging of capillary ultrastructure revealed that BBB permeability was associated with an increase in endothelial transcytosis rather than a loss of tight junctions. Pharmacological inhibition (initiated 2.5 days post-stroke) or vascular-specific knockdown of VEGF-R2 after stroke attenuated BBB permeability, loss of synaptic structure in peri-infarct regions, and improved recovery of forepaw function. However, the beneficial effects of VEGF-R2 inhibition on stroke recovery were restricted to diabetic mice, and appeared to worsen BBB permeability in non-diabetic mice. Collectively, these results suggest that aberrant VEGF signaling and BBB dysfunction after stroke plays a crucial role in limiting functional recover

1-C-84 Leucine-rich repeat kinase 2 knockout prevents behavioral deficits and promotes survival in Parkinson's disease model

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor and non-motor behavioral deficits. While the vast majority of PD cases arise from unknown origins, it is now well established that chronic neuroinflammation and oxidative stress contribute to disease etiology. Much attention has been afforded to the commonly used pesticide, paraquat (PQ), as an environmental risk factor for the development of PD. Indeed, animal models using PQ have shown to recapitulate many of the hallmark features of the disease. The leucine rich repeat kinase 2 (LRRK2), a kinase mutated in both autosomal-dominantly inherited and sporadic PD cases, has been shown to modulate inflammation in

response to different stimuli. However, whether or not LRRK2 plays a role in PQ-induced brain and behavioral changes has not been determined. Accordingly, we investigated the effect of PQ exposure in male LRRK2 knockout (KO) and wild-type (WT) mice on behavioral functioning. Mice were given 6 injections of PQ (10 mg/kg; 3X/week) and sacrificed 1 hour following the final injection. Consistent with previous findings, PQ induced signs of sickness and decreased motor and affiliative behavior in WT but not KO mice. Additionally, we assessed striatal levels of the neuroplastic cytokine, brain-derived neurotrophic factor (BDNF). We found that, irrespective of genotype, PQ decreased BDNF levels. Taken together, these data suggest that LRRK2 may play a role in select aspects of PQ induced pathology and may be relevant for primary motor PD pathology, as well as other comorbid aspects of the disease.

1-C-85 Investigation of Pannexin 1 in the response of developing neurons to stroke

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After stroke, developing neurons traveling along the rostral migratory stream are activated and diverted to the peri-infarct cortex. The factors involved in their activation are poorly understood, but their ablation worsens stroke outcomes. Here we investigated the hypothesis that a protein called pannexin 1 (Panx1) plays a role in their activation. Our data suggest that Panx1 has location-specific effects on developing neurons following stroke. Panx1 is a non-selective ion and metabolite permeable channel enriched in the brain. It was originally detected in mature neurons and has been implicated in their anoxic depolarization in stroke. We recently detected Panx1 in developing neurons where it promotes proliferation and migration and negatively regulates neurite outgrowth. Using the photothrombotic focal cortical stroke model, we investigated the role of Panx1 in developing neuron activation. Stroke increased Panx1 expression in developing neurons within 4 hours. Treatment with the Panx1 blocker probenecid reduced their proliferation. We next selectively ablated Panx1 in the developing neuron population at the time of stroke. Within the ventricular zone 2 days after stroke, there were appreciable numbers of Panx1-null cells. At 10 days, Panx1 null developing neurons were absent suggesting its presence is critical for maintaining these cells. Conversely, in the peri-infarct cortex at 10 days, Panx1-null cells were selectively spared in comparison with Panx1-expressing cells, suggesting that the presence of Panx1 is deleterious to developing neurons in this region.

1-C-86 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 & and 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.

1-C-87 Concurrent assessment of forelimb function and mesoscopic cortical networks in mouse stroke models

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We developed an automated forelimb motor task that allows simultaneous imaging of brain activity using the GCaMP6s calcium indicator, and behavioural assessment during stroke recovery. The task requires head-fixed mice to pull and hold a lever mounted on a rotary encoder, thus allowing limb position to be tracked throughout each trial. The apparatus is compatible with wide-field imaging, enabling longitudinal assessment of cortical activity and forelimb function during stroke recovery. Imaging was performed transcranially through a chronic window that did not require removal or thinning of the skull. Transgenic mice (Ai94; Jackson Labs) expressing the GCaMP6s calcium indicator completed an average of 128±27 successful trials per 20-minute session (n=7 mice). Ca2%2B imaging revealed a time-locked increase in activity of the forelimb sensorimotor region (8.5% ∆F/F) while barrel cortex and other unrelated sensory areas showed less activity (1.8% ∆F/F) during pulling. After a photothrombotic stroke in motor cortex, successful pulls decreased significantly during the first week (48% of baseline, p=0.022), indicating that motor cortex at least in part contributes to task performance. The Ca2%2B activity within the forelimb region was also substantially diminished during the first week (1.5% ∆F/F), however peri-infarct motor regions such as the rostral forelimb area maintained their baseline level of activity (5% ∆F/F). Our results demonstrate the feasibility to monitor post-stroke cortical reorganization with optogenetic tools in awake mice engaged in a forelimb motor task.

1-C-88 Transcription regulation of the human USP25 gene

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Dysregulation of the ubiquitin proteasome pathway has been implicated in the pathogenesis of neurodegenerative diseases and Down Syndrome. The ubiquitin-specific proteases 25 (USP25) gene spans over 150kb and located in Chr21q11.2. To define the molecular mechanism of USP25 gene transcriptional regulation, we isolated a 2.2-kb 5'UTR of USP25 gene. A series of nested deletions of the 5'UTR fragments were subcloned into a luciferase reporter plasmid pGL3-Basic. HEK293 cells were transfected with the USP25 promoter constructs and luciferase activity was measured to assay its promoter activity. We identified a 104-bp fragment containing the transcription initiation site as the minimal region necessary for USP25 gene promoter activity. Several putative cis-acting element including SP1 are found in the 5' flanking region of USP25 gene and it regulates the promoter activity of the human USP25 gene.

1-C-89 VEGF protects against blood brain barrier disruption, dendritic spine loss and spatial memory impairment in an experimental model of diabetes

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Clinical and experimental studies have shown a clear association between diabetes, vascular dysfunction and cognitive impairment. However, the molecular underpinnings of this association remain unclear. Since vascular endothelial growth factor (VEGF) signaling is important for maintaining vascular integrity and function, we hypothesized that cognitive impairment and vascular dysfunction in the diabetic brain could be caused by a primary deficiency in VEGF signaling. Here we show that chronic hyperglycemia in the streptozotocin model of type 1 diabetes leads to a selective reduction in the expression of VEGF and its cognate receptor (VEGF-R2) in the hippocampus. Correlating with these changes, diabetic mice showed selective deficits in spatial memory in the Morris water maze, increased vessel density, width and permeability in the dentate gyrus/CA1 region of the hippocampus and reduced spine densities in the apical dendrites of CA1 neurons. Chronic intracerebroventricular infusion of VEGF in diabetic mice was sufficient to protect them from memory deficits, as well as vascular and synaptic abnormalities in the

hippocampus. These findings suggest that a hippocampal specific reduction in VEGF signaling and resultant vascular/neuronal defects may underlie early manifestations of cognitive impairment commonly associated with diabetes. Furthermore, restoring VEGF signaling may be a useful strategy for preserving hippocampal-related brain circuitry and functions in degenerative vascular diseases.

1-C-90 Transcriptomic approach to cellular composition changes in psychiatric disorders

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Increasing evidence has accumulated regarding the involvement of cellular death and neuroinflammation in neuropsychiatric disorders (e.g., bipolar disorder and schizophrenia). Identifying the affected cell-types is crucial for understanding the pathophysiology of a disorder, providing new directions for future studies, and assisting in the analyses and interpretation of the gene expression data. Since different cell-types express distinct sets of genes, it plausible that changes in cellular populations would result in observed transcriptional alterations in the bulk tissue. For example, loss of dopaminergic neurons in substantia nigra would result in decreased transcript levels of tyrosine hydroxylase in that region. Thus, given that the cell-type specific transcripts are known, changes in specific cellular populations could potentially be inferred from the bulk tissue expression data. Publicly available brain expression datasets of patients and healthy subjects provide the opportunity to accomplish this task without the need for additional experiments.

We used a cell-type enriched marker gene database compiled by our group to infer changes in different neuronal and glial populations in four expression datasets of dorsolateral prefrontal cortex from a similar cohort of psychiatric patients and healthy subjects. Using statistical methods, we infered changes in several cell types , some of which were shown by others using direct cell counting methods (e.g. pyramidal cells). The inferred changes were similar across the datasets, supporting the robustness of our approach.

1-C-91 Effect of chronic minocycline treatment on restoring dendritic atrophy in a Murine Fragile X Model

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Fragile X Syndrome (FXS) is the most common single gene cause of inherited intellectual disability. FXS occurs when the CGG trinucleotide sequence expands to >200 repeats in the Fmr1 gene on the X chromosome. This leads to gene silencing and a loss of its protein product, Fragile X Mental Retardation Protein (FMRP). The Fmr1 knockout (Fmr1-/y) mouse also lacks FMRP, and displays mental impairments similar to those in humans. It also shows an increased proportion of immature dendritic spines, similar to that reported in humans. Recent clinical trials indicate that the tetracycline derivative minocycline may be a promising treatment for FXS patients; however, its underlying mechanism remains unclear. We investigated the effect of chronic minocycline treatment on dendritic morphology in the dentate granule neurons of the hippocampus of wildtype (WT) and Fmr1-/y mice. Minocycline (30mg/kg/day) was administered in drinking water to newborn mice until they were two-months old. Biocytin-filled dentate neurons were obtained using whole cell patch technique and visualized by immunostaining. Sholl analysis revealed that Fmr1-/y mice exhibited a decrease in both dendritic length and intersections with Sholl radii in the dentate gyrus when compared to WT mice. Chronic minocycline treatment restored such structural deficits in the dentate region of Fmr1-/y mice. Our results suggest that this beneficial effect of minocycline in rescuing dendritic atrophy in the dentate region of the hippocampus of FXS mice may partially underlie its therapeutic effect on cognitive improvement in FXS.

1-C-92 Amyloid-β oligomers induce autophagy and inhibit axonal transport of autophagosomes in cultured hippocampal neurons

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Autophagy, a lysosomal degradative process used to recycle cellular constituents and eliminate damaged organelles and proteins, plays a vital role in cellular

quality control. Mounting evidence has implicated defective autophagy in the pathogenesis of numerous neurodegenerative diseases, including Alzheimer's disease (AD). Indeed, AD brains, which are characterized by increased amounts of amyloid-beta plaques and neurofibrillary tangles, show a buildup of faulty autophagosomes (APs) in dystrophic neurites. Surprisingly, very little is known about how amyloid-beta oligomers (AβOs) - the main toxic species in AD - affect the autophagic process. For example, although it is known that autophagy is responsible for the degradation of intracellular AβOs, it is not known what effects extracellular AβOs exert on autophagy. Here we show that application of AβOs to rat hippocampal neurons increases autophagic flux initially. However, as treatment time increases, AβOs induce transport defects, resulting in less AP movement from the distal axon (where APs form) to the cell body (where APs mature). These results suggest that extracellular AβOs play an important role in altering autophagy dynamics in neurons, and this research will hopefully aid in the designing of AD therapeutics specifically targeting autophagy.

1-C-93 The Unfolded Protein Response and cholesterol biosynthesis link Luman/CREB3 to regenerative axon growth in sensory neurons

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Luman/CREB3 (herein called Luman) is an endoplasmic reticulum (ER) transmembrane basic leucine zipper transcription factor whose mRNA and protein localize to adult sensory neurons and axons, the latter with axonal ER along the axon length. We have recently shown that this axon ER transmembrane protein is a unique retrograde regeneration signal in axotomized sensory neurons and that it plays a critical role in axon regrowth. Here, we provide evidence that Luman contributes to axonal regeneration by regulating the Unfolded Protein Response (UPR) and cholesterol biosynthesis. siRNA knockdown of Luman expression in injury-conditioned neurons reduced axonal outgrowth to 48% of control injured neurons, and was concomitant with reduced UPR- and cholesterol biosynthesis-associated gene expression. UPR PCR-array analysis coupled with qRT-PCR, identified and confirmed that 4 mRNAs involved in cholesterol regulation were also downregulated >2 fold by the Luman siRNA treatment of injury-conditioned neurons. Further, the Luman siRNA-reduced outgrowth could be significantly rescued by either cholesterol supplementation or 2ng/ml tunicamycin-induced elevation of the depressed UPR gene expression to a level equivalent to that observed with crush injury, with outgrowth increasing to 74% or 69% that of injury-conditioned controls respectively. The identification of Luman as an injury-induced UPR and cholesterol regulator at levels that benefit the intrinsic ability of axotomized adult rat sensory neurons to undergo axonal regeneration reveals new therapeutic targets for nerve repair.

1-C-94 Altered precision of Purkinje cell firing in a mouse model of spinocerebellar ataxia type 6

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Spinocerebellar ataxia type 6 (SCA6) is a midlife-onset disease that affects motor control and gait. SCA6 is caused by a poly-Q expansion in the Cacna1a gene that encodes the α1Α subunit of the P/Q calcium channel and the α1ΑCT transcriptional regulator that are enriched in cerebellar Purkinje cells. Since P/Q channels and the α1ΑCT transcriptional regulator are both implicated in neuronal development, we wondered if changes occur prior to disease onset in the developing cerebellum in a transgenic SCA6 knock-in (SCA6 KI) mice. We found that firing rate and precision is altered in Purkinje cells during postnatal development, and that these changes are accompanied by alterations in excitatory, but not inhibitory input. Thus, there are changes in the SCA6 brain well before disease symptoms are observed. However, by the time cerebellar development is complete in young adult mice, differences in Purkinje cell firing and excitatory input can no longer be observed in SCA6 KI mice, suggesting that the SCA6 brain homeostatic recovers cerebellar function during development. These young adult SCA6 KI mice also display no detectable motor deficits, in line with the late age of onset observed in people. Finally, we found that in 7-month-old SCA6 KI mice that display motor deficits on Rotarod, the precision of cerebellar Purkinje cell firing is lost. These results suggest that breakdown of Purkinje cell firing

precision may contribute to the pathophysiology of SCA6.

1-C-95 Development of a primate model of Alzheimer's Disease II: Characterization of behavioural phenotype

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Alzheimer's disease (AD) is a devastating neurodegenerative disease and there is an urgent need to develop new therapeutics. Promising drugs developed in rodents have failed to work in AD patients in clinical trials. To bridge this translational gap, our laboratory has developed a non-human primate (NHP) model of AD via intracerebroventricular injection of neurotoxic amyloid beta oligomers (AβOs). This model recapitulates the molecular aspects of human AD pathology, such as tau hyperphosphorylation coupled with tangle formation, synaptic loss, and astrocytic activation (Forny-Germano et al., J. Neurosci, 2014; Boehnke et al. at this meeting). To characterize the behavioural deficits associated with these molecular pathologies, we have developed a behavioural phenotyping platform for primates. General home-cage activity levels including circadian rhythms are measured using activity monitors and 24/7 video recording. Cognitive performance is measured using eye movement tasks (including step and memory-guided saccade tasks), as well as performance on classic CANTAB touchscreen-based tasks including self-ordered spatial search, delayed match to sample, and paired associate learning. Here, we present preliminary results of the changes in behavioural parameters from 3 male rhesus macaques before and after AβO administration to provide behavioural validation of our primate Alzheimer's disease model.

1-C-96 Adaptation of a naturalistic motor learning task to assess behaviour and drug interventions in the YAC128 model of Huntington's disease

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Behavioural testing of disease models in rodents can be useful in assessing the extent of cognitive and physical impairment, as well as evaluating the efficacy of potential drug treatments. However, many traditional behavioural paradigms involve putting the animal in stressful or unfamiliar environments, often during times of the day when they would typically be sleeping, and this can cause stress responses that introduce variability into the data. To address this problem, we have adapted a new paradigm to identify behavioural phenotypes in models of chronic, progressive neurodegenerative disease. This paradigm involves the incorporation of a lever pull task into the mouse home-cage environment, allowing continuous access to the task and automated collection of data. Animals can be tested at different disease stages, and advancement through the levels of task difficulty can be personalized to individual mice within the same cage. In addition, continuous testing over long periods can be used to more accurately determine the change in behavioural phenotype over time in a particular animal model. So far, we have used this paradigm to test procedural learning, cognitive flexibility, and fine motor ability in the YAC128 mouse model of Huntington's disease at 2 months (presymptomatic) and 4 months (early symptomatic) of age. We plan to use this paradigm as a tool to screen therapies for efficacy in reversing the HD behavioural phenotype in these mice.

1-C-97 Effects of exercise on the basal ganglia morphology in schizophrenia.

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Schizophrenia has been associated with morphological brain deficits, including alterations in hippocampal and basal ganglia volume (McClure et al., 2013). Abnormalities in these key regions are thought to give rise to both neurocognitive deficits and to the severity of psychosis. Psychotropic medications ameliorate psychosis, however they adversely affect metabolism and cardiovascular functioning and further increase changes in striatal volume (Stassnig, Brar & Ganguli, 2011). In contrast, physical activity is also known to mediate some of the negative metabolic effects of antipsychotic medication, including weight gain, hypertension and decreased vascular compliance in chronic patients (Bredin, Warburton & Lang, 2013). Of particular

interest, exercise may have ameliorative effects on regional brain volumes, including the basal ganglia. Using high resolution imaging, morphology, connectivity and local neurovascular integrity of psychosis patients may be examined. It is expected that physical activity would result in recuperation of basal ganglia volume and reduced symptom severity. Better understanding of the beneficial effects of exercise on basal ganglia volume, as well as clinical symptoms, would be critical in understanding the potential for physical activity as a non-pharmaceutical intervention in these patients. Further work would investigate whether these effects are seen in other regions of the brain implicated in psychosis, including the frontal grey matter and the thalamus.

1-C-98 Synaptic dysfunction by an Alzheimer-associated mutation A713T in the APP gene

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Alzheimer's Disease (AD) is the most common neurodegenerative disease. A number of amyloid β precursor protein (APP) mutations were found in AD patients and studies of these mutations profoundly improved the current understanding of AD pathogensis and its drug development. The APP A713T mutation was identified in two independent families of AD and a strong genetic linkage between this mutation and AD pathology was confirmed by screening the gene sequence of the family members. However, how this single mutation of APP causes AD remains undefined. In the present study, we found that APP A713T mutation impaired APP protein's ER to Golgi transportation, its maturation, turnover and suppressed APP proteolytic processing by the secretases. Although the Aβ generation was reduced by A713T mutation, the Aβ harboring this mutation exacerbated the neuronal toxicity compared with the wildtype Aβ. More importantly, the A713T mutation significantly impaired the synaptogenesis. Our study provides a new insight into the function of APP protein and suggests a novel effect of the APP mutation on AD pathogenesis.

1-C-99 Using induced pluripotent stem cell-derived neurons to uncover effects of autism-linked mutations on neuronal function.

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De novo deletions and nonsense mutations of SHANK2 have been implicated in several autism spectrum disorder (ASD) cases. To determine how ASD-associated SHANK2 mutations affect human neuronal function, we have generated induced pluripotent stem cells (iPSC) from two children with ASD, one with a nonsense mutation and the other with a deletion. We have also knocked out SHANK2 in iPSCs from neurotypic individuals using CRISPR/Cas9 technology. As controls, we are generating iPSCs from unaffected family members and unrelated individuals, as well as correcting the nonsense SHANK2 mutation in patient iPSCs. To obtain cortical neurons, we first generate neural precursor cells with dorsal forebrain identity by inhibiting BMP, Nodal/Activin and Wnt signaling. To date, we have generated NPCs from the patient with the nonsense mutation, as well as two controls. To assess dendritic arborization, 6 week-old neurons are transfected with a GFP plasmid and tracked on a live-imaging platform. KCl stimulation causes neurite extension in control, but not patient-derived neurons. To assess synaptogenesis, four week-old neurons are re-seeded on mouse astrocytes to promote synaptogenesis. Five weeks after re-seeding, electrophysiological and imaging approaches are used in parallel to assess synaptic connectivity. Specifically, we are currently recording miniature excitatory postsynaptic currents and quantifying synapse number per cell. Using these phenotyping assays, we aim to determine how development and function are perturbed in neurons specific to children with ASD and SHANK2 mutations.

1-C-100 Electrophysiological investigation in neurons derived from human induced pluripotent stem cells with deletions of PTCHD1 locus

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Autism is an early onset neurodevelopmental disorder. The pathogenesis of autism remains unclear and genetic contributions are critically important in the occurrence of this disorder. The X-linked PTCHD1 locus is one of the most frequently disrupted loci in autism. However, the molecular mechanisms underlying autism due to the disruption of PTCHD1 are poorly understood. To address dysfunction of this gene in autism we utilized neurons derived from patient induced-pluripotent stem (iPS) cells. We characterized electrophysiological function of neurons derived from iPS cells of an unaffected control and two probands with autism: proband 1 has a deletion of PTCHD1 and proband 2 has a deletion disrupting the autism-associated long non-coding RNA PTCHD-antisense. Patch-clamp recordings showed that neurons from the unaffected control, proband 1, or proband 2 displayed spontaneous and/or evoked action potentials and miniature excitatory postsynaptic currents, indicating that they have similar features to those neurons in the central nervous system. Statistical analysis uncovered that there were not defects in the resting membrane potential, input resistance, or action potential waveform for iPS cell-derived neurons from proband 1 or proband 2, compared with the unaffected control. However, results from electrophysiology revealed dysfunction of NMDA receptor-mediated currents in proband 2, compared with the unaffected control. These findings are the first functional data characterizing the roles of PTCHD1 in neuronal activity in humans and its relevance to autism.

D - Sensory and Motor Systems

1-D-101 Time course 'dose' of cross-education of strength after handgrip training

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Cross-education is a neural adaptation defined as the increase in strength of the untrained contralateral limb after unilateral training. It occurs in both neurologically intact and clinical (e.g. stroke, orthopedic injury) populations. Limitations for clinical translation include knowledge on the minimum time course for emergence of the crossed effects. Therefore, the major purpose of this study was to characterize the time-course of strength changes in both the trained and untrained limbs during unilateral handgrip training. Neurologically intact participants completed 6 weeks of unilateral handgrip training 3 times/week. During each training session, participants performed 5 sets of 5 maximal isometric contractions (MVC) using a handgrip dynamometer in their dominant right hand. Changes in strength and muscle function were evaluated by MVC force and electromyography (EMG) during handgrip in both trained and untrained limbs before and after training. During training, handgrip force was recorded for all handgrip repetitions while EMG was measured weekly. For the untrained limb, handgrip force and EMG were measured once a week during a single contraction. After six weeks of training, handgrip force significantly increased in both trained and untrained limbs. Interestingly, the increase in strength occurred at a similar rate between limbs. These results emphasize the importance of establishing a "dose" for the time course of functional and neurological adaptations in strength for effective translation to rehabilitative interventions.

1-D-102 Repetitive transcranial direct current stimulation (tDCS) of the primary visual cortex induces long-lasting enhancement of contrast perception

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Background: The primary visual cortex (V1) plays a key role in humans' faculty to process contrasts that is crucial for almost all tasks of daily routine. Understanding the type of processes performed by an intact visual cortex and developing methods that enhance visual perception are of great scientific interest. Objective: In this study we tested whether contrast sensitivity can be enhanced in healthy subjects via non-invasive, anodal transcranial direct current stimulation (tDCS) over V1. Methods: The double-blind study comprised 24 healthy subjects (mean age 24.5 years, SD = 3.53), 12 subjects received anodal tDCS, 12 received placebo stimulation (sham). The polarization (P) electrode was placed over V1 for 20 minutes daily over five consecutive days. Contrast perception was tested

using threshold perimetry before and immediately after stimulation, as well as at 2- and 4-week follow-up (days 19 and 33). Results: Compared to sham, the tDCS group showed with 23% a significantly higher improvement of contrast perception (sham: 5%) (day 5 to baseline, p = 0.045). tDCS-effects lasted for 24 hours (days 1-5, p = 0.020) within the tested visual field (2-10°) and within the central 2-4° at follow-up dates (day 19 to baseline: p = 0.013, day 33 to baseline, p = 0.021). Conclusion: Long-term effects suggest that repeated anodal tDCS induces plasticity on the healthy human primary visual cortex. The effect-size was higher in the central visual field. Regarding the retinotopic neuronal organization of V1, this may indicate a stronger enhancement in the brain region closer to P.

1-D-103 Cell-type specific reorganization of inhibitory circuits during motor learning

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Motor learning is a basic form of learning that is crucial for survival in all animals. However, our understanding of precise microcircuit plasticity in motor cortex during motor learning, especially with regards to different neuron types, is limited. To address this question, we utilized in vivo two-photon imaging to chronically monitor synaptic structures of excitatory neurons and two inhibitory neuron types in head-fixed awake mice as they learned a forelimb lever-press task over weeks. We found that, while motor learning induces new dendritic spine formation in the layer 2/3 excitatory neurons, it also causes a reorganization of inhibitory circuits in a neuron-type specific manner. Somatostatin (SOM) inhibitory neurons that mainly inhibit the distal dendrites of excitatory neurons showed a decrease in axonal boutons immediately after the training begins, whereas parvalbumin (PV) inhibitory neurons that mainly inhibit the perisomatic region of excitatory neurons exhibited a gradual increase in the axonal boutons during training. Such structural reorganizations were restricted only to the learning phase and did not occur when the animals performed a previously learned task. Optogenetically manipulating SOM inhibition affected the stabilization of newly formed spines and thus prohibited the animal from learning and forming stereotyped lever-press movements. These findings demonstrate that motor learning induces neuron type-specific synaptic changes of local excitatory and inhibitory circuits in the motor cortex, which is essential for acquiring a new motor skill

1-D-104 Cortical substrates for allocentric vs. egocentric representation of remembered saccade targets in the human

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The location of a remembered target can be defined in egocentric or allocentric reference frames, but the neural mechanisms for allocentric saccade representation in humans are essentially unknown. Here we employed an event-related fMRI design same as our recent reach study (Chen et al. Journal of Neuroscience 2014) to investigate the brain areas supporting these two types of coding in twelve participants. The target and the landmark were always presented briefly, but at the beginning of each trial, participants were instructed to ignore the landmark and remember target location (Ego) or remember target location relative to the landmark (Allo). During the delay phase participants had to remember the target location in the appropriate reference frame. In a non-spatial Control participants remembered and reported the target color. We found that during the delay phase Ego and Allo elicited higher activation as compared to the Control in bilateral precuneus, midposterior intraparietal sulcus, dorsal premotor cortex and left extrastriate cortex. Inferior parietal lobes showed higher activation for Ego vs. Allo, whereas temporal and occipital cortex showed higher activation for Allo vs. Ego. Egocentric directional selectivity was observed in superior and inferior occipital cortex (IOG). Allocentric directional selectivity was observed in calcarine, IOG and precuneus. These results confirm different cortical mechanisms for egocentric vs. allocentric target memory, but comparing this to our previous study, the detailed mechanisms also depend on the motor effector (eye vs. hand).

1-D-105 Wii Balance Board and electromyography to assess postural adjustment after perturbation

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The Nintendo Wii Balance Board (WBB) is reliable in measuring balance when compared to a force plate. As a cost effective and portable system, the WBB may be used efficiently in clinical and research settings. Previous studies on balance assessment tools measured ranges of center of pressure (COP) and recovery time after perturbation. The current study combined a customized WBB computer game with electromyography (EMG) to determine feasibility to assess COP and recovery time in healthy individuals. Participants' COP was presented as a dot on a laptop using a customized LabVIEW program. In each trial (n=5), a target moved in random sequence among eight cardinal and ordinal directions where N was to the front. Participants met the target with their COP by redistributing their weight on the WBB. In each direction, time used to reach the target and return to centre was recorded along with bilateral EMG recordings of medial gastrocnemius (MG), peroneus longus (PL) and tibialis anterior (TA). Preliminary results showed increased bilateral MG and PL activity when recovering from perturbation signals to the N, NW and NE. When recovering from the virtual perturbation to the S, SW and SE, an increase in bilateral TA activity was observed. This study demonstrates that a simple device and computer software can be used as a tool to evaluate muscle synergies in balance control. Future research aims to use this combined WBB and LabVIEW program to assess balance deficits and recovery status in people with neurological disorders.

1-D-106 More than a feeling: Passive somatosensory priming facilitates processing of graspable objects

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Cognitive embodiment refers to idea that cognitive processes are rooted in perception and action experience. Although much research has focused on motor involvement in response to graspable object stimuli (e.g., Tucker & Ellis, 1998), somatosensory contributions to accessing motor knowledge have remained relatively unexplored. In the somatosensory domain, haptic object exploration has been shown to facilitate object processing when the prime object is congruent with the target object (e.g., James et al., 2002), however, passive sensory contributions have received less attention. Therefore, we employed a passive haptic vibratory prime in order to pre-engage the somatosensory cortex, followed by a picture stimulus of either a graspable (e.g., a spoon) or a non-graspable (e.g., an airplane) object. On half of the trials, presentation of the picture stimulus was preceded by the vibratory prime, whereas on the other half of the trials no vibration was received. Participants were then required to report how they would interact with the presented object while visualizing themselves performing that action. Results indicated that the graspable objects showed processing benefits on RT when preceded by the passive vibratory prime as opposed when they received no prime, while there were no differences in RTs found in the vibration versus no-vibration conditions for the non-graspable objects. These results suggest that general somatosensory stimulation has the ability to impact motor representations for graspable objects. Pilot results from an fMRI variant will also be discussed.

1-D-107 Potentiation of phase II formalin responses in zinc transporter-3 (ZnT-3) knockout mice

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Zinc is abundant in the central nervous system, and its activity is implicated in neuropathic and inflammatory pain. Expression of zinc is high in synaptic vesicles, the localization of which is tightly regulated by the vesicular zinc transporter 3 (ZnT-3). In the present study, we asked whether ZnT-3 is critically involved in the sequelae of inflammatory pain. To test this, we examined the behavioural response of ZnT-3 knockout mice to a single intraplantar injection of formalin, which evoked a biphasic nociceptive response characterized by licking and shaking of the injected paw. We found that phase I of the formalin response (attributed to c-fibre activation) was comparable in ZnT-3 knockout and wild-type mice. By contrast, phase II nociceptive behaviours (associated with central sensitization) were significantly greater in the ZnT-3 knockout mice. We also determined that the deletion of ZnT-3 was associated with lower expression of the microglial and astrocytic markers, ionized calcium-binding adapter molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP), respectively. Collectively, our findings suggest that zinc transport mediated by ZnT-

3 plays a critical role in the central sensitization associated with phase 2 of the formalin response.

1-D-108 Central and peripheral afferent processing of natural and artificial vestibular inputs

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Electrical vestibular stimulation (EVS) is thought to artificially modulate the firing rate of vestibular afferents, yielding a virtual sensation of head motion and reflexive responses in muscles throughout the body. The vestibulocollic reflex (VCR) provides robust control over neck muscles across a wide bandwidth (0-70 Hz), even in the absence of head postural control. In this study, we explore how vestibular inputs from electrical and kinematic stimuli interact and affect the VCR. We applied stochastic vestibular stimulation (0-75 Hz) to seated subjects who were rotated about a vertical axis at various frequencies (0.4-4 Hz) and peak amplitudes (0-150 °/s). Bilateral EMG of the sternocleidomastoid was collected. The electrically-evoked VCR (i.e. stimulus-EMG correlations) decreased with increasing angular velocity at all motion frequencies. This VCR attenuation occurred across the entire bandwidth of electrical stimulation but varied within the motion cycle: maximum attenuation led the input velocity signal by ~40-60°, irrespective of motion-induced muscle activity. Furthermore, the attenuation of the electrically-evoked VCR occurred in phase with the estimated natural motion-induced excitation of vestibular afferents (leading head angular velocity by 10-60°). In other words, increased natural afferent activity due to motion decreases the relative contribution of electrical stimulation to the total vestibular input to neck muscles, an integration process that likely occurs peripherally, prior to central processing of vestibular signals.

1-D-109 CD11b+Ly6G- myeloid cells drive mechanical inflammatory pain hypersensitivity

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Inflammatory pain is a common medical condition characterized by pain hypersensitivity at the site of inflammation due to chronic immune diseases, pathogenic infection and tissue injury. However, the specific contributions of the innate and adaptive immune system to the generation of pain during inflammation have not been clearly or systematically elucidated. We therefore set out to characterize the cellular and molecular immune response in two widely used preclinical models of inflammatory pain: 1) intraplantar injection of complete Freund's adjuvant (CFA) as a model of adjuvant- and pathogen-based inflammation and 2) a plantar incisional wound as a model of tissue injury-based inflammation. Our findings shed light on the differences in temporal patterns of immune cell recruitment and activation states, cytokine production, and pain in these two animal models, finding that CFA induced non-resolving granulomatous inflammation while tissue incision induced resolving immune and pain responses. Using various cell-depletion strategies, we find that CD11b+Ly6G- monocytes/macrophages were necessary for mechanical hypersensitivity during incisional pain, and to a lesser extent CFA-induced inflammation. In contrast, CD11b+Ly6G+ neutrophils and TCRβ+ T cells do not contribute to the development of thermal or mechanical pain hypersensitivity in either model. This work specifically identifies a population of CD11b+ Ly6G- myeloid cells as contributors to and potential targets for the treatment of mechanical inflammatory pain.

1-D-110 Cholinergic receptors expression in the visual cortex following long-term enhancement of visual cortical activity by cholinergic stimulation

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The cholinergic transmission in the primary visual cortex (V1) is involved in the enhancement of specific visual stimuli as well as long-term modifications of the neuronal processing. We investigated the involvement of cholinergic receptors subtypes underlying this long-term functional plasticity. Perceptual learning-like test

was performed by exposing awaken rats to a visual stimulation(VS) for 10min/day during 14days. This VS was provided alone or coupled to an electrical stimulation of the basal forebrain(HDB/VS) or paired with donepezil, a cholinesterase inhibitor(DONEP/VS). One week after the last training session, V1 were collected to determine the expression level of mRNA of muscarinic(M1-5) and nicotinic(á/â) receptors sub-units by RT-PCR. Two weeks of VS treatment alone caused an increase of the expression of M3 and M5 mRNA suggesting an increase of their production and activity during long-term visual stimulation. In the HDB/VS group, á3 sub-unit was decreased suggesting its involvement in phasic cholinergic stimulation. The DONEP/VS treatment presented a decrease in the expression of M2, suggesting a down regulation of mRNA synthesis. This could indicate an increased cortico-cortical inhibition, as M2 receptors are located massively on the GABAergic neurons. Therefore, even with similar functional enhancement, the cholinergic receptors regulation differs between an electrical and a pharmacological treatment. These results are crucial for determining which receptors are the most involved in the pharmacological cholinergic stimulation to enhance visual perception.

1-D-111 A computational approach to decipher the network topology of the dorsal root ganglion

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Understanding the network responsible for treating sensory inputs in the dorsal horn is necessary to grasp how disinhibition in this network leads to allodynia and hyperalgesia. Despite reasonable hypotheses, the topology of the network connections linking afferent fibres to projection neurons remains unresolved. We use computational modelling in order to investigate the vast space of possible networks and identify those compatible with experimental findings. Our approach provides a rigorous way to rule out hypotheses as well as to identify possibilities that have been overlooked by experimentalists.

1-D-112 Primary motor cortical neurons reflect vector sum of ipsilateral and contralateral feedback modulation

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It is commonly accepted that the primary motor cortex (M1) is involved in controlling the contralateral side of the body, but recent studies have noted some M1 neurons are active during ipsilateral limb movements. We use mechanical perturbations to quantify how M1 activity was related to ipsilateral and contralateral motor activity. A non-human primate (NHP) stabilized a cursor representing the location of its right or left hand at a central virtual target. After 1000-1500ms, flexor or extensor step torques were applied to the shoulder, the elbow, or both (9 load conditions). The NHP had 1000ms to return the cursor to, and stop at, the target. We recorded and examined the activity of 167 M1 neurons using a micro-electrode array and 29 neurons from single electrodes. We found that 73% of M1 neurons modulated their responses to contralateral perturbations, 40% modulated to ipsilateral perturbations and 34% modulated to either. When torques were applied to both arms at once, the modulation of each neuron's activity corresponded to the vector sum of its modulation to torques on either arm alone. When we considered neural activity over time during perturbations, we found that the vector sum predicted modulation throughout the perturbation, even for neurons with very different response onsets to ipsi- and contralateral perturbations. Our results indicate that M1 shows fast and substantial perturbation-related activity from both limbs which interacts in a very lawful manner.

1-D-113 Prediction of future sensory states requires self-generated motor commands

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Predicting the sensory consequences of limb movement has been thought to influence current decisions, however it is unclear whether these predictions are based on knowledge of upcoming movements or based directly on motor planning signals. The present experiment examines how forward modelling can influence limb localization. It

has been previously shown that crossing the arms induces a subjective reversal of spatially-defined cutaneous temporal order judgments. By applying brief vibrations to the hands during the planning stages of an arm-crossing movement, we observed how forward modelling systematically influences temporal order judgements. We tested this by having subjects either actively moving their arms into a crossed posture or having a robot passively move their arms into a crossed posture. In the active condition, error rates increased as the planning process evolved, mirroring the errors that were observed when the limbs were physically crossed. However, in the passive condition, error rates remained constant across the planning period. This data suggest that the brain uses motor planning signals to predict sensations from impending movements, and not necessarily the context of future limb postures.

1-D-114 Multisensory integration in 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. Recent studies have shown pupil dilation induced by presentation of visual and auditory stimuli, and the timing and size of the evoked responses are systematically modulated by stimulus salience. Moreover, microstimulation of the superior colliculus (SC), a midbrain structure involved in eye movements, attention, and multisensory integration, evokes similar transient pupil dilation, suggesting a coordinated role of the SC on the pupil orienting response. Although pupil dilation is evoked by presentation of single modality stimuli, stimuli in everyday life are often comprised of multisensory inputs. Here, we examined how human pupil dynamics are modulated by multisensory stimuli and hypothesized that sensory signals induced by salient stimuli presented from different modalities should be integrated in the SC to produce coordinated transient pupil responses. While requiring participants to maintain central fixation, we presented a visual, auditory, or combined audiovisual stimulus. Transient pupil dilation was elicited after presentation of visual or auditory stimuli. More importantly, presentation of audiovisual stimuli induced similar pupil responses with greater response magnitude. Together, the results demonstrated multisensory integration in pupil orienting responses, further arguing that the SC is the likely neural substrate coordinating these pupil orienting responses.

1-D-115 Reversible inactivation mapping of cortical sites required for voluntary forelimb movements in VGAT-ChR2 transgenic mice.

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VGAT-ChR2 mice were used to induce reversible local loss of function (by activation of GABA neurons) while mice were engaged in a lever-pulling task to map cortical regions that were required for voluntary forelimb movement. Awake water restricted mice with transcranial windows were head-fixed and trained to pull a robotic lever to get water rewards. Blue laser light was targeted to points within the contralateral and ipsilateral hemispheres to produce focal inhibition and probe their role in directed movements. Different frequencies (10, 50, 100Hz) of the blue laser light were targeted to the regions of interest for 1min intervals. Our preliminary results show a 3mW train of 5ms pulses delivered at 100Hz to the contralateral primary motor cortex(M1) reduced the number of rewarded lever pulls by 95±9% relative to a control site located outside of the cranial window, 10 Hz was less effective. This extent of inhibition was not seen while photo-activating other contralateral points such as M2, visual cortex, and even ipsilateral M1, showing (as expected) that contralateral M1 is required for the execution of voluntary movement. Putative hubs such as the retrosplenial cortex and the parietal association area were not required to initiate pulling. M1 inhibition was fully reversible as the mouse started pulling the lever within seconds once the laser was off the contralateral M1 site. Future experiments will require mice to correct for a perturbation in lever position after the initiation of a trial, revealing sites of potential sensory-motor feedback.

1-D-116 From chaos to control: Using oscillations to harness neuronal networks

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Synchronized (or zero time-lagged) activity carries functional benefits by dampening variability and enhancing the reliability of neuronal responses to stimuli. On the other hand, theoretical work has shown that neurons can generate asynchronous (or time-lagged) activity. Here, we employed numerical simulations to explore the benefits of synchronous versus asynchronous oscillations in balanced neuronal networks. Starting from a model of sparsely connected integrate-and-fire neurons that generates spontaneous spiking, we injected oscillations by adding a sine wave to the membrane potential of all cells. We induced synchronized spiking by tuning the waves such that the phase was the same across all neurons; we elicited asynchronous activity by setting each neuron at a unique phase (Fig. 1A). We then examined the ability of networks to respond reliably and discriminate between large depolarizing events delivered to random subsets of the population. We show that synchronized cells responded with greater reliability than asynchronous and hybrid (i.e., 50/50 mix of synchrony and asynchrony) networks to repeated presentations of the same stimulus (Fig. 1B). Asynchronous networks, however, responded with the highest degree of heterogeneity (i.e., distinct responses to different stimuli). These results highlight a functional trade-off between the reliability and heterogeneity of neuronal responses that is dependent on the nature of upstream activity. Neuronal networks may utilize both synchronous and asynchronous activity depending on the requisites of information processing.

1-D-117 A Computational Model of Updating and Integration of Remembered Visual Stimuli across Eye Movements

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Despite the ever-changing visual scene on the retina between eye movements, our perception of the visual world is constant and unified. It is generally believed that this space constancy is due to two internal processes; spatial updating and trans-saccadic visual feature integration. In this study, we aim to model and integrate both of these phenomena. We developed a state space model for updating gaze-centered spatial information. To explore spatial updating, we considered two kinds of eye movements, saccade and smooth pursuit. The inputs to our proposed model are: a corollary discharge signal, an eye position signal and 2D visual topographic maps of visual stimuli. The state space is represented by a radial basis function neural network and we can obtain a topographic map of the remembered visual target in its hidden layer. Finally, the decoded location of the remembered target is the model output. Training this model revealed that the receptive fields of state-space units are remapped predictively during saccades and updated continuously during smooth pursuit. Moreover, during saccades, receptive fields also expanded (to our knowledge, this predicted expansion has not yet been reported in the published literature). Subsequently, we construct a computational model for integration of visual feature information across saccades using recent discoveries in hierarchical processing as well as different feature information channels of primate visual system. We believe that incorporating these two models can shed light on the neural mechanism of Trans-saccadic perception.

1-D-118 Phosphatase 2B mediates NMDAR plasticity and metaplasticity in early odor preference learning in rats

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Previously we have demonstrated NMDA receptor (NMDAR) plasticity in the anterior piriform cortex (aPC) of rat pups following early odor preference learning. Down-regulation of NMDAR at 3 hr post-training results in metaplasticity with decreased LTP and inducible LTD at the aPC synapse. Re-training at 3 hr with the same odor abolishes preference memory. Here, we investigated the molecular mechanisms of NMDAR plasticity and metaplasticity using quantitative immunoblot of synaptic fractions of the aPC following training and pharmacological interventions. Intra-animal control was achieved through unilateral drug infusion. NMDAR down-regulation was prevented by aPC infusion of group I/II mGluR antagonist MCPG (100 mM), while neither NMDAR (D-APV, 500 µM or 5 mM) nor mGluR1

(AIDA, 500 µM or 5 mM) blockage had such an effect. Down-regulation of NMDAR was also blocked by FK-506 (5mM), a phosphatase 2B inhibitor. Bilateral infusion of either MCGP or FK-506 during the initial training restored odor preference memory when pups were re-trained at 3 hr. These results suggest mGluR (likely mGluR5) mediated phosphatase pathway leads to NMDAR down-regulation at 3 hr and unlearning upon re-training. It has been shown metaplasticity is mediated by NMDAR itself. Blocking NMDARs during the re-training prevents unlearning. Here we further demonstrated that unlearning was mediated by phosphatase 2B as FK-506 infusion during the re-training restored odor preference memory. Together, de-phosphorylation by phosphatase 2B results in both NMDAR down-regulation and NMDAR-mediated metaplasticity.

1-D-119 Lack of adenylate cyclase 1 (AC1) affect corticospinal tract development and locomotor recovery after spinal cord injury

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Cyclic AMP(cAMP) signalling pathways are involved in axonal growth and regeneration. The calcium-calmodulin-stimulated adenylate cyclase1 (AC1),a regulator of cAMP levels,is strongly expressed in the corticospinal motorneurons (CSMN) in cerebral cortex layer V during development, but its role in the development of the corticospinal tract (CST) is unknown. In our study, we analyse the organization of the CST pathway using anterograde and retrograde tracers in the barrelless (brl) mouse that carries an inactivating mutation of the AC1 gene. We show that in brl mice the general organization of the CST is normal but there is an increase in the number of axons in the ipsilateral contingent in the dorsal and ventral medial funiculi of the cervical spinal cord. The density of CSMN in layer V of the motor cortex is increased in brl compared to wild-type mice. Thus,lack of AC1 likely perturbs late phases of CSMN and CST development. Moreover, our study analyses the motor recovery after a spinal cord injury (SCI). We find that brl mice show enhanced locomotor functions as assessed by the BMS (Basso mouse scale) as early as 6h and up to 6 weeks after SCI, indicating a smaller responsiveness of brl mice to SCI. It is therefore possible that developmental effects on motor systems might decrease the locomotor effects consecutive to a SCI. This point is particularly important with regards to the use of transgenic animals for testing SCI recovery.

1-D-120 Spatiotemporal profiles of receptive fields of area 21a neurons in the cat

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In cats, cortical area 21a is considered a homolog of primate's area V4 and is involved in high-level cortical processing of visual information such as form detection. Basic properties of 21a neuron receptive fields (RFs) have been described in numerous studies but their spatiotemporal dynamics has not yet been described. In order to address this issue, extracellular recordings were undertaken in area 21a of anesthetized adult cats. Visual stimulation consisted in a pseudo-randomized sequence of bright and dark squares (4x4 deg) briefly presented (35 ms) against a gray background. To date, first-order spatiotemporal profiles of 27 neurons were obtained by reverse correlation analysis. Spatial analysis revealed that bright subfields were in average larger than dark subfields (316.7 ± 42.9 vs 168.8 ± 32.5 deg2) and, in most cells (25/27), subfields were overlapping (38 ± 7% overlap). Moreover, the average maximal spike probability of bright subfields was greater than for dark subfields (0.04 ± 0.008 vs 0.02 ± 0.003). Temporal analysis showed that subfields activity was highly concurrent but also that bright subfield's activity peaked earlier than their dark counterpart (58.3 ± 11.2 vs 87.5 ± 16.9 ms). Our laboratory has previously characterized RF profiles of cells in regions providing major input to area 21a. Thus far, the profiles obtained in area 21a differ substantially from the ones found in lateral suprasylvian areas and in pulvinar, supporting a distinct role of this cortical area in visual processing. Supp by CIHR grant to CC.

1-D-121 Precise coding of ankle rotation by lower-limb muscle spindle afferents

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Owing to the recent observation of paradoxical muscle movements of the plantar flexors during quiet stance, the contribution of calf muscle spindles during standing balance has recently been questioned. To investigate whether calf muscle spindles can provide useful feedback during the small ankle movements associated with quiet stance, we collected microneurographic recordings from human lower-limb muscle spindles. Muscle spindle afferents were recorded from the tibial (n=12) and common peroneal (n=3) nerves in 9 healthy, young adults. Five muscle receptors from the lateral gastrocnemius, 1 from the medial gastrocnemius, 4 from the soleus, 2 from the intrinsic foot muscles, and 3 from the tibialis anterior were sampled. When a muscle spindle was identified, the participant's foot was loaded into an actuated ankle platform instrumented with a load cell. Participants were exposed to continuous ankle plantarflexion-dorsiflexion movements with a power spectrum replicating that of quiet stance (peak-to-peak amplitude = 0.7°; bandwidth = 0 to 0.5 Hz; mean power frequency = 0.28 Hz). Three-quarters of the muscle spindles (9/12), particularly those located in the soleus muscle (4/4), displayed instantaneous firing rates that correlated with ankle angle. Overall, the soleus exhibited the highest level and broadest bandwidth of significant coherence calculated between spike times and ankle angle. Thus, a sub-population of calf muscle spindles likely play an important role in the standing balance control loop.

1-D-122 Dopamine exerts concentration-dependent bidirectional modulation and evokes state-dependent rhythmicity in motor networks of the neonatal mouse spinal cord

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Motor networks that produce rhythmic patterns of motor activity in the hind limbs reside primarily in the spinal cord and are under the influence of a number of descending modulatory inputs. Neuromodulators, such as dopamine, have the capacity to reconfigure neuronal networks creating diversity in the range of outputs that a fixed circuit can produce. However, evidence from invertebrate systems has demonstrated that the effect of neuromodulators is dependent on the pre-existing state of the network on which it is acting. Work in our lab using the neonatal mouse isolated spinal cord has demonstrated that spinal dopamine acts as a potent modulator of locomotor activity. Here we present data demonstrating the capacity of dopamine to evoke different patterns of motor activity is not only concentration dependent, but also dependent on the excitability state of motor networks in the spinal cord. Specifically, we have found that low concentrations of dopamine inhibit motor activity by acting on D2 receptors where as higher concentrations of dopamine are capable of evoking patterns of rhythmic motor activity and are mediated by excitatory D1 receptors. At constant concentrations of dopamine, the different patterns of activity can be recapitulated by altering global motor network excitability by manipulating the extracellular potassium or NMDA concentration. Using dopamine as an example, this suggests that in vertebrate rhythmically-active motor systems, excitability state may also play an important role in determining the influence of a neuromodulator on motor output.

1-D-123 Plasticity of Binocularity and Visual Acuity are Differentially Limited by Nogo Receptor

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The closure of developmental critical periods consolidates neural circuitry but also limits recovery from early abnormal sensory experience. Degrading vision by one eye throughout a critical period both perturbs ocular dominance (OD) in primary visual cortex and impairs visual acuity permanently. Yet understanding how binocularity and visual acuity interrelate has proven elusive. Here we demonstrate the plasticity of binocularity and acuity are separable, and differentially regulated by the neuronal nogo receptor 1 (NgR1). Mice lacking NgR1

display developmental OD plasticity as adults. Furthermore, their visual acuity and OD spontaneously recover after prolonged monocular deprivation throughout the critical period. Restricting deletion of NgR1 to either cortical interneurons or a subclass of parvalbumin-positive interneurons alters interlaminar synaptic connectivity in visual cortex and prevents closure of the critical period for OD plasticity. However, loss of NgR1 in PV neurons does not rescue deficits in acuity induced by chronic visual deprivation. Thus, NgR1 functions with PV interneurons to limit plasticity of binocularity, but its expression is required more extensively within brain circuitry to limit improvement of visual acuity following chronic deprivation.

1-D-124 Wide field calcium imaging of resting state activity in mice reveals both motif level, as well larger sensory clusters

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Strong reciprocal connections exist between the primary (S1) and secondary somatosensory cortex (S2) and primary motor cortex (area M1). In this study, the topology of functional connections between these areas was explored by using wide field calcium imaging in GCaMP3 or GCaMP6 mice expressing genetically encoded calcium indicator in cortical neurons. Wide field fluorescence imaging measured cortical calcium responses in anesthetized and awake mice. Spontaneous activity was recorded to establish connectivity relations between cortical points using a "seed pixel" approach. During sequences of spontaneous activity, calcium signals recorded of each location of the area S1 were correlated with localized activity in region of homotopic contralateral area S1, ipsilateral area S2 and bilateral areas of M1. Comparably, activity within each location of area S2 was correlated with activity localized in ipsilateral area S1 and M1. Activity within each location of area M1 was correlated with localized activity in region of homotopic contralateral area M1, bilateral areas of S1, and ipsilateral area S2. The K-means clustering revealed 3 mains clusters of brain areas corresponding to locomotion, oro-facial activity and vision as well as the symmetries existing between areas S1 and M1. This study demonstrated that several degrees of imbricated mesoscopic cortical functional organization co-exist. We anticipate that calcium imaging of functional connections using spontaneous activity will enable longitudinal studies during plasticity paradigms or after models of disease such as stroke.

1-D-125 Decoding the encoding strategy of primary sensory neurons by in vivo calcium imaging

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Dorsal root ganglion (DRG) neurons are the primary sensory neurons in the somatosensory pathways. The properties of individual DRG neuron have been thoroughly studied and many types of DRG neurons with distinct sensitivity have been identified. However, the responsiveness of an isolated sensory fiber to a particular stimulus cannot predict the sensory modality it is associated with. Moreover, in vivo, how a specific stimulus is represented by different types of DRG neurons simultaneously remains poorly understood. Here, we used AAV9 virus to specifically label DRG neurons with GCaMP6s, a genetically encoded calcium indicator, in wildtype mice. Then we conducted in vivo calcium imaging from small populations of DRG neurons using video rate laser scanning two-photon microscopy. The responsiveness of the neurons to various thermal and mechanical stimuli were recorded and analyzed. Our results showed that the majority of DRG neurons were polymodal and responded to multiple stimulations. The largest population is the DRG neurons which respond to hot and noxious hot stimulations. The activation patterns of different neurons by various thermal stimuli were also studied to reveal the encoding of stimuli. Principle component analysis will be applied for further neuronal clustering and decoding analysis. Our findings could help understand the underlying coding principle for ambient information by DRGs and by sensory systems in general.

1-D-126 Unbiased estimate of the spinal cord neuronal population involved in non-human primate motor control

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The spinal cord plays an essential role in the sensory and motor functions that give rise to behaviours ranging from simple reflexes to complex goal directed movements. The ratio between motoneurons and interneurons in the spinal cord has been commonly computed to understand the relative computational capabilities of spinal motor networks (Walloe, 2011). Currently, there are few accurate estimates of the number of motoneurons and interneurons within the non-human primate spinal cord and this ratio is not well defined. 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. The spinal cords of rhesus monkeys (Macaca mulatta) were examined from segments C2 to T1 using stereological methods to estimate the total number of neurons in specific areas of the grey matter. These areas included both those containing motor neurons (Lamina IX) as well as those containing interneurons associated with motor pathways (Laminae V - VIII). A ratio of 1:25 in the rhesus monkey was calculated using motoneuron and interneuron estimates. This ratio is higher than that of other mammalian and non-mammalian vertebrates, indicating that a more robust spinal network may underlie the ability of primates to generate complex upper limb movements.

1-D-127 Dissociation of parietal cortex contributions to obstacle memory in walking cats

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A working memory of environmental obstacles is essential for avoidance in walking mammals. In quadrupeds, vision is unavailable to guide hindleg stepping over an obstacle previously cleared by its forelegs. Instead, visual information about an obstacle and motor information about foreleg clearance are held in memory to modify hindleg movements. Previous studies suggest that this memory system relies on parietal areas associated with sensorimotor integration. To examine parietal contributions to obstacle memory, cortical cooling was used to reversibly deactivate areas 5 or 7 in cats trained to step over an obstacle with their forelegs and pause for a variable delay period before resuming locomotion. Hindleg step height and trajectory over the obstacle were measured to assess memory. While hindleg stepping was unaffected when area 7 was bilaterally cooled, bilateral cooling of area 5 resulted in significantly lower steps and altered trajectories, demonstrating a disregard for the obstacle. When bilateral cooling was restricted to the delay phase during which obstacle memory must be maintained, similar stepping deficits were observed. Additionally, cooling of area 5 in one hemisphere produced stepping deficits in the contralateral hindleg only. Finally, when area 5 cooling was stopped immediately after the memory acquisition phase (approach and foreleg clearance), high hindleg steps were restored, suggesting reactivation of obstacle memory. These results suggest that area 5 is critical for maintaining the memory of an obstacle encountered in the contralateral hemispace.

1-D-128 Imbalance of Excitation and Inhibition at Threshold Level In the Auditory Cortex

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The interplay of cortical excitation and inhibition is a fundamental feature of cortical information processing. Excitation and inhibition in single cortical neurons are balanced in their response to optimal sensory stimulation due to thalamocortical feedforward microcircuitry. It is unclear whether the balance between cortical excitation and inhibition is maintained at the threshold stimulus level. Using in vivo whole-cell patch-clamp recording of thalamocortical recipient neurons in the primary auditory cortex of mice, we examined the tone-evoked excitatory and inhibitory postsynaptic currents at threshold levels. Similar to previous reports, tone induced excitatory postsynaptic currents when the membrane potentials were held at - 70 mV and inhibitory postsynaptic currents when the membrane potentials were held at 0 mV on single cortical neurons. This coupled excitation and inhibition is not demonstrated when threshold-level tone stimuli are presented. In most cases, tone induced only excitatory postsynaptic potential. The best frequencies of excitatory and inhibitory responses were often different and thresholds of inhibitory responses were mostly higher than those

of excitatory responses. Our data suggest that the excitatory and inhibitory inputs to single cortical neurons are imbalanced at the threshold level. This imbalance may result from the inherent dynamics of thalamocortical feedforward microcircuitry. Based on our and other findings, a neural model is proposed to broaden our view of thalamocortical feedforward circuitry (see attached figure).

E - Homeostatic and Neuroendocrine Systems

1-E-129 Effects of ANA-12, a selective tyrosine-related kinase B (TrkB) antagonist, on anxiety, exploration, locomotion and fear avoidance learning following a repeated stress regimen in male Wistar rats

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In both human and animal studies, repeated exposure to stressors can have negative impacts on brain health, cognition, and mood. Stress can promote the expression of brain-derived neurotrophic factor (BDNF) and its primary receptor tyrosine-related kinase B (TrkB), and increased levels in the brain reward circuitry (e.g. nucleus accumbens (NAc)) can predict susceptibility to anxiety and depression. This study aims to assess the effects of repeated stress on the activity of BDNF/TrkB in the reward circuitry, and whether the inhibition of this system can promote anxiolytic effects in dopamine-related behaviors. 4 groups of male Wistar rats (n=10 /group) were implanted with guide cannulas in the NAc shell and treated with ANA-12 or Vehicle 30 minutes prior to undergoing a ten-day repeated stress/no stress regimen. A 5th group was added as non-treatment controls (n=6). Rats were subjected to the open field test (OFT), elevated plus maze (EPM) and Y maze passive avoidance test (YM-PAT). Findings indicate that ANA-12 significantly reduced anxiety-like behavior in the OFT and EPM in stressed rats. In contrast, ANA-12 increased anxiety-like behavior in the no stress group, indicated by a higher index of open arm avoidance in the EPM. Finally, in the YM-PAT, we observed an increased latency to re-enter the aversive arm in the ANA-12 (stress) group compared to other groups. This study demonstrates a beneficial effect of TrkB inhibition in stressed rats, and suggests that in a normal state, reduced TrkB activation in the reward circuitry can negatively impact the emotional response.

1-E-130 Dopamine acts directly on arcuate nucleus neurons to alter expression of neuropeptide genes

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The arcuate nucleus (ARC) of the hypothalamus acts as a central coordinator in the homeostatic regulation of energy balance. ARC neurons exhibit remarkable plasticity in response to changes in peripheral and central energy balance signals. The neurotransmitter dopamine (DA) appears to play a role in the regulation of food intake through alteration of the ARC neuropeptide Y/melanocortin circuit. Of particular interest is the D2 receptor, which has been implicated in mediating this effect. However, it is currently unclear what effects result from direct versus indirect action of DA on individual ARC neurons. In order to assess the direct effect of DA on ARC neurons, we utilized a dissociated ARC neuron preparation that effectively isolates neurons from interacting with one another. Cultures were treated with the selective D2 agonist quinpirole (10 M), or selective D2 antagonist sulpiride (10 M) for 5-6 days and changes in morphology and gene expression examined. No significant difference in the total number of neurite branches, total branch length or maximum branch length was found between treatments and control. However, qPCR analysis revealed a robust increase in pro-opiomelanocortin expression in agonist treated groups. These results indicate that DA acts directly on ARC neurons to favour a decrease in food intake.

1-E-131 Intra-VTA insulin decreases nucleus accumbens dopamine release in vivo

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Mesolimbic dopamine (DA) circuits, implicated in incentive motivation, are an important target for metabolic hormones such as leptin, ghrelin and insulin. We have previously demonstrated that insulin induces long-term depression of excitatory synapses onto ventral tegmental area (VTA) DA neurons and reduces food reward behaviours, including food anticipatory behaviour and

conditioned place preference for food. Whether intra-VTA insulin reduces DA release in projection areas of the VTA remains unknown. Using in vivo fast scan cyclic voltammetry in anesthetized rats, we show that peripheral and intra-VTA administration of insulin reduces stimulated DA release in the NAc, an effect that is blocked by the insulin receptor antagonist, S961. Furthermore, intra-VTA insulin reduces cocaine-evoked increases in NAc DA. Together, these results suggest that insulin action in the VTA decreases DA release in the NAc. Furthermore, because intra-VTA insulin decreases cocaine-evoked DA in the NAc, CNS delivery of insulin may provide clinical utility for decreased drug-induced craving.

1-E-132 Alteration to one carbon metabolism may underlie glutathione deficiencies in the rat dentate gyrus after prenatal ethanol exposure

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Fetal alcohol spectrum disorder (FASD) describes the wide scope of symptoms that can arise in a child when a mother consumes alcohol during pregnancy, and is one of the leading causes of preventable mental disorder in Canada and the United States. Unfortunately, consensus is vague with regards to core cognitive defects and behavioural temperaments associated with FASD, making diagnosis and treatment a considerable challenge. The varying degree symptoms that arise after prenatal ethanol exposure (PNEE) points towards a globally deleterious effect that fluctuates with timing, dosage, and pattern of exposure. For this reason, a considerable amount of attention has been invested into understanding the inhibitory effects of alcohol on one-carbon metabolism (OCM). OCM is a central pathway existing in all tissue types that regulates the metabolites necessary for epigenetic modification of gene expression, and is very tightly associated with cellular signaling and homeostasis. Furthermore, conditions of oxidative insult and damage are prevented when OCM metabolites are converted to cysteine and used in the synthesis of glutathione (GSH), an anti-oxidant molecule depleted in the brain after PNEE. Long-term deficits in OCM could alter cellular homeostasis and gene expression, and affect GSH levels by restricting the limiting substrate for GSH synthesis. The aim of this study is to investigate the primary metabolites associated with OCM after PNEE in a manner that would elude to the point of OCM truncation, and aid in the delivery of public understanding of the detriments of PNEE.

1-E-133 4(5)-methylimidazole, found in caramel colouring, alters gene expression in arcuate nucleus neurons.

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Evidence indicates environmental and synthetic contaminants may contribute to the obesity epidemic via endocrine disruption. Recent concern has focused on 4(5)-methylimidazole (4-MEI), a chemical byproduct found in caramel colouring, with confirmed carcinogenic and suspected neuroendocrine disrupting activity; high levels of 4-MEI are often found in foods despite stringent regulations. The goal of this study was to determine levels of 4-MEI in various beverages and evaluate possible effects on morphological parameters and gene expression of arcuate nucleus neurons, neurons that regulate energy balance. Levels of 4-MEI were determined using isotope-dilution reverse-phase liquid chromatography tandem mass spectrometry. Of 19 beer and soda beverages containing caramel colouring tested, 10 exceeded the maximum allowed dose of 29 μg per day; notably Waterloo Original Dark Lager contained 226.49 μg in a 355 mL serving. Based on these observations, we investigated whether 4-MEI could influence morphology and gene expression of arcuate nucleus neurons maintained in culture for 5 days in media that contained 0.3 μg/mL of 4-MEI or control media. Total neurite length, maximum neurite length, total branch number, and generalized morphological subtype were not altered by 4-MEI treatment. Interestingly however, qPCR analysis of RNA from treated cultures revealed that 4-MEI reduced expression of TH, NPY and POMC transcripts, which play a key role in the regulation of energy balance. These data indicate that 4-MEI has the capacity to act as a neuroendocrine disrupter.

F - Cognition and Behavior

1-F-134 Circuit principles of neuronal processing in larval Drosophila melanogaster thermotaxis

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The goal of systems neuroscience is to understand the computational process by which neural circuits use sensory information to generate adaptive behaviors. Drosophila larvae avoid excessively cool temperatures using a small set of sensorimotor transformations regulating the frequency and outcome of navigational decisions. During each navigational decision, larvae sweep their head from side to side, gathering thermal information that informs the choice of a new direction for forward movement. Automated trajectory and posture analysis of individual animals navigating temperature gradients enables us to quantify navigational decisions of each animal. Statistical analysis of an inactivation screen using the Gal4/UAS system with Janelia collection allowed us to identify neurons in the thermotactic circuit. We have identified two distinct groups of projection neurons that when inactivated exclusively modulate individual navigational decisions, such as turn direction and acceptance of larval headsweep. We mapped all partners using EM and found they receive direct synaptic inputs from cold sensing neurons. We are currently characterizing the computational dynamics of these neurons by measuring and manipulating neuronal activity in freely moving and/or restrained animals using novel methods in optical neurophysiology. Combining behavioral analysis, EM reconstruction of behaviorally relevant neurons and functional imaging will allow the complete identification of circuits underlying thermotaxis from sensory inputs to motor outputs.

1-F-135 Cognitive impairments in a touchscreen-based visual discrimination and reversal learning procedure in a rat model of absence epilepsy

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Absence epilepsy is a seizure disorder characterized by generalized spike-and-wave discharges (SWDs). Although epidemiological studies report that epilepsy syndromes with absence seizures are more likely to occur in females, potential sex differences in the comorbid cognitive functions are unknown. Here, adult GAERS (Genetic Absence Epilepsy Rats from Strasbourg), a well-validated rat model with absence seizures, and their paired strain, non-epileptic control (NEC) rats, were tested on visual discrimination learning using a paired-choice test in touchscreen-equipped operant chambers. Results showed that GAERS males made more errors and took more trials to reach criterion during visual discrimination. In contrast, no statistical difference was found between GAERS and NEC females, although GAERS females tended to spend more days in the pre-training stage. During the last two discrimination test days, reward latencies were altered in both male and female GAERS rats. GAERS males were slower to collect the reward, while GAERS females were faster. During the reversal learning phase, male GAERS took significantly more days and more trials to reach criterion than NECs. Our data suggest that a sex difference in visual discrimination and reversal learning may exist in the GAERS with males performing poorer on visual discrimination and reversal learning than females. Further studies are necessary to explore the mechanisms underlying these effects.

1-F-136 Training experience affects the selectivity of neurons and the pattern of noise correlations in primate lateral prefrontal cortex

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Recent studies have proposed that the primate lateral prefrontal cortex (LPFC) plays an important role in attention. How neurons in this area interact during attentional tasks and how that depends on prior experiences remains unclear. We recorded responses of neurons in LPFC area 8a of two macaque monkeys using 96-channel microelectrode arrays. The animals had to identify one of two moving random dot patterns as the target based on its color and indicate a change in its direction, while

ignoring the other stimulus. Monkey R was exclusively trained in this task, while monkey S had previously been extensively trained on a pure motion discrimination task. In monkey R, ~1/3 of the neurons were selective for the target location and few neurons were selective for motion direction. In monkey S, ~1/3 of the neurons were tuned for motion direction and few neurons were tuned for the target location. We computed noise correlations between pairs of neurons and found that similarly tuned neurons showed higher correlations than dissimilarly tuned ones. Using a linear classifier, we further assessed if correlations influence the neurons' ability to encode the stimulus features. In monkey S, removing noise correlations increased decoding performance. In monkey R, removing them either decreased or increased performance. Our results suggest that training shapes the tuning of neurons in prefrontal area 8a. Moreover, noise correlations vary with the similarity between the neurons coding preferences. The effect of noise correlations in information coding by LPFC ensembles seems to be complex.

1-F-137 Pattern separation deficits in a patient with bilateral dentate gyrus lesions

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Neuroimaging and behavioural studies support various theories on the nature of hippocampal contributions to episodic memory, the ability to remember past personal events in one's life that are specific to time and place as well as items that have been previously encountered in a laboratory setting. In order to represent episodic memories as distinct, a mechanism of pattern separation is needed to reduce interference among similar neural inputs by using non-overlapping representations. In recent years, computational models and animal studies have prompted speculation that pattern separation can be localized to the dentate gyrus (DG) and CA3 subfields at the core of the hippocampus. A challenge with studying pattern separation in humans, however, is the fact that individuals with hippocampal damage typically have lesions that extend into and beyond the hippocampus, making it difficult to localize pattern separation to the DG. In the current study, we investigated pattern separation in a rare person, B.L., who, in relation to electrocution and cardiac arrest, suffered bilateral hippocampal damage localized to the dentate gyrus. B.L.'s performance was compared to that of age-matched controls on a widely used pattern separation task. Results of impaired pattern separation provide the first evidence in humans of a selective role of the DG in creating and maintaining distinct visual memories.

1-F-138 Simulation of embodied and the large-scale neuronal systems with the iqr software

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The notions that cognition is embodied, and that function arises through the large-scale connectivity at least as much as the computational power of individual units, are now widely accepted as core to understanding the human brain. Hence, neuronal simulations must incorporate these paradigms, and to do so we have -- over the past more than 10 years -- developed the large-scale neuronal systems simulator iqr. Embodiment of cognition is addressed in iqr by providing the means to interface neuronal simulations to "real-world" devices such as mobile robots, cameras, microphones etc. While the need for sophisticated connectivity is met by providing powerful, yet elegant ways of specifying the connectivity between large groups of neurons. In iqr large-scale neuronal models are developed using a graphical user interface, and the simulation can be controlled on-line by changing parameters of the model at run-time. Built-in on-line tools allow to visualize, record, and analysis of data without the need for additional tools. iqr provides both, pre-defined neuron and synapse types, and an open architecture for new, user-defined neurons, synapses, and hardware interfaces. The simulation software has been successfully used in research and teaching in number of projects, is fully documented, and the source code of is publicly accessible under the GNU General Public License (GPL).

1-F-139 The Word on the Beat: Behaviour and Brain Interactions of Reading and Rhythm

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Like musical rhythm, speech rhythm involves organized acoustic sequences and complex cognitive

and motor processes. More specifically, speech rhythm refers to the patterns of stressed and unstressed syllables that build up the meter of spoken utterances. The current study used fMRI to identify common brain regions that are shared during rhythm processing and reading aloud. A behavioral study revealed a connection between rhythm and reading, whereby reading was enhanced by the presentation of a rhythmic prime that was on-beat compared to off-beat with the syllabic stress of the bisyllabic target letter string. We used words vs. pseudohomophones (PH; which must be phonetically decoded; eg praktis), to investigate whether sublexical reading would show a greater effect of the rhythmic prime given recent findings (Cason & Schön, 2012). The results revealed a Beat by Stimulus Type interaction, indicating that reading sublexically (PHs) was more enhanced by preceding the target with 'on-beat' primes than lexical reading (words). In an fMRI study, we examined overlap in brain activation in regions such as the brain stem, cerebellum, thalamus, basal ganglia, motor regions, prefrontal cortex and temporal cortex, as these regions have been implicated as important for speech rhythm (Fujii & Wan, 2014). Furthermore, the putamen was a region of interest given that it has recently been implicated in sublexical processing (Oberhuber et al. 2013), as well as rhythm processing (Grahn, 2009). These regions of shared activation are discussed in terms of models of reading and rhythm processing.

1-F-140 The effect of fendiline on cocaine self-administration and reinstatement of cocaine-seeking behaviour in the rat.

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GABAB receptors have been a recent target in the development of new pharmacological compounds for cocaine addiction. GABAB agonists like baclofen lower breakpoints in cocaine self-administration studies and prevent reinstatement of cocaine-seeking behaviour in rats, but produce negative side effects in clinical populations. Researchers have now begun testing drugs such as fendiline that act indirectly on the GABAB receptor. In the present investigation, the effect of fendiline on cocaine self-administration and on cue- and drug-induced reinstatement was assessed. In the first study male Wistar rats were trained to self-administer cocaine under a progressive ratio schedule of reinforcement and then pretreated with fendiline (vehicle, 1.78, 3.16, or 5.62 mg/kg, IP). A separate group of rats received baclofen (vehicle, 0.56, 1.78, 3.16, or 5.62 mg/kg, IP) 30 minutes before a 5.62 mg/kg dose of fendiline directly before their self-administration session. In the next study, the effect of fendiline pretreatment (vehicle, 3.16 or 5.62 mg/kg, IP) on cue-induced (light and tone) or drug-induced (10 mg/kg, cocaine, IP) reinstatement was assessed in separate groups of rats that underwent 10 days of extinction training after cocaine self-administration had been established. Results indicated that fendiline only reduced breakpoints when it was combined with 3.16 or 5.62 mg/kg baclofen; fendiline alone reduced both cue- and drug-induced reinstatement of lever pressing. Results suggest that fendiline, and other drugs that indirectly target the GABAB receptor, warrant further investigation

1-F-141 Younger age of onset of cannabis use is associated with thalamic dysconnectivity in youth at clinical high risk of psychosis

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Dysconnectivity in thalamic circuits has been observed in schizophrenia and in youth at clinical high risk (CHR) of psychosis. The impact of environmental risk factors for psychosis on thalamic connectivity is unknown. We evaluated the impact of cannabis use patterns on thalamic connectivity in a CHR sample. Cannabis use including lifetime use, severity and frequency of use, and age at onset of first use was collected in 162 CHR and 105 controls. Resting-state fMRI scans were used to create whole-brain thalamic functional connectivity maps generated using individual subjects' anatomically defined thalamic seeds. Results indicated that

thalamic connectivity did not differentiate those with or without a lifetime history of cannabis use nor significantly correlate with current use severity or frequency in either group. In CHR cannabis users, attenuated thalamo-cerebellar connectivity significantly correlated with a younger age of onset of cannabis use. CHR who used cannabis before age 15 did not differ on thalamic connectivity compared to CHR who used after age 15 or cannabis naive CHR. CHR converters and CHR non-converters separated according to positive/negative lifetime history of cannabis use did not differ on thalamic connectivity. These data indicate that although a younger age at onset of cannabis use is associated with disrupted thalamo-cerebellar coupling, cannabis use does not appear to be an identifying characteristic for thalamic circuitry in CHR early-onset users or CHR who convert to psychosis compared to non-converters.

1-F-142 A novel Presenilin 1 mutation causes Alzheimer's Disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder leading to dementia. A majority of AD cases are sporadic with late onset. Missense mutations in Presenilin 1 (PS1), Presenilin 2 (PS2) and Amyloid β Precursor Protein (APP) cause early onset familial AD (FAD). PS1 mutations account for the majority of early-onset FAD. PS1 is the catalytic core of y-secretase complex to process APP to generate amyloid β protein (Aβ), the central component of neuritic plaques in AD brain. Pathogenic mutations in PS1 gene has been shown to contribute to AD pathogenesis via impaired processing of APP. A novel PS1 mutation has been identified in the AD patients with early onset in a Chinese family. We extensively characterized the function of the PS1 mutation in mammalian cells and established a transgenic mouse strain carrying the mutation. We found that this PS1 mutation altered y-cleavage of APP to produce Aβ and promoted AD pathogenesis in PS1/APP double transgenic mouse model.

1-F-143 Effect of T-type calcium channel blockade on the induction and reinstatement of morphine-induced conditioned place preference

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Z944 is a Ca2+ channel antagonist for the low-voltage activated T-type channel. Rhythmic burst-firing in the ventral tegmental area (VTA) is partially driven by T-type channels. The VTA is responsible for dopamine (DA) release in the mesolimbic DA system, implicated in the rewarding properties of drugs of abuse. Accordingly, pharmacological blockade of T-type channels may attenuate the rewarding properties of a drug by reducing DA efflux. We evaluate the ability of a novel T-type Ca2+ antagonist, Z944, to attenuate the rewarding properties of morphine (MOR) in a conditioned place preference (CPP) paradigm. Rats were given injections of MOR (5mg/kg) or saline prior to being placed in one of two contextually distinct chambers. Conditioning trials alternated between MOR or saline treatment for 4 days each. Z944 (5, 7.5mg/kg) or vehicle were administered 5 min. prior to each 45 min. MOR conditioning session. Expression of MOR CPP was assessed after conditioning, and after at least 12 days of extinction, MOR-induced (5mg/kg) reinstatement of CPP was assessed. We observed a dose-dependent effect of Z944 on the induction of MOR CPP. Both experimental groups extinguished their preference for the 'MOR chamber' significantly faster (5 d) compared to vehicle controls (10 d). Reinstatement was blocked in the group that received 7.5mg/kg of Z944 during conditioning.

Induction and MOR-induced reinstatement of MOR CPP is attenuated by the T-type Ca2+ channel antagonist Z944. Based on the findings, Z944 may provide a novel pharmacotherapy for modulating the rewarding properties of MOR.

1-F-144 Knowledge of haptic feedback availability does not influence size information supporting pantomime grasping

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Delayed grasp of a visual target entails the use of absolute size information. In addition, receiving haptic feedback via target grasp plays an important role in the neural control of action. More specifically, our previous work showed that pantomime grasp of a target in delayed conditions (PH- condition) requires the use of relative size information. However, when participants were given haptic feedback from the object following pantomime grasping to the target's empty location (PH+ condition) absolute size cues were used. Notably, in this earlier work subjects were given instructions on how to perform the task prior to initiating the experiment. As such, we wanted to investigate whether the knowledge of receiving haptic feedback influences the distinct processing pathways supporting PH- and PH+ tasks. Participants grasped differently sized objects in separate blocks of PH+ and PH- and instructions about the upcoming task were given before the start of the each block. In a third condition PH+ and PH- trials were randomly presented (PR condition) meaning that participants did not know what trial type they were about to perform. To determine whether responses adhered to/or violated Weber's psychophysical law just noticeable difference scores (JNDs) were computed. Comparable to the PH- trials, JNDs in the PR condition scaled to target size irrespective of the availability of haptic feedback. Our findings indicate that participant's anticipation about the upcoming task does not influence the nature of the processes controlling grip aperture formation in pantomime conditions.

1-F-145 Functional interaction between medial prefrontal cortex and dorsomedial striatum is necessary for odour memory span in rats: role of GluN2B-containing NMDA receptors

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Working memory is a type of short-term memory for storage and manipulation of information necessary for higher order cognition. Working memory capacity is often assessed using span tasks. Span performance is linked to activation of medial prefrontal cortex (mPFC). The present experiments examined whether inactivation or GluN2B NMDA receptor blockade in mPFC, dorsomedial striatum (dmSTR), or disconnection of these areas reduced span using the odour span task (OST). The OST requires rats to remember an increasing span of odours to receive reward. Rats were cannulated bilaterally in mPFC and dmSTR and trained on a delayed non-match to sample procedure with scented bowls containing food reward. Subsequent "span" tests involved adding bowls with novel odours one at a time until an error occurred. The number of cups correctly chosen before an error was the span. Infusions of the GABA receptor agonists muscimol and baclofen (M/B) were used for inactivation or Ro25-6981 was used to block GluN2B NMDARs. Bilateral inactivation of mPFC significantly impaired span (span after inactivation = 2.7 odours; vehicle = 7.8). Bilateral dmSTR inactivation or blockade of GluN2B NMDARs significantly reduced span (inactivation = 5.6; vehicle = 11.0; GluN2B NMDAR blockade = 6.2; vehicle = 10.2). Disconnection of mPFC and dmSTR significantly impaired span (inactivation = 1.7; vehicle = 6.8; mPFC inactivation, dmSTR GluN2B NMDAR blockade = 2.4; vehicle = 9.0). The present results demonstrate a critical role of the direct projection from the mPFC to the dmSTR in performance of the OST in rats.

1-F-146 Moxifloxacin Induced Psychosis: A Case Report Study

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Background: Prior studies have demonstrated that the floroquinolones can cause drug induced mental status changes (Farrington et al. 1995). Discussion: The reported patient is a 91 year old lady with prior medical history of chronic obstructive pulmonary disease (COPD), hypertension, hypelipidemia , coronary artery disease, peripheral artery disease and no prior psychiatric history. The patient was admitted to the inpatient medical unit for COPD exacerbation , for which she was started on oxygen therapy, prednisone 40mg tab PO daily (the patient was on long term prednisone regimen), monteleukast 10mg tab PO every evening at 7pm, as well as Moxifloxacin 400mg tab PO daily. On the following evening after her admission, the patient became acutely agitated, displaying paranoid delusions comprised of patient believing that the inpatient nurses and staff members were trying to steal her belonging and kill her. Despite multiple verbal redirection and reassurancefrom the inpatient staff members, the patient remained acutely

agitated.Given the fact that this was the first time that Moxifloxacin was administered for the patient and there was no prior history of exposure to this regimen, and in light of prior clinical reports regarding the possible psychiatric manifestations regarding various floroquinolones, the CL team recommended discontinuation of this medication. Conclusion:Given prior reports of floroquinolone induced mental status changes and with rate but possible psychiatric manifestations, due clinical diligence is advised specially in the elderly population.

1-F-147 Approach and Avoidance Processing: Investigating a Rostrocaudal Gradient in the Nucleus Accumbens Core

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The nucleus accumbens is a site of integration of positively and negatively valenced information and action selection. While a rostrocaudal topographical gradient in valence processing has been found in the accumbens shell subregion primarily in terms of unconditioned behaviours, a potential gradient has not fully been explored in the core in relation to its role in motivation in response to conditioned cues. In the current study, rats were trained to associate visuo-tactile cues with appetitive sucrose, aversive foot-shocks and neutral outcomes. In a test of motivational bias, the aversive and appetitive cues were superimposed in a maze arm and rats' exploratory bias was measured for this arm vs. a neutral cued arm. Animals receiving GABA receptor agonists in the caudal core region displayed a bias in the direction of aversion, whereas those undergoing disruption of activity in the rostral core displayed an ambivalence similar to controls, with an additional behavioural difference of augmented vacuous chewing. The results suggest a rostrocaudal differentiation in valence processing of conditioned cues in the accumbens core.

1-F-148 Impoverished Descriptions of Familiar Routes in Three Cases of Medial Temporal Lobe/Hippocampal Amnesia

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Recent research has challenged classic theories of hippocampal function in spatial memory with findings that the hippocampus may be necessary for detailed representations of environments learned long ago, but not for remembering the gist or schematic aspects that are sufficient for navigating within those environments (Rosenbaum et al., 2000; 2012). We aimed to probe further distinctions between detailed and schematic representations of familiar environments with three medial temporal amnesic patients with hippocampal damage by testing them on a route description task and mental navigation tasks that assess the identity and location of landmarks, and distances and directions between them. The amnesic cases could describe basic directions along known, imagined routes, estimate distance and direction between well-known landmarks, and produce sketch maps with accurate layouts, suggestive of intact schematic representations. However, findings that patients' route descriptions lack richness of detail, along with impoverished sketch maps and poor landmark recognition, substantiates previous findings that detailed representations are hippocampus-dependent.

1-F-149 Decoding Phenomenal Experience in Vegetative State Patients

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The objective of this project is to investigate the epistemological implications of recent neuroimaging studies, which offer a "neural index" for conscious human experience. Neuroscientists have long suspected that the network of brain regions involved in "executive function" may provide a window into conscious experience. However the numerous processes involved in conscious experience make it difficult to relate patterns of brain activity to specific higher or lower-order functions. A recent study addressed this problem by having subjects watch an engaging and suspenseful movie, providing viewers with a shared conscious experience (Naci el al. 2014). The results of initial investigations on healthy controls showed that the timing of activity in the relevant regions of the brain was predicable based

on participants' highly similar qualitative experience of the movie's moment-to-moment executive demands. The neural activity across participants was synchronized, indicating a similar conscious experience. Moreover, in a patient who was thought to be in a vegetative state (VS), highly similar moment-to-moment executive processes as healthy individuals' were detected. This project aims to answer the following question: does Naci and colleagues' "neural index" truly offer novel insight into the phenomenology of conscious experience?  Answering this question will significantly impact a number of areas of inquiry, including philosophy of mind and epistemology.

1-F-150 Does place field repetition impair spatial learning?

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Place cells are pyramidal neurons found in regions CA1 and CA3 of the hippocampus. They fire in a particular area of an environment, referred to as a place field, and are generally thought to support spatial cognition. Recent research has shown that place cells exhibit repeating place fields in an environment comprised of visually identical, parallel compartments. Through two experiments, we set out to determine the significance of place cell activity in supporting the discriminatory ability of rats. In our first experiment, we tested whether a group of rats (n=6) could learn a spatially-guided odour discrimination task in an environment composed of 4 parallel compartments. A second group (n=6) was trained in the same 4 compartments arranged in a semi-circular (radial) formation; in which we would not predict place field repetition. Our results concluded that learning was significantly impaired in the parallel environment. Next, we performed single-unit recordings from CA1 place cells as rats foraged in the parallel and radial environments. We observed repeating place fields across the parallel compartments. To quantify this we performed correlative analyses between compartments of each maze configuration and calculated a significantly higher correlation between place cell activity in the parallel compartments as compared to activity in the radial compartments, supporting the observation of place field repetition. Overall, these results suggest that rats have difficulty discriminating environments in which place field repetition occurs.

1-F-151 Differential Effects of Dopamine and Selective Dopamine Agonists on Spatial Working Memory, Attention, Learning and Reaction Time in Healthy Controls

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Dopamine (DA) plays a critical role in working memory and cognitive control. However, DA has been shown to both improve and/or impair cognitive performance across different subjects, tasks, and studies. This complex relationship may be due to the differential effects of dopamine at D1 and D2 receptors in basal ganglia cognitive loop nuclei (striatum and the dorsolateral prefrontal cortex). In order to address this question, we have investigated the effects of DA manipulation on cognitive performance in healthy monkeys using a touch screen running the CAmbridge Neuropsychological Test Automated Battery (CANTAB). One of three DA drugs or placebo were administered prior to each daily CANTAB session: Sinemet (Levadopa/Carbidopa), Dihydrexidine hydrochloride (selective D1 like receptor agonist) or Sumanirole maleate (selective D2 agonist). Three CANTAB tasks were tested at each session: (1) 'visually guided reaching task', which tests reaction time and reaching accuracy, (2) 'reversal learning task', which tests association learning, cognitive flexibility and attention, and (3) 'spatial ordered sequential search task' which tests spatial working memory. Metrics from these tasks were compared between control and each DA drug condition. Results demonstrated that the D1 agonist most strongly impaired reversal learning whereby the D2 agonist most strongly impaired reaction time and spatial working memory. These results provide insight into the relationships between DA and cognition and provide baseline behavioural data for future physiological recordings in the basal ganglia.

1-F-152 Increased spontaneous somatic-patterned cortical activity in a mouse model of depression

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Background: Individuals with depression commonly present with medically unexplained somatic complaints. The neurobiology of somatisation, however, is poorly understood. In mice, we have shown that spontaneous cortical activity contains patterns resembling evoked sensory activity. We hypothesized that depression would induce alterations in spontaneous somatic-patterned activity. Method: Using the Chronic Social Defeat (CSD) model, 8 week old C57/B6 males were exposed to 10 days of defeat. The depressive phenotype was assessed using the Forced Swim Test (FST) and the Sucrose Preference Test (SPT). A large craniotomy exposed both hemispheres and voltage-sensitive dye (VSD; Rh1692) was incubated over the cortex. We imaged with 6.67ms temporal resolution and 8.6*8.6mm field of view. We recorded 6 minutes of spontaneous cortical activity after which we recorded somatic patterns of neuronal depolarization using forelimb, hindlimb and whisker stimulation. These were used as templates to identify somatic-patterned activity in spontaneous brain activity. Results: Mice exposed to CSD had a depressive phenotype on the FST and SPT. Cortical representation of sensory stimulation did not differ between 'depressed' and control mice. Yet, 'depressed' mice had a higher frequency of somatic-patterned matches in spontaneous cortical activity, and the frequency of these was related to the FST and SPT. Discussion: A mouse model of depression is associated with increased frequency of spontaneous somatic-patterned cortical activity, a putative neurobiological underpinning of somatisation.

1-F-153 Cadherin adhesion complexes and cocaine-mediated synaptic plasticity

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Memory disorders are typically associated with the loss of memory, however pathological conditions can also arise when the brain retains maladaptive associations too powerfully. Exposure to addictive drugs elicits pathologic adaptations in many parts of the brain, specifically an LTP-like potentiation of excitatory synapses onto dopaminergic (DA) neurons in the ventral tegmental area (VTA). Cadherin cell adhesion complexes are well suited to provide a link between structural changes of the synapse and synaptic plasticity. Emerging evidence suggests that cadherins and their intracellular binding partners, may be of importance in the study of addiction. Indeed, several genome-wide allelic association studies have reported a high incidence of mutations in cadherin genes in individuals with substance abuse problems. We have demonstrated that cadherins are localized to excitatory and inhibitory synapses being formed onto DA neurons within the VTA. We have used immunoelectron microscopy to quantify cadherin localization at synapses within the VTA after wildtype mice develop preference for cocaine in a conditioned place preference (CPP) paradigm. With this method, we have shown that cadherin localization is significantly increased at excitatory synapses onto dopaminergic neurons of the VTA after mice develop preference for cocaine. Moreover, inhibiting intercellular N-cadherin interactions abolishes spike-timing dependent plasticity in the VTA, indicating that cadherin adhesion complexes play an important role in synaptic plasticity in this region.

1-F-154 Synaptic Plasticity and Reversal Learning are Impaired following B-catenin Stabilization in Hippocampal Neurons

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The cadherin/β-catenin adhesion complex is a key mediator of the bidirectional changes in synapse strength which are believed to underlie complex learning and memory. In the present study, we demonstrate that stabilization of β-catenin in the hippocampus of adult mice results in significant impairments in cognitive flexibility and spatial reversal learning, including impaired extinction during the reversal phase of the Morris Water maze and deficits in a delayed non-match to place T-maze task. In accordance with this, β-catenin stabilization was found to abolish long-term depression (LTD) by stabilizing cadherin at the synaptic membrane and impairing AMPA receptor endocytosis, while leaving basal synaptic transmission and long-term potentiation (LTP) unaffected. These results demonstrate that the β-catenin/cadherin adhesion complex plays an important role in learning and memory, and that

aberrant increases in synaptic adhesion can have deleterious effects on cognitive function.

1-F-155 Disrupted docosahexaenoic acid metabolism in carriers of apolipoprotein E epsilon 4 allele: is there a link with the risk of developing cognitive decline?

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Background: Over the last five years, our group investigated imbalance in the metabolism of docosahexaenoic acid (DHA) in humans and in transgenic mice carrying human apolipoprotein E epsilon 4 (APOE4%2B) genotype. One of our hypotheses is that rebalancing DHA metabolism could contribute to lower the risk of cognitive decline in APOE4%2B. Objective: To overview evidences collected from human and mice studies on disturbed DHA metabolism in APOE4%2B compared to APOE4-. Results: In APOE4%2B, DHA concentration in plasma triglycerides was higher than APOE4-, but after a n-3 fatty acid supplementation, increase of DHA was lower than APOE4-. Using 13C-DHA, APOE4%2B had 31% lower postprandial 13C-DHA compared to APOE4-. Our recent results in 4 and 13 month-old transgenic mice carrying human APOE4%2B showed that brain uptake of 14C-DHA was 24% lower in APOE4%2B than APOE2 but cortex DHA was significantly lower in 13 month-old mice only. Plasma DHA was significantly higher in APOE4 mice than APOE2 suggesting that lower brain uptake was not because of lower availability of DHA in plasma. Recently, we prevented spatial and visual cognitive deficits in APOE4 mice by feeding a DHA diet compared to a control diet. Conclusion: Disturbed DHA metabolism in APOE4%2B seems highly age-dependant. However, a diet rich in DHA seems to prevent cognitive deficits in APOE4%2B mice. Therefore, understanding why DHA metabolism if disturbed in APOE4%2B will help in finding strategies for preventing or delaying onset of cognitive decline in this population.

1-F-156 Suppression of simple visual hallucinations from occipital stroke using TMS

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We present the case of a 31-year old patient who perceived continuous simple hallucinations in a hemianopic field defect immediately following right occipital cortex stroke, which have remained unchanged over 2 years. We performed 1 Hz repetitive transcranial magnetic stimulation (TMS) to the lesioned area for 30 minutes per day over 5 days in an attempt to suppress the perpetual hallucinations. fMRI was performed prior to and after TMS treatment to assess plasticity changes. Pre-TMS, the patient showed greater immediate activation at the boundary of the lesion compared to healthy controls; in the cuneus, lingual gyrus and surrounding areas. The associated "hyperactivity" corresponded to a reported perceptual increase in visual hallucinations. During daily TMS sessions, the perception of hallucinations was greatly reduced. Post-TMS fMRI showed not only a suppression of activity in the previously associated regions of "hyperactivity", but a redistribution of this activity to surrounding regions, to a level similar to that of controls. A decrease in occipital activation with TMS resulted in a decrease of frontal activity that is consistent with our previous work, indicating connections between ventral regions and the frontal lobe. This case provides evidence of an infarct resulting in excitatory discharges at the border of the lesioned area which stimulate neighbouring areas, and thus result in abnormal visual perception. We causally demonstrate that repetitive TMS provides a valuable method of modulating hallucinations from occipital injury or infarct.

1-F-157 Navigational strategies in young and older adult Inuit hunters

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Inuit hunters exhibit exceptional navigational skills. Although researchers have described the ways in which Inuit hunters navigate, such as by using the direction of winds, stars, landmarks, and snowdrifts, we do not know the specific cognitive strategies used. The use of these ever-changing features implies that they are constantly forming relationships between their current location, environmental features, and their destination. This process is the hallmark of the "spatial strategy". Different strategies can be used to navigate, but we hypothesized that Inuit hunters use the spatial strategy to a greater extent. We also hypothesize

that young Inuit hunters, because they rely more on technology, use the spatial strategy to a lesser extent than older Inuit hunters. Nine healthy older Inuit men hunters and seven healthy young Inuit men hunters, tested in Igloolik, Nunavut, took part in the 4-on-8 virtual maze, an 8-arm radial maze. Spatial learners use landmarks to learn the location of the objects in the maze, while response learners use a sequence of right and left turns from a given starting position or stimulus. As per our hypotheses, 87.5% of the elderly Inuit participants used a spatial strategy in contrast to only 39% of Southern Canadians. Further, elderly Inuit participants used spatial strategies to a greater extent than young adult Inuit hunters. Traditional occupations such as hunting seem to promote hippocampus-dependent spatial memory in the Inuit, a strategy associated with healthy cognition.

1-F-158 Different measures of decisions yield distinct information: Explicit and implicit measures reveal independent biases during economic decision making in a reaching task

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Decision making involves the neural accumulation and integration of evidence to make a choice between available options. Even the simplest decisions, such as deciding between an apple and an orange, involve complex computations involving sensory inputs, valuation, and memory; however, real life decisions are often more complex and can involve additional factors, including risk and ambiguity. That is, options that can have multiple outcomes (risk) and the likelihood of outcomes can be uncertain (ambiguity). We investigated choice biases during risky and ambiguous decision making using a simple reaching task, allowing us to collect several behavioural measures. Participants reached to choose shapes that rewarded points with differing probabilities (25%, 50%, and 75%), some of which they knew, and some they did not. Both explicit (choices, questionnaires, and probability estimates) and implicit (reaction times and reach trajectories) measures were recorded. Each measure revealed distinct results: probability estimates were relatively accurate; early choices were biased by novelty-seeking and later choices were sensitive to ambiguity aversion; and reaction times and reaching movements were primarily driven by reward and expected value, respectively. In sum, our results demonstrate that different measures within the same economic decision making task can reveal distinct behavioural biases. Our research sheds light on an often overlooked aspect of decision making research-it is as important to consider how people physically make a choice as it is to measure what they chose.

1-F-159 The role of parvalbumin-positive interneurons in memory consolidation

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After a memory is initially encoded, it undergoes consolidation, during which it becomes less reliant on the hippocampus (HPC) and more dependent on the medial prefrontal cortex (mPFC). HPC oscillations, called ripples, coincide with mPFC oscillations, called spindles, and the spindle-ripple coupling is thought to facilitate memory consolidation. While parvalbumin-positive interneurons (PVNs) support spindles and ripples, whether they regulate the spindle-ripple coupling, and by doing so, modulate memory consolidation, is unclear. Here, we combine the designer receptor approach with behavior experiments and in vivo recording in freely behaving mice, to manipulate PVNs and investigate their role in memory consolidation. PV-Cre mice were infused with Cre-recombinase-dependent virus carrying the hM4Di receptor, allowing PVNs to be silenced by the designer drug clozapine-N-oxide (CNO). Then mice were trained using contextual fear conditioning, and we silenced PVNs during the consolidation period. When mPFC PVNs were selectively silenced, mice showed memory deficits. To explore the electrophysiological mechanisms, we performed simultaneous local field potential recordings in the mPFC and HPC. Our preliminary data shows that inhibiting mPFC PVNs does not alter the density or duration of spindles or ripples; however, spindle-ripple coupling may be reduced. This suggests that the mPFC PVNs are important in synchronizing the oscillations between HPC and mPFC, and interfering with their activity may disrupt the coherence between the two regions, and impair memory consolidation.

1-F-160 Prenatal Marginal Vitamin A Deficiency Facilitates Alzheimer's Disease Pathogenesis

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Deposition of amyloid β protein (Aβ) to form neuritic plaques in the brain is the pathological hallmark of Alzheimer's disease (AD). Mild or marginal vitamin A deficiency (MVAD) is a serious and widespread public health problem in pregnant women and children in developing countries. There has been increasing evidence for the involvement of vitamin A in AD pathogenesis. However, the role of vitamin A in the development of AD is not well defined. Our studies in an elderly population have shown that vitamin A deficiency (VAD) could enhance the risk to develop AD, and retinoic acid receptors (RARs) plays an important role in the amyloid β precursor protein (APP) metabolic pathway. To further examine vitamin A's effect on AD pathogenesis, we established a prenatal MVAD model in APP/PS1 double-transgenic AD model mice. Our study showed that MVAD significantly increases Aβ production by inhibiting ADAM10-mediated α-secretase cleavage and increasing β-secretase (BACE1) cleavage of APP. MVAD significantly increased neuritic plaque formation and aggravated spatial learning and memory deficits. Our findings provide a mechanistic explanation for the role of MVAD in AD pathogenesis and demonstrate the importance of retinoic acid signaling as a target for AD therapy.

G - Novel Methods and Technology Development

1-G-161 Expanding the toolbox of genetically encoded voltage indicators

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Optical imaging using voltage indicators based on green fluorescent proteins (FPs) has emerged as a powerful approach for detecting the activity of many individual neurons with high spatial and temporal resolution. To expand the toolbox of genetically encoded voltage indicators, we engineered two FP-based voltage indicators: A bright Red fluorescent voltage indicator (FlicR1) and a green to red highlightable voltage indicator (FlicGR). We used directed protein evolution to screen libraries of thousands of variants to identify clones with sufficient brightness and response amplitude that would report membrane potential changes in mammalian cells. FlicR1 has voltage sensing properties that are comparable to the best available green indicators. It reports single action potentials in neurons in single trial recordings. Furthermore, FlicR1 can be imaged with wide field fluorescence microscopy using a typical mercury arc lamp, and faithfully reports spontaneous activity in cultured hippocampal neurons and rat brain organotypic. We also demonstrate that FlicR1 can be used in conjunction with PsChR, a blue-shifted channelrhodopsin, for all-optical neuronal activation and activity recording. FlicGR is the first example of a highlightable voltage indicator. A powerful application of fluorescent proteins is their use as highlighters where they can be converted from one color to another by light. Using blue light, we can photoconvert FlicGR from green to red. We demonstrate that both the green and red forms are sensitive to membrane potential changes in mammalian cells.

1-G-162 RNA-Lipid Nanoparticles: A Robust and Potent Tool for Gene Knockdown and Expression in Primary Neurons

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The use of RNA to manipulate gene expression in neuroscience research is limited due to the lack of an effective delivery tool. Issues surrounding the use of viral vectors, electroporation, and lipofection reinforce the demand for a more durable and less toxic method to administer RNA into primary neurons. Here, we describe a solid-core lipid nanoparticle (LNP) system, developed by employing the microfluidics-based NanoAssemblr platform, capable of delivering RNA into neurons in vitro and in vivo with high efficiency and low toxicity.

Treatment of primary neurons with siRNA-LNP results in > 85% gene knockdown which is sustained for 21 days after administration, even at a dose as low as 100 ng/ml of siRNA-LNP. This system affects gene silencing even at DIV 2, providing flexibility over experimental design/setup. When exposed to mRNA-LNP, primary neurons exhibit >90% reporter gene expression. The performance of these RNA-LNP systems is further characterized by looking at their effect on individual cells, by using a microfluidic device capable of conducting high-throughput single-cell digital PCR. These single-cell studies are important to understand cell-to-cell heterogeneity in terms of quantity of delivered RNA, and its correlation within a cell to changes in gene expression. The kinetics of LNP uptake and subsequent alterations in gene expression demonstrate the potency of the developed RNA-LNP system. This technology, thereby, provides a simple and versatile solution to enable loss- and gain-of-function studies in difficult-to-transfect primary neurons.

1-G-163 Epidural fiber optic implant for spinal optogenetics in freely behaving animals

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Optogenetic tools enable the use of light to detect and control neuronal activity with remarkable temporal and cellular precision. Yet, the difficulty in delivering light to the spinal cord of awake, behaving animals has hampered the use of optogenetics in the study of spinal sensory processing. Accordingly, we have developed an epidural optic fiber implant that allows the delivery of light to the spinal cord dorsal horn and dorsal roots of sensory afferents. The epidural optic fiber was implanted in adult mice that expressed the light-activated inhibitory opsin ArchT in Nav1.8-expressing nociceptive afferents (Nav1.8-ArchT) and in wildtype C57Bl/6 mice. Amber light (592 nm) inhibited acute thermal nociception in the Nav1.8-ArchT mice, but did not affect nociception in C57Bl/6 mice. The acute delivery of blue light (488 nm) to the lumbar spinal cord of mice that selectively express channelrhodopsin (ChR2) in Nav1.8+ afferents (Nav1.8-ChR2) produced nocifensive behaviour, while repeated activation of Nav1.8+ afferents in these mice induced long-lasting mechanical hyperalgesia. Finally, inhibition of ArchT-expressing GABAergic interneurons in the lumbar spinal cord induced mechanical but not thermal hyperalgesia. Together, these results demonstrate the utility of our epidural optic fiber for the optogenetic modulation of both sensory afferents and intrinsic spinal cord neurons, permitting studies of activity-dependent nociceptive sensitization and the manipulation of sensory processing pathways within the spinal cord dorsal horn.

1-G-164 Optical Guidance for Deep Brain Stimulation Electrode Placement in the Treatment of Parkinson?s Disease

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Deep Brain Stimulation (DBS) surgery is a very effective way to treat the motor symptoms of Parkinson's disease after standard medications, such as Levodopa, no longer have a positive effect. The surgery's effectiveness relies on the placing of a stimulating electrode inside the brain with its tip placed very precisely within a millimeter-sized region called subthalamic nucleus (STN). Currently there is no onboard guidance for the stimulating electrode during the surgery, with only an x-ray projection as a means for navigation after the STN is located. Onboard guidance would lead to decreased surgery time, and increased accuracy. With this in mind, we have designed a cheap, optical fiber-based device that is small enough to be placed within commercially available DBS stimulating electrodes' hollow cores and is capable of sensing biological information from the surrounding tissue using diffuse reflectance spectroscopy. Using our device, we have shown the ability to sense the difference between white and grey matter both in vitro, in human and primate brain tissue, as well as in vivo, in rats. With the current probe, we can already theoretically provide increased accuracy and efficiency during neurosurgery; however, there is a high ceiling for increased utility using optical enhancements. We are currently designing probes with micro-optical components that can drastically increase the axial resolution of our device and thus, will give it the ability to identify even smaller structures within the brain, with increased precision.

1-G-165 Modeling by finite element method of ion concentration fluctuations in dendritic spines and the extracellular space

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To understand the principles that govern the changes in electrical potential and ion concentrations in dendritic spines and the extracellular space, we propose mathematical modeling using the finite element method that provides outstanding spatial and temporal resolution. We will consider the Poisson-Nernst-Planck equations in order to understand how the phenomenon of 'crosstalk' comes regulate synaptic spines and dendritic impact the integration of signals. This model will also allow us to study the impact of the extracellular geometry on the accumulation of ions.

1-G-166 Label-free microscopy to infer nerve fibers morphology and myelination in structurally complex samples

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Understanding mechanisms of neurodegenerative diseases, developing effective diagnostic strategies and comparing treatment methods often require the investigation of nervous system cells morphology. Widely used magnetic resonance imaging techniques constantly report new alterations of white matter in various brain regions. Although very successful, they lack the spatial resolution to infer myelin integrity at the cellular level. Recently demonstrated Coherent anti-Stokes Raman Scattering (CARS) microscopy has accomplished cellular level, label-free imaging of myelin sheaths in the nervous system. However, a quantitative morphometric analysis of nerve fibers still remains a challenge especially in brain tissue where fibers exhibit very small diameters (~1-3µm). In this work, we developed an automated myelin identification and analysis method that is capable of providing a complete picture of axonal myelination and morphology at the microscopic scale in structurally complex samples. This method performs three main procedures - extracts molecular anisotropy of membrane phospholipids using CARS images, identifies regions of different molecular organization, and finally calculates myelin thickness, axon numbers and diameters. We demonstrated this method with lesions in the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Next, we analyzed the local organization and structure of myelinated axons in the splenium of human corpus collosum. We demonstrated that our method provides an accurate description of nerve fiber morphology and myelination.

1-G-167 AAV-compatible MiniPromoters Target Specific Cell Types of the Central Nervous System

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Purpose: Small promoters that drive cell-type restricted expression in the CNS are important tools for basic and clinical research, and may be critical for future gene therapies. Here, we have focused on the development of such promoters for use in recombinant adeno-associated virus (rAAV). We tested the hypothesis that CNS-restricted Pleiades Promoters, which were developed using single-copy site-specific knock-in mice, would retain selectivity when used in rAAV. Methods: Promoters were typically cloned into a "plug and play" rAAV genome plasmid driving: icre (improved), emGFP (emerald), or MiniSOG; with or without WPRE (a transcript stabilizer). 50 μL of rAAV2/9 at 10E13 genome copies/mL was injected into the temporal vein of neonatal mice. For icre, the injected mice carried the Gt(ROSA)26Sortm1Sor allele, which responds to cre by rearranging the genome to express lacZ. Expression was analysed in adults using whole mount or cryosections for lacZ, and cryosections for immuno detection of emGFP or MiniSOG. Results: We tested 29 experimental viruses carrying 19 different MiniPromoters designed from 17 genes. Four MiniPromoters were tested with both icre and emGFP, and three with and without WPRE. Of the experimental viruses, 24 (83%) showed specific CNS expression. Positive results were independent of reporter and WPRE, although the WPRE strengthened expression. Conclusions: The Pleiades MiniPromoters represent a rich resource of human-

DNA promoters, compatible with rAAV, able to express in the CNS, and useful for research and clinical applications.

1-G-168 Hyperspectral imaging to track simultaneously the spatial dynamics of multiple subtypes of individual proteins on live neurons

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The efficacy of existing therapies and the discovery of innovative treatments for Central Nervous System (CNS) diseases have been limited by the lack of appropriate methods to investigate complex molecular processes at the synaptic level. In order to better understand the fundamental mechanisms that regulate synaptic signaling and remodeling, we designed a fluorescence hyperspectral imaging platform to track simultaneously different subtypes of individual neurotransmitter receptors trafficking in and out of synapses. This new imaging platform allows fast simultaneous image acquisition of at least five fluorescent markers in living neurons with a high spatial resolution. We used quantum dots of different emission wavelengths, functionalized to specifically bind single receptors on the membrane of living neurons, and quantified their mobility following treatments with various pharmacological agents. This hyperspectral imaging platform enabled the simultaneous optical tracking of five different synaptic proteins, including subtypes of glutamate receptors (mGluR, NMDAR, AMPAR), postsynaptic density proteins, and signaling proteins. This technique provides an effective method to monitor several synaptic proteins at the same time, which should accelerate the screening of effective compounds for treatment of CNS disorders.

1-G-169 Development of a novel tissue engineered model of the cerebrovasculature

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The complex interrelationship between cerebrovascular dysfunction and Alzheimer's Disease (AD) is increasingly appreciated. Indeed 30% of AD patients have cerebral infarcts at autopsy, and up to 90% demonstrate evidence of cerebral small vessel dysfunction including deposition of amyloid beta (Ab) within the vascular wall. This Aβ deposition is associated with cerebrovascular and blood-brain barrier dysfunction. Little is known about the mechanisms by which vascular risk factors for AD lead to cerebral small vessel dysfunction. The potential magnitude of this knowledge gap is put into perspective when one considers that every neuron is partnered with its own capillary. The need to better understand cerebral small vessel function and dysfunction in dementia appears therefore of particular interest.

We recently developed a unique human based tissue engineering approach to develop a 3D functional model of the cerebrovasculature, which is composed of either vascular smooth muscle (SMC) and endothelial cells (EC) or astrocytes, SMC and EC culture under native like flow conditions in vitro. Upon anteluminal injection, Aβ42 is accumulated within the tissue in a time and dose dependent manner. A subsequent activation of the endothelium is measured by adherence of circulating monocytes. In conclusion, the described tissue engineered human vascular equivalent model represents a significant step towards a relevant in vitro platform for the systematic assessment of pathogenic processes in AD independently of any systemic factors.

1-G-170 Two-photon optogenetics with near-infrared light-activated cyclases for studying the role of cAMP and cGMP in living neurons

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Optogenetics provides powerful tools to non-invasively manipulate neural activity by light. However, for studying intracellular signaling mechanisms involved in synaptic function, these tools are not yet well established. Here, we describe two-photon optogenetics with far-red light-activated cyclases for elucidating the role of intracellular messengers cAMP and cGMP in the synapses of living neurons. To dissect the role of cAMP in synaptic plasticity, we utilized a bacterial photoactivatable adenylate cyclase (PAC) which produces cAMP in response to blue light. We demonstrated photoactivation of PAC at the single

synapse level by two-photon excitation light in cultured hippocampal slices. We also examined a far-red light-sensitive adenylate cyclase (NIR-PAC), a fusion of the photosensory PAS-GAF-PHY domain and the catalytic domain of adenylate cyclase (Ryu et al., 2014). By mutating the enzymatic pocket of NIR-PAC, we engineered a far-red light-sensitive guanylate cyclase (NIR-PGC). We excited each cyclase with LED light (660 nm peak) in vitro and measured cAMP or cGMP levels by ELISA. Intracellular messenger levels were raised within seconds after light stimulation, indicating rapid photoactivation. We then examined enzymatic activity across the two-photon excitation spectra in vitro. Longer wavelengths (1040 nm) activated both NIR-PAC and NIR-PGC, suggesting the possibility for co-application with PAC (750 nm peak). We will discuss how these two-photon optogenetic technologies serve as valuable tools for studying cAMP and cGMP in the synapses of living neurons.

1-G-171 Point source networks: correlation between local firing properties and regional cortical imaging in mouse cortex

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A more complete understanding of brain function will require measurement techniques which monitor large-scale neuronal activity across multiple brain areas and relate this to single neuron properties. We present a step towards achieving this aim: by simultaneous in vivo recording of cortical/subcortical single unit activity and wide-field cortical imaging during spontaneous activity. We use glass pipettes to record single unit activity in target sites, and use wide-field cortical imaging (GCaMP) to determine large-scale neuronal activity. By employing spike-triggered averaging we established correlation between single neuron firing and regional cortical calcium imaging. We apply the method in cortex and establish expected regional maps that are associated with activity at single cortical points. Extension of the electrophysiological assessment to sub-cortical structures permits the relations between these regions and maps of cortical activity to be determined. We find that wide-field regional calcium imaging is highly correlated with spiking activity from neurons. By examining axonal projections maps in the Allen Mouse Brain Connectivity Atlas and comparing these to the spike evoked maps, we observed strong spatial co-localization. Within a functional domain, single neuron firing is linearly correlated with regional calcium imaging, indicating how single neurons relate to large networks. Spike evoked maps should be useful for assessing the functional relationship between single neurons and wide-field activity in in vivo models of neurological disease

1-G-172 High yield and purity of primary astrocyte and microglia cultures from embryonic mouse and rat cerebral cortex

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The study of disease-related processes and pathways is often done using primary cultures to best preserve characteristics seen in vivo. This study presents a cost-effective and simple protocol to culture both astrocytes and microglia from E18 primary cortical neural progenitor cells of rat or mouse origin with high yield and purity. Cortical cells are isolated aseptically according to Aiga et al. (2011). Single cell suspensions were plated at 250,000 cells/cm2 on poly-L-lysine coated plates, and non-adherent cells removed 24h later. Astrocytes at days 14-21 in vitro (DIV) are enriched by media exchange every 3 days and expanded until confluent and cells exhibit elongated/stellate morphology. For microglia, media exchange occurred DIV3 with or without addition of granulocyte-macrophage colony stimulating factor. Microglia appear by DIV14 and enrich to DIV21. Astrocytic layers show >95% purity with positivity for glial fibrillary acidic protein. Harvested microglia yield ~400,000 cells per 25 cm2 flask. Flow cytometry shows >99% purity by OX42 (rat) and CD45/CD11b (mouse) staining. Murine microglia were further characterized as CD45lo, CD80%2B, MHC I,IIlo, CD40%2B, CD11b%2B, CD11clo and CD86%2B. Lipopolysaccharide enhanced expression of OX42 in rat, and CD80, CD40, CD11b, MHC I and CD86 in mice microglia, along with precise temporal morphological changes. Experiments are ongoing to establish the relative activation states and immunological profile of these cells. This method provides a reliable, simple and cost-effective means to obtain highly purified glial cultures in high yield.

IBRO – International Brain Research Organisation

1-IBRO-173 Peer rescue of autism-related behavior after prenatal exposure to valproic acid

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Autism is a severe neurodevelopmental disorder characterized by impediments in social interaction, a reduction in communicative skills and by stereotyped behaviors. Symptoms appear in early childhood and persist in adulthood. Currently there are no pharmacological treatments, but several clinical studies suggest early social stimulation as the most effective treatment of autistic children, who show significant improvements in social behavior through these approaches. It was previously shown that the mouse prenatal exposure to valproic acid (VPA) at gestational day 12.5 results in reduced social interaction in the adult offspring. In those experiments VPA-treated mice were weaned with other VPA mice. Here, we compared VPA mice weaned with VPA mice (VPA-VPA mice) with VPA-Saline groups (VPA-Sal), containing 2-3 VPA-exposed mice per cage along with 2-3 Saline mice. This design allowed VPA and Sal mice to interact in the home cage from postnatal day (P)21 to P60. At P60, VPA-Sal mice showed higher levels of sociability than VPA-VPA, showing that this treatment can rescue at least some of the behavioral alterations observed in our model. On the other hand, there are evidences to suggest that patients with autism have alterations in stress and inflammatory responses. We have previously identified alterations in inflammatory and stress response after inflammatory stimuli in VPA mice. We therefore studied whether these alterations are reversed in rescued animals.

1-IBRO-174 Proteolytic processing of CXCL12 transforms CXCL12 into a death factor for neural stem cells

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The neural stem cells (NSC) resident in mammalian adult central nervous system (CNS) represent an encouraging potential for neurodegenerative and acute brain diseases treatment. NSC can migrate to CNS injury answering to chemotactic factors like CXCL12. Despite the NSC capacity to generate newborn neurons to different CNS areas, the most of neural stem cells undergo apoptosis after arrive at lesion site. CXCL12 or SDF-1 (stromal cell-derived factor 1) is a chemotactic cytokine and together with its CXCR4 receptor are constitutively and broadly expressed in many tissues. Together with cytokines and chemokines, matrix proteases are also released in brain injury states and activated to contribute for breakdown of the extracellular matrix and consequent cell migration. Among those, matrix metalloproteases 2 and 9 (MMP-2 and 9), gelatinase A and B, respectively, are secreted locally and could cleave CXCL12 removing the first four N-terminal aminoacids generating CXCL12(5-67). We produced CXCL12 and CXCL12(5-67) recombinant proteins, and evaluated its effect on mice astrocytes and NSC in vitro. We identified that CXCL12(5-67) impairs neural stem cell migration, reduces astrocytes viability and induces apoptosis through caspases 9 and 3 activation in adult neural stem cell. A possible CXCL12(5-67) involvement in a low CNS regeneration capacity could became it a target for trauma and neurodegenerative disease treatment.

1-IBRO-175 Acute stress increases FMRP levels in hippocampus and promotes Akt-mTOR and MAPK1/2 pathways activation in rats

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Acute stress enhances learning and memory acquisition probably mediated by stress released corticoids. These hormones promote modifications in morphology, cellular excitability and synaptic efficacy in hippocampus. These processes involve mRNA transport to dendrites and local control of translation process. In line with this, the Fragile X Mental Retardation Protein (FMRP) acts suppressing translation of mRNAs encoding proteins involved in the regulation of synapsis. FMRP is phosphorylated by S6K1 at Ser499 (p-FMRP), a modification required for mRNA translational repression and its association with stalled polyribosomes. In neuronal cultures, the

activation of mGluR1/5 type glutamatergic receptors promote FMRP Ser499 phosphorylation by S6K1 trough PI3K-AKT-mTORc1 and MAPK ERK1/2 pathways in a different temporal pattern. We proposed that acute restraint stress regulates FMRP phosphorylation in rat hippocampus, event related to activation of PI3K-AKT-mTORc1 and MAPK ERK1/2 pathways. Adult male rats were stressed by restraint during 0.5 or 2.5 h, and to evaluate the posterior effects of stress another group was sacrificed after 1.5; 6 and 24 h post stress. It was determined that restraint stress did not change p-FMRP levels. However at 0.5 hours of stress session there was an increase in FMRP protein levels, which was coincident with the AKT activation. These findings suggest that stress activates translational processes and FMRP acts as a negative modulator in hippocampus.

1-IBRO-176 Cognitive studies and a direct cell reprogramming protocol for the aging rat brain

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We use the aging rat (AR) as a model of age-related cognitive decline and plan to use cell reprogramming as a restorative intervention in their hippocampus. We assessed the extent of spatial memory (SM) impairment in 26 (old), 31 (senile) and 5 (young) months female rats as well as the age-related changes in their dorsal hippocampus. Age changes in SM performance were assessed with the Barnes maze test. We employed two probe trials, 1 and 5 days after training, in order to evaluate "short-term" and "middle-term" SM. The results revealed that old rats perform better than senile rats in acquisition trials and young rats perform better than both aging groups. Morphological analysis of the dorsal hippocampus showed a marked decrease (94-97%) in doublecortin neuron number in the dentate gyrus in both aging groups. It has been recently demonstrated that transient induction of the four pluripotency genes oct4, sox2, klf4 and c-myc (the Yamanaka genes), followed by appropriate signalling inputs, can efficiently transdifferentiate fibroblasts into neural stem/progenitor cells (NPCs). We report here the ongoing construction of a helper-dependent recombinant adenovector for the simultaneous expression of hGFP and the Yamanaka genes all under the control of a bidirectional Tet-Off regulatable promoter. This vector will be used to implement direct cell reprogramming in a non-integrative fashion. We describe the direct reprogramming protocol that we plan to use to convert fibroblasts into NPCs, which will be subsequently used for experimental cell therapy in the hippocampus of AR.

1-IBRO-177 Relative Expression of Odorant Binding Proteins in the Forest Tsetse fly Species Glossina brevipalpis

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Glossina brevipalpis is a forest Tsetse fly species that vectors Animal African Trypanosomiais. Olfaction is crucial for vital tsetse fly behaviours such as feeding, con specific mate selection and identification of larviposition sites. Odorant binding proteins (OBPs) are involved in the peri-receptor events in odour transmission. Identification of OBPs in various tsetse fly species has been achieved through annotation. However, little expression studies have been carried out.This study reports for the first time the expression profile of selected G. brevipalpis OBPs in 3rd instar larvae, pupae and wild females starved at different periods; 2h, 24h,48h,72h and 96h post blood meal. Expression profiles were analysed by quantitative real time PCR. Anternnal OBP expression in the wild female G.brevipalpis was significantly high at 24h starvation period. Some OBPs were expressed in the larva and pupal stages suggesting that they might be involved in the flys' development. The findings from this study could give insight on genes that could be targeted in the development of specific and environmentally friendly approaches targeting the tsetse fly.
Keywords: Glossina brevipalpis, olfaction, odorant binding proteins, quantitative real time PCR

1-IBRO-178 Impaired autophagy associated with glucose deprivation induces neuronal death through subsequent autophagy activation.

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Insufficient glucose supply to the brain results in the impairment of neuronal function and neuronal death. Hypoglycemia occurs as a complication of insulin treatment in diabetic patients, leading to brain glucose deprivation (GD). The cellular mechanisms that ultimately lead to neuronal death have not been completely elucidated and the role of autophagy remains unknown. Autophagy is a lysosome-mediated catabolic mechanism characterized by the appearance of double-membrane vesicles and the conversion of LC3-I to LC3-II. The role of autophagy is to maintain the cellular homeostasis and supply energy in response to starvation. However, excessive autophagy can lead to cell death. In this study we analyzed the progress of autophagy along GD and glucose reperfusion periods (GR) and investigated its role on neuronal death. Cortical cultures exposed to GD and GR showed enhancement of LC3-II and accumulation of autophagosomes during GD. When glucose is reintroduced, LC3-II and p62 levels decrease at first stages of GR, suggesting the completion of the autophagic flux, but during late stages of GR a second peak of autophagosome accumulation occurs. Results suggest that autophagy is impaired during GD, but the autophagic degradation occurs as soon as ATP recovers during GR. Partial inhibition of autophagy by 3-MA or Atg7 siRNA reduces GD/GR damage. Moreover, inhibition of autophagic flux during GR increases cell survival. Results suggest that autophagosome overload during GD triggers a subsequent degradation and autophagy induction during GR that contribute to neuronal damage.

1-IBRO-179 Evidence for a decrease of pyramidal cells dendrites in neonatal thalamic lesioned rat?s prefrontal cortex: implication in Schizophrenia

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Normal development of the cerebral cortex is dependent upon reciprocal connections with the thalamus. Therefore, understanding the role MD plays in the development of the PFC is important and may help in understanding the progression of psychiatric disorders that have their root in development. The present study assessed whether early postnatal damage to mediodorsal nucleus of the thalamus (MD) causes behavioral and cognitive alterations in young adult rats and examined the hypothesis that the MD plays a role in the dendritic development of pyramidal cells in the PFC. Rat pups at postnatal day four were randomized in 3 groups: group 1 received bilateral electrolytic lesion of MD, group 2 with MD Sham lesion and group 3 without any surgery Seven weeks later, all rats were tested with the following behavioral and cognitive paradigms: open field test, elevated plus maze test, social interactions test, passive avoidance test and dorsal immobility. After the behavioral testing, then we examined dendritic morphology, apical dendrite number and spine density in several cortex areas, which receive afferents from the MD by using Golgi technique. Our findings suggest a functional role for MD in the postnatal maturation of affective behavior. Further some of the behavioral and cognitive alterations observed in these young adult rats after early MD lesion are reminiscent of those present in major psycho-affective disorders, such as schizophrenia in humans

1-IBRO-180 Role of IL-10 in macrophage polarization and recovery after peripheral nerve injury

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Wallerian degeneration (WD) is tightly regulated in injured axons and is crucial to provide a permissive microenvironment for axon regrowth. Macrophages enter the distal segment of injured nerve within about 2-3 days, remove tissue debris by 7-14 days, and are cleared from the tissue by 3-4 weeks. An important feature of the peripheral nerve is the rapid switching-off of the pro-inflammatory response, likely by anti-inflammatory cytokines in the first 2 weeks after injury. However, the role of these "brakes" has not been fully studied after nerve injury. The aim of this study was to assess the expression profile of IL-10 after nerve injury and to analyze how this cytokine affects the macrophage responses and resolution of nerve inflammation. WT and IL-10 null mice underwent sciatic nerve crush and the nerves were collected after varying lengths of time after injury. Macrophage polarization was assessed by using a panel of M1 (CD16/32, CD86) and M2 markers (CD163, Arg1, CD206). We observed

that the lack of IL-10 resulted in higher number of macrophages in the distal nerve segment at 3, 14 and 21 dpi. Moreover, in the IL-10 null mice, there was greater M1 and reduced M2 polarization in vitro and in vivo. A chemokine/cytokine PCR array of the nerves obtained from IL-10 null mice showed 2 to 4-fold increase in the levels of ten pro-inflammatory mediators. Moreover, our data show that in the absence of IL-10, the increased pro-inflammatory milieu of the injured nerve leads to impairment of axon regeneration and loss of functional recovery.

POSTER SESSION 2

A – Development

2-A-1 TALEN- and CRISPR/Cas9-mediated disruption of the robo3 gene in Xenopus tropicalis

Anteneh Argaw[1], Dietmar Schmucker[1]

[1]VIB

Developing axons, tipped at their distal end by the growth cone (GC), navigate through relatively long distances in a highly directed manner to establish functional synapses with their targets. Intermediate targets like the ventral midline play an important role by steering the GC by means of attractive and/or repulsive mechanisms. In mammals, netrin-1/DCC which attracts axons and slit/ROBO which repels GCs play a major role by regulating commissural axon midline crossing. Commissures fail to develop in mice and humans in the absence of the transmembrane receptor ROBO3 which appears to be the master regulator of midline crossing in mammals. To investigate in detail the function of robo3 in lower vertebrates, we have generated the first Xenopus tropicalis mutant robo3 alleles using TALENs and CRISPR/Cas9 based genome engineering techniques. After germline transmission multiple F1 animals were obtained and are currently interbred. Phenotypic analysis will be performed in F2 homozygous mutant animals where hindbrain and spinal commissural neurons will be sparsely labelled by electroporating reporter transgenes. To compare robo3 function in lower vertebrate and mammals, we will perform rescue experiments using ROBO3 chimera with specific substitutions in the first immunoglobulin domain unique to mammalian ROBO3. Taken together these experiments will provide detailed function of the robo3 gene in Xenopus and highlight functional differences between lower vertebrate and mammalian ROBO3.

2-A-2 Survival and maturation of the developmentally-born cell population in the rat dentate gyrus.

Shaina Cahill[1], Ru Qi Yu[1], Dylan Green[1], Jason Snyder[1]

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In the dentate gyrus, adult-born neurons have enhanced plasticity and contribute to the mnemonic and emotional functions of the hippocampus. While the stages of adult neurogenesis and the factors that regulate neuron addition in adulthood have been relatively well-characterized, less is known about how developmentally born cells integrate and survive over time. Since the dentate gyrus is comprised of large numbers of adult-born and developmentally-born cells, identifying the properties of these populations is essential for understanding how the dentate gyrus contributes to behavior. To address this question we used BrdU to label dentate gyrus neurons born on postnatal day 6 in rat pups, the peak of dentate gyrus development. We quantified BrdU%2B cells in 2 and 6 month-old rats and found 20% fewer BrdU%2B neurons at the 6 months, indicating substantial loss of mature, developmentally-born cells throughout adulthood. This contrasts with adult-born cells, which show stable survival after reaching maturity. We additionally examined the survival and maturation of developmentally-born cells at earlier timepoints, similar to past work done in characterizing adult neurogenesis. We found that developmentally-born cells are stable during development and have a prolonged period of loss into adulthood. Collectively, our findings indicate that the survival and turnover of developmentally-born cells influences the composition of the adult dentate gyrus. These findings are relevant for understanding the role of the dentate gyrus in learning and memory and in diseases where neurons are lost.

2-A-3 Role of Cux factors in cerebellar development

Emily Capaldo[1], Angelo Iulianella[1]

[1]Dalhousie University

The cerebellum has been known to be highly involved in motor coordination, but more recently it has been implicated in various congenital and acquired developmental conditions, including autism spectrum disorders. Although the anatomy and connectivity of the adult cerebellum have been known for decades, the developmental origin of each cell type is only beginning to be understood. The numerous conditions associated with aberrant cerebellar development, such as the growth of medulloblastomas, highlights the importance of understanding the mechanisms controlling the development of this highly proliferative brain region. The transcription factor Cux2 has been hypothesized to define a specific subset of neural progenitors in various regions of the nervous system, including the cortex, hippocampus and spinal cord, but its role in the cerebellum is unknown. We used an inducible transgenic mouse line that labels Cux2-expressing progenitors to delineate the ontogeny of cerebellar precursors. We discovered that Cux2 activity was restricted to the developing rhombic lip region of the mid-hindbrain junction, and subsequently fate-mapped their descendants to the external granule layer and granule cells that radially migrate inwards during cerebellar development. These findings support the view that Cux2 expression delineates a fate-restricted neural progenitor, and within the cerebellar primordia its activity is largely restricted to granule cell formation.

2-A-4 Prenatal Alcohol Exposure Alters Expression of Glucocorticoid and Mineralocorticoid Receptor Levels in the Placenta and Fetal Brain at Gestational Day 21

Melissa Chiu[1], Ni Lan[1], Joanne Weinberg[1]

[1]University of British Columbia

The hypothalamic-pituitary-adrenal (HPA) axis plays a key role in the mediation of the stress response. Prenatal alcohol exposure (PAE) can result in a hyperresponsive HPA axis, which could leave an individual vulnerable to stress-related disorders such as depression and anxiety later in life. The activation of the HPA axis causes the secretion of glucocorticoids, which feed back on the stress-responsive neurocircuitry via mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). Currently, the mechanism by which PAE results in HPA hyperresponsiveness is not fully understood. Utilizing an animal model, we investigated how PAE may affect the expression level of MR and GR proteins of control (C), pair-fed (PF), and PAE fetal brains and placentae at gestational day 21. We found that PAE resulted in differential expression of MR and GR proteins compared to that of C and PF animals. PAE resulted in increased GR protein levels and an increased GR/MR ratio in the medial prefrontal cortex and amygdala, respectively. Furthermore, PAE induced a loss of sexually dimorphic expression of MR in the amygdala, hippocampus, and placenta, suggesting that PAE may result in a decreased sensitivity to the organizational effects of androgens. Our results indicated that PAE altered important regulatory components of the HPA axis, setting the stage for increased risks for mental and behavioural issues later on in life. Funded by NIH/NIAAA R37 AA007789, R01AA022460 and NeuroDevNet to JW; NSFC (China) 31100793 and IMPART (CIHR) Fellowship to NL.

2-A-5 Inflammation dysregulates neural circuit formation in vivo via microglial activation and IL-1β

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Converging evidence implicates immune activation in the etiology of neuropsychiatric disease. Little is known about the links between immune activation and neural circuit formation. We investigate these links by exposing larval zebrafish to bacterial lipopolysaccharide (LPS) to induce inflammation and subsequently observing neuronal development. LPS induces inflammatory cytokine (TNFα, IL-1β, IL-6) transcription within two hours and induces an amoeboid microglial morphology suggesting microglial activation. In vivo two-photon microscopy of retinal ganglion cells (RGCs) demonstrates that two-hour LPS exposure acutely increases rates of axonal branch addition and retraction. This hyperdynamic remodelling may reflect failure to stabilise synapses. Daily imaging of axons after LPS exposure reveals a shift in developmental trajectory with smaller, simpler arbors and fewer presynaptic puncta. Microglia are key immune regulators of the CNS and we hypothesised that they mediate inflammatory effects on neurons. Morpholino knockdown of the SpI1/Pu.1 transcription factor depletes the myeloid lineage; morphant animals lack microglia and macrophages. RGCs in Spi1/Pu.1 morphants exposed to LPS do not demonstrate the acute or lasting

perturbations seen in control animals. Morpholino knockdown of IL-1β phenocopies the effect of myeloid depletion. Microglial activation and/or IL-1β may therefore mediate developmental effects of inflammation. We are currently analysing whether IL-1β acts upstream or downstream of microglia in this process. Funding: CIHR Vanier CGS (NAIF); CIHR (ESR).

2-A-6 The requirement for the Rb Family during Adult Neurogenesis

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During adult neurogenesis, the regulatory function of the Rb family - pRb, p107 and p130 - remains presently unclear. While p107 is required for neural precursor cell (NPC) self-renewal and commitment, and Rb is required for neuronal quiescence and survival, complications in data interpretation arise from compensation between family members. In order to address the function of the Rb family in regulating NPC proliferation and differentiation, all three pocket proteins were inducibly deleted from the adult subgranular zone (SGZ) in the hippocampus using the NestinCreERT2 mouse model. Four weeks following protein deletion, brains were analyzed using immunohistochemistry. Triple knockout (TKO) animals demonstrate a dramatic expansion of the recombined cell population as marked using YFP. Within this recombined population, there is a significant increase in the number of cells expressing markers for self-renewal and proliferation, concomitant with a decreased in the number of cells expressing markers for neuronal commitment and maturation. This suggests that loss of the Rb family leads to the development of an increased neural precursor pool, unable to commit to a mature neuronal fate: demonstrating a requirement for the Rb family in maintaining a balance between NPC proliferation and commitment. This study will define the mechanisms by which the Rb family pocket proteins regulate the NPC population. This understanding is crucially important in developing future therapeutic tools for regeneration therapies. This research is supported by a CIHR grant to RSS.

2-A-7 Examining the Role of DIXDC1 in Neural Connectivity and Autism Spectrum Disorders

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Emerging studies suggest that molecules in the Wnt signalling pathway are important for the development of neural connectivity and are associated with autism spectrum disorders (ASD). We are studying a Wnt signalling molecule named DIX domain containing-1 (DIXDC1), a homolog of Disheveled. Using mouse in vitro and in vivo models, preliminary results show that decreasing expression of DIXDC1 reduces dendritic outgrowth, and spine formation. We also found that MAP/microtubule affinity-regulating kinase 1 (MARK1), a kinase associated with ASD, phosphorylates DIXDC1 to mediate these effects. Preliminary data indicates that the MARK1-DIXDC1 pathway regulates neural connectivity by directly modulating actin dynamics. We found that the loss of DIXDC1 inhibits actin dynamics, and application of an actin polymerization drug rescues abnormal dendritic spine phenotypes. Furthermore, through exome sequencing, we discovered two rare inherited genetic variants in DIXDC1 in patients with ASDs. Interestingly, expression of these variants in neurons causes impaired dendrite outgrowth and spine morphology. This suggests the MARK1-DIXDC1 interaction is involved in a novel pathway for synaptic development and its function may be disrupted in ASDs. We are interested in identifying drugs and other compounds that target the actin cytoskeleton network to rescue spine defects when the MARK1-DIXDC1 pathway is inhibited. We are developing a high-throughput imaging assay to perform drug screens to identify chemicals that modulate synaptic phenotypes for the development of novel therapeutics for ASDs.

2-A-8 Inter-neuronal interaction defines topographic synaptic innervation in C. elegans

Kota Mizumoto[1]

[1]University of British Columbia

Cellular interactions between neighboring axons are essential for the stereotyped positioning of individual axons and topographic map formation. So far however, it is not known how axons communicate with each other at the level of synapse

formation. In other words, how do axons divide up the target area when they choose synaptic targets? To answer this question, we focus on two closely related cholinergic motor neurons, DA9 and DA8. Both DA8 and DA9 neurons extend their axons through a commissure into the dorsal nerve cord, where they proceed anteriorly to form a series of en passant synapses. Although those axons largely overlap in the dorsal cord, they form synapses only at the specific area. DA9 forms about 25 synapses onto the dorsal muscles in its axon in the posterior-most domain (DA9 synaptic domain). Interestingly, DA8 axon has no synapses in DA9 synaptic domain, and starts to form synapses just anterior to DA9 synaptic domain. Therefore, DA8 and DA9 form "tiled" synaptic domains leaving no apparent gap or overlap between them. Recently, we developed a genetic marker to visualize synaptic tiling between DA8 and DA9, and showed that Plexin-dependent axon-axon interaction as well as gradients of Wnt morphogens are critical for establishing stereotyped tiled synaptic innervation (Mizumoto and Shen, 2013 Neuron, Cell Reports). To fully understand the molecular mechanisms of synaptic tiling, we are working on additional mutants that show synaptic tiling like plexin mutants.

2-A-9 Regulation of synaptic connectivity by a novel FMR1-TAO2 pathway and its disruption in autism spectrum disorders.

Nadeem Murtaza[1], Melanie Richter[2], Pritha Paul[1], Vickie Kwan[1], Emanuela Pasciuto[3], Susan Walker[4], Stephen Scherer[4], Claudia Bagni[3], Froylan Calderon de Anda[2], Karun Singh[1]

[1]Mcmaster University/Stem Cell and Cancer Research Center, [2]University of Hamburg/Center for Molecular Neurobiology Hamburg, [3]University of Leuven/Flanders Institute for Biotechnology, [4]University of Toronto/Hospital for Sick Children

Abnormal neuronal communication is hypothesized to contribute to the pathogenesis of autism spectrum disorders (ASDs). Studies have discovered numerous ASD-associated genes controlling synapse formation and function. One potential gene of significance is the thousand and one amino acid kinase 2 (TAO2) located on 16p11.2, a common ASD-linked copy number variation. Initial studies have shown a role for TAO2 in neuronal circuitry, but an underlying mechanism has not been elucidated. We found that loss of TAO2 resulted in decreased dendritic spine density due to decreased spine stability, and translated into decreased synaptic connectivity. While exploring mechanisms by which TAO2 controls these processes, we found that TAO2 mRNA is a target of the fragile x mental retardation protein (FMRP). In this regard, TAO2 protein levels were higher in brains of FMR1 KO mice, suggesting that elevated TAO2 plays a role in the abnormal synaptic phenotypes observed in the KO mice. By overexpressing TAO2 to simulate the elevated protein levels, we found it resembled some aspects of FMR1 KO spine/synapse pathology. This suggests that reducing TAO2 levels in the FMR1 KO brain may rescue its synaptic deficits. We then asked if TAO2 itself was genetically disrupted in patients with ASDs, and discovered three genetic variants in TAO2. Overexpression of two TAO2 variants (a de novo kinase dead mutant and a regulatory domain mutant) resulted in impaired spine formation and abnormal morphology. These results suggest a FMR1-TAO2 pathway regulates synaptic connectivity, which may be disrupted in ASDs.

2-A-10 A FASD Mouse Model: Biochemically Mimicking Alcohol Exposure using Gsc promoter driven Cyp26A1 cDNA.

Berardino Petrelli[1], Arzu Ozturk[1], Molly Pind[1], Abraham Fainsod[1], Geoff Hicks[1]

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Prenatal alcohol exposure resulting in Fetal Alcohol Spectrum Disorder (FASD) has an estimated prevalence of 1% in Canada. FASD is a spectrum of disorders, including Fetal Alcohol Syndrome (FAS), partial FAS, and alcohol related neurodevelopmental disorder (ARND). It is well established in FASD rodent and Xenopus models that even a single exposure to alcohol during the earliest stage of gastrulation is sufficient to induce the developmental defects associated with FAS. We hypothesize that acute ethanol exposure overwhelms the aldehyde metabolic enzymes that also normally convert retinol (Vitamin A) to retinoic acid (RA); and moreover, the reduction of RA levels during gastrulation drives the cranio-facial defects associated with FAS. To test our hypothesis, a murine model was established to biochemically mimic the alcohol effect in vivo using a genetically engineered mouse expressing Cyp26A1-eGFP from the endogenous Goosecoid (Gsc) promoter. The Gsc promoter is utilized to dictate spatio-temporal

expression of the Cyp26A1-eGFP cassette during development. Cyp26A1, the normal down regulator of endogenous RA, mimics the reduced RA levels induced by acute alcohol exposure at early gastrulation. F1 mice were born with a normal Mendelian ratio of het:wt (0.95, n=127). Newborn F1 mice were phenotypically assessed (blinded) for cranial-facial defects characteristic of FASD, compared to normal pups. Pups were later genotyped at weaning and analysis shows 88% (15/17) of mutant mice assessed had a discernable FAS phenotype. 100% of wild-type mice had been assessed as normal (14/14).

2-A-11 Collaborative regulation of Prostaglandin E2 and Wnt signalling pathways in neuroectodermal stem cells: implication in autism

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[1]York University

Prostaglandin E2 (PGE2) is an endogenous lipid-derived signalling molecule important in early brain development and function. We have previously shown that PGE2 is important for calcium regulation in growth cones, and migration, proliferation, and differentiation in neural stem cells. Exogenous factors, such as diet and exposure to infections, inflammation, and chemicals, can disrupt the levels of PGE2 and disturb its normal role in brain development. Moreover, irregular PGE2 signalling has been associated with pathologies of the nervous system, including Autism Spectrum Disorders (ASDs). In this study, we examined the potential dose-dependent interaction between two key developmental pathways, PGE2 and the crucial morphogen Wnt, in neuroectodermal (NE-4C) stem cells. We report that concentration-dependent PGE2 alters the expression of Wnt-target genes including ligands, Wnt3, 3a, 5a, 8a, and downstream genes, cyclooxygenase-2 (Ptgs2), cyclin d1 (Ccnd1), and matrix metalloproteinase 9 (Mmp9). Interestingly, the Wnt pathway has also been previously implicated in ASDs, including Wnt-target genes affected in this study. We also found that the expression of Wnt-target protein, Ccnd1, increased with higher concentrations of PGE2. Furthermore, we found that Wnt agonist treatment elevates the expression of Ptgs2, which encodes the enzyme responsible for PGE2 production. Overall, our study provides novel molecular evidence for the interactive regulation of PGE2 and Wnt neurodevelopmental pathways and for a potential link between abnormal signalling of these pathways in ASDs.

2-A-12 The ASD-associated gene Glyoxalase1 integrates the fetal-maternal metabolism of the diabetes risk factor methylglyoxal to regulate embryonic neurogenesis

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[1]The Hospital for Sick Children

Genetic perturbations and prenatal environmental insults, such as maternal metabolic conditions, are important sources of causality for ASD and other mental illnesses. However, little is known about how these two components interact at the molecular and cellular levels to influence early brain development. Here, we report that the deregulation of a circulating metabolite methylglyoxal (MG) that is elevated in maternal diabetic condition as well as a MG detoxification enzyme glyoxalase 1 (Glo1) that is perturbed in ASD, synergistically disrupt neural precursor cells (NPCs) and embryonic neurogenesis in the developing mouse cortex. We found Glo1expression is high in NPCs to keep the steady-level of MG low. Acute knockdown of Glo1 in vivo depletes NPCs as a result of premature neurogenesis. Lateral ventricular injections of either exogenous MG or a Glo1 inhibitor similarly increase neurogenesis. In contrast, ectopic expression of Glo1 promotes NPC maintenance. Mechanistically, accumulation of MG causes NPC apical endfeet detachment and basal-apical movement retardation, leading to aberrant neurogenesis. Maternal injections of MG, which mimic observed metabolic insults in diabetic patients, also increase neurogenesis at the expense of NPC maintenance. These results highlight an important role of the Glo1-MG metabolic pathway in embryonic cortical development. Fetuses with genetic perturbations in this pathway may be particularly vulnerable to maternal metabolic insults, and susceptible to neurogenesis deregulation, leading to the abnormal development of cortical circuitry.

2-A-174 Effects of prenatal alcohol exposure and early life stress on the immune response to challenge: Profiling cytokine expression patterns in multiple compartments

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[1]The University of British Columbia

Prenatal alcohol exposure (PAE) affects development and function of many physiological systems resulting in a range of deficits, collectively known as Fetal Alcohol Spectrum Disorders (FASD). Among these deficits are changes in immune function, such as increased infections, as well as increased responses to stress. To investigate possible mechanisms underlying altered immunity, pregnant rats were assigned to: PAE, Pair-fed (control for reduced food intake in PAE) or controls. At puberty, offspring were exposed to Chronic Mild Stress (CMS), a series of stressors (2/d for 10 d) to induce mild stress, or remained undisturbed. In adulthood, animals received an adjuvant (inflammatory) challenge (adjuvant-induced arthritis; AA). Preliminary data show that plasma cytokine levels are low and may not significantly change with AA. Interestingly, in the hind paw, while PAE CMS animals with AA show a blunted cytokine response, in the absence of AA, these animals show heightened cytokine expression, compared to controls. Overall, this suggests that exposure to stress may alter the ability of PAE animals to mount appropriate immune responses to challenge. Therefore, changes in cytokine expression may play a significant role in immune abnormalities seen in PAE offspring. Investigation into cytokine responses in other tissues (brain and spleen) is currently underway to increase our understanding of stress-immune interactions that may alter immune function in children with PAE. Support: NIH/NIAAA RO1 AA022460, R37 AA007789, NeuroDevNet, & CFFAR to JW.

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

2-B-13 Modulation of synaptic fidelity by post-synaptic Pannexin-1

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Ananamide (AEA) is an endogenous fatty acid that modulates synaptic activity though its activity as both an endocannabinoid and endovanilloid. In order for AEA to act as an endovanilloid, it must reach a concentration 10x greater than needed for it to activate cannabinoid 1 (CB1) receptors. To regulate synaptic concentrations of AEA, an unidentified protein transporter is hypothesized to shuttle AEA from the synapse to the post-synaptic cell to be degraded. Pannexin-1 is a large pore ion channel which is expressed post-synaptically in CA1 hippocampal neurons and conducts large molecules. We hypothesized that Panx1 acts as an AEA transporter. We tested this by blocking post-synaptic Panx1 with an intracellular Panx1 antibody and observed an increase in the frequency of neurotransmitter release following stimulation. The same result was seen with bath application of AEA without Panx1 blocking. Application of AEA in the presence of a Panx1 block produced no further increase in neurotransmitter release. Since AEA is known to act at TRPV1 channels, we tested a TRPV1 receptor antagonist, capsazepine, which prevented any increase in neurotransmitter release seen with exogenous AEA or during Panx1 block. Finally, application of AM-404, an AEA transporter antagonist, mimicked increased glutamate release similar to that that seen with Panx1 block. We conclude that Panx1 is an important pathway for AEA clearance from CA3-CA1 synapses. Furthermore, our data suggests that Panx1 may play a role in regulation of synaptic efficacy.

2-B-14 Two-photon FRET and optogenetics for studying post-synaptic cGMP during plasticity

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Cyclic GMP (cGMP) is a crucial intracellular messenger which underlies synaptic plasticity. However, the role of post-synaptic cGMP is poorly understood due to a lack of established techniques for its spatiotemporal visualization and manipulation. Here, we report two-photon live imaging and optogenetics to non-invasively monitor and manipulate cGMP at the level of the dendritic spine. To monitor post-synaptic cGMP dynamics in living hippocampal neurons, we prepared genetically engineered cGMP sensors utilizing two-photon FRET (Fӧrster resonance energy transfer) and FLIM (fluorescence lifetime imaging microscopy). We expressed these sensors in pyramidal neurons of cultured organotypic hippocampal slices and observed post-synaptic cGMP dynamics during synaptic plasticity. We detected transient cGMP production in neurons during long-term depression

(LTD), but not long term potentiation (LTP), suggesting a role of postsynaptic cGMP in LTD. Since LTD is associated with dendritic spine shrinkage (structural plasticity), we next examined the role of cGMP in structural changes at the dendritic spine. To mimic cGMP production in living hippocampal neurons, we characterized a mutant bacterial photoactivatable adenylate cyclase (BlgC), which generates cGMP when exposed to light. We confirmed two-photon photoactivation of BlgC at target dendritic spines by cGMP FRET/FLIM sensors, and observed the effect of postsynaptic cGMP production on structural plasticity. These two-photon optogenetics and live imaging techniques will provide new tools for studying cGMP signaling pathways.

2-B-15 ATP-evoked internalization of Pannexin 1 channels

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Pannexin 1 (Panx1) is a large pore ion and metabolite channel permeable to molecules up to 1kDa (including ATP), which is widely expressed in the brain, primarily in neurons. To date, exploration of its function has focused on its role at the plasma membrane; however, populations of mature Panx1 also localize to intracellular compartments. Interestingly, recent evidence suggests that high levels of extracellular ATP inhibit Panx1 currents. This is physiologically relevant because ATP is released as a signaling molecule in both regulated and constitutive contexts from multiple cell types in several brain regions, leading to transiently high levels of extracellular ATP. Here we hypothesize that ATP inhibition of Panx1 occurs by ligand-stimulated endocytosis of the Panx1 channel from the plasma membrane, akin to receptor-mediated endocytosis. To explore the influence of ATP on Panx1 plasma membrane localization, we transiently inhibited de novo protein synthesis (to stabilize the Panx1 population) then stimulated with ATP and other stimuli known to modulate Panx1 activity and/or cellular excitability as controls. Using both live and fixed cell confocal imaging, and cell surface luminometry, we show striking evidence for dose-dependent ATP-induced internalization of Panx1 from the plasma membrane with concurrent increase in early endosome distribution. Further, we present evidence that implicates purinergic receptors and extracellular residues of Panx1 in the internalization mechanism. These results have important implications for the regulation of Panx1 signaling in the brain.

2-B-16 Nemo kinase is a transcriptional target of the BMP signaling cascade in motorneurons

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Coordinated growth of synaptic structures and synaptic strength are regulated by signaling cascades across pre- and post-synaptic terminals. Despite great progress, the molecular players that modulate signal transduction pathways remain largely unknown. We have used the genetic and molecular tools in Drosophila to investigate how the retrograde bone morphogenic protein (BMP) cascade is regulated at the larval neuromuscular junction. BMP signaling is initiated in the muscles by postsynaptic release of the ligand Gbb, and results in a cascade in the presynaptic motorneuron upon binding to a receptor complex that includes the type II receptor Wit and a combination of Type I receptors. This results in an increase in phosphorylation and nuclear translocation of the BMP transcription factor, Mad. Here we describe the identification of Nemo (Nmo) kinase as a transcriptional target of this pathway. Using an in vivo enhancer trap reporter for nmo, we found that nmo transcription responds to BMP signaling manipulation. Transgenic RNAi knockdown of Wit in motorneurons or Gbb in muscles led to a significant reduction in nmo mRNA expression. Finally, chromatin immunoprecipitation and in vitro reporter gene assays of the nmo gene region revealed a site that binds and responds to Mad. Interestingly, our previous findings demonstrated that Nmo genetically and physically interacts with Mad. Therefore, we propose a new model in which BMP signaling self-regulates its action in motorneurons through transcriptional regulation of its own modulator, ensuring normal synaptic growth and function.

2-B-17 Distinct functional roles for P/Q- and N-types voltage-gated calcium channels in neurotransmitter release at mossy fiber to CA3 pyramidal cell synapses

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Neurotransmitter release from the presynaptic terminal is ensured by calcium entering through several types of VGCCs. Although the spatial distribution of distinct VGCCs impacts calcium dynamics and neurotransmitter release, the specific contribution of individual types of VGCCs to neurotransmitter release remains poorly defined. To dissect the roles of P/Q and N-types VGCCs, we used random-access two-photon calcium imaging, electrophysiology and electron microscopy. Our results show that P/Q- and N-types VGCCs support different calcium dynamics with specialized functions in triggering glutamate release. First, bouton calcium imaging revealed that P/Q-type VGCCs contributed a larger but more spatially homogeneous fraction of calcium than N-type VGCCs. Consistent with a global calcium increase, blockade of P/Q-type VGCCs prevented the recruitment of additional release sites during trains of activity. This effect could be mimicked by EGTA-AM, indicating that P/Q-type VGCCs can be loosely-coupled to the sensor. In contrast, antagonizing N-type VGCCs decreased the overall amplitude of EPSCs but had no effect on short-term facilitation. Finally, the spatial distribution of P/Q- and N-type VGCCs was investigated. Altogether, our results demonstrate the highly specialized roles of P/Q- and N-type VGCCs in neurotransmitter release. While N-type VGCCs are tightly-coupled to calcium sensor and provide local calcium elevations, P/Q-type VGCCs are well-suited to support global calcium elevations and promote the recruitment of additional release sites during trains of activity.

2-B-18 Pathway specific depolarization-induced suppression of inhibition in hypothalamic parvocellular neuroendocrine neurons

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Activation of parvocellular neurosecretory cells (PNCs) within the paraventricular nucleus of the hypothalamus (PVN) represents the final integrative step in recruiting the stress axis. Output from these neurons is constrained by GABAergic synapses, which constitute well over 60% of total synaptic input onto corticotropin releasing hormone (CRH) PNCs in the PVN. Postsynaptic depolarization induced suppression of inhibition (DSI) can regulate the efficacy of GABAergic transmission in the PVN, representing a mechanism by which PNCs may modulate their own afferent inputs. However, the extent to which eCB signalling can regulate specific afferents is unclear. The fusiform nucleus of the bed nucleus of the stria terminalis (BNSTfu) integrates specific stress- and anxiety-associated information from a variety of nuclei, which it then relays to the PVN through a dense GABAergic projection. Therefore, we assayed PNC DSI with pathway specificity by expressing ChR2 in VGAT positive BNSTfu neurons. PNCs in hypothalamic slices exhibited repeatable and robust DSI of light evoked currents that underwent a prolonged depression. Both the DSI and the depression could be blocked by AM251, indicating a role for eCBs in self-regulating GABAergic tone. These results taken together show that stress-associated signalling from the BNSTfu can be silenced for a prolonged period, underlining the plasticity present in this system. Understanding the conditions in which eCBs regulate GABAergic signalling highlights how CRH PNCs can modulate their own output along with that of the HPA axis.

2-B-19 Absence of MDGA1 Enhances Inhibitory Drive and Confers Resistance to Increased Excitation in Mouse Hippocampus

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Dysregulation of synapse development is emerging as a key cellular pathology underlying neurological disorders. MDGAs (MAM domain containing glycosylphosphatidylinositol anchor proteins) are neuronal surface proteins that have been genetically linked to schizophrenia and autism. Given that imbalance between inhibitory and excitatory synapses may contribute to these disorders, we sought to characterize the role of MDGAs in mediating synaptic function and structure. Recently, we determined that MDGA1 acts as a rare negative regulator of synapse development. We found that MDGA1 directly binds neuroligin-2 to selectively repress inhibitory synapse formation. To further probe the role of MDGA1 in synapse dynamics, we

studied MDGA1 knockout mice (MDGA1-/-). Whole-cell recordings taken from MDGA1-/- mouse pyramidal cells in CA1 of the hippocampus showed increased mIPSC frequency, whereas mEPSCs were unaltered. Similarly, symmetric perisomatic synapse density and GAD-65 punctate immunofluorescence were increased in CA1, with no change in asymmetric synapse density or VGlut1 puncta. To determine if network excitability was altered, we measured the frequency of spontaneous excitatory events in MDGA1-/- slices in response to increased extracellular potassium. Slices taken from MDGA1-/- mice showed resistance to excitation treatments consistent with enhanced inhibitory drive. Our results bolster our understanding of the molecular processes governing inhibitory synapse formation and function and provide the basis for more efficiently targeted therapeutics for brain-based diseases.

2-B-20 Investigating the regulation and function of mitochondrial remodelling in rat cortical astrocytes

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Mitochondria are dynamic organelles that drive cellular activity and maintenance in neurons and glial cells. Investigation of mito-morphology has allowed for a greater understanding of their functional roles in eukaryotic cells and in neurodegeneration. To date, mito-morphological changes have mostly been attributed to fission and fusion. Mito-remodelling/rounding, however, is a process that regulates mito-length without division or fusion of the mito-membranes. We show that remodelling can occur concomitantly with fission and have provided evidence that the mechanism of mito-remodelling is distinct from fission. In our study, we used mito-targeted yellow fluorescent protein, ROS-sensitive GFPs and live cell fluorescence microscopy to show that ROS generating agents such as rotenone and Ca2+ caused mito-remodelling and fission. We used antioxidants to block mito-remodelling without attenuating fission, indicating that remodelling is a distinct process from fission and is regulated by ROS whereas fission is regulated by Ca2+. Furthermore, we provide evidence that glycogen synthase kinase 3β (GSK3β) may be involved in the signaling mechanism of remodelling. Although the process of remodelling has been described in previous reports, the regulation mechanisms and functional significance are unclear. Through membrane potential and cell viability experiments we show that remodelling may be a protective mechanism. Exploration of the mechanism and function of mito-remodelling will provide a greater understanding into the role of mitochondria in cell maintenance and survival.

2-B-21 Inducible Deletion of Myelin Regulatory Factor is a Cell Selective Mechanism to Impair Oligodendrocyte Remyelination

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During remyelination, oligodendrocyte precursor cells (OPCs) are recruited to the lesion epicenter where they differentiate and mature into new myelinating oligodendrocytes, a process requiring intricate transcriptional regulation. Therapies that promote myelin repair hold enormous promise, but require a greater understanding of the key factors regulating transcription during oligodendrocyte maturation and subsequent remyelination. One gene, myelin regulatory factor (Myrf) is essential for the maturation of oligodendrocytes and the formation of central nervous system (CNS) myelin during development. However, its role in remyelination has not been elucidated. We crossed Myrf floxed mice with PDGFRα Cre ERT2 mice to remove Myrf from OPCs at the time of demyelination. Mice were then injected with lysolecithin to induce a focal demyelinating lesion in the corpus callosum, and examined at different time points post lesion. Myrf was not required for recruitment of OPCs following demyelination, but there was a marked reduction in the total number of CC1-Olig2+ mature oligodendrocytes at ten days post lesion (dpl). Recombined cells fail to robustly express GST-π, oligodendrocytes by 14 dpl. Recombined cells visualized with a membrane tethered reporter reveal that when Myrf is deleted in recombined cells, they rarely contributed to forming new myelin internodes. Thus, the inducible deletion of Myrf from OPCs prior to demyelination mimics aspects of chronically demyelinated MS lesions: the inability of OPCs to mature and successfully remyelinate.

2-B-22 Reduced Hyperpolarization-Activated Current Contributes to an Enhanced Intrinsic Excitability in Hippocampal Neurons from PrP-87-/- Mice

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Background: Hyperpolarization-activated cation (HCN) channels are highly expressed in the hippocampus where they act as key regulators of firing properties. Recordings from hippocampal neurons from prion protein (PrP) knockout mice reveal a variety of electrophysiological abnormalities including increased neuronal excitability. We thus wanted to determine whether PrP regulates neuronal firing properties via modulation of HCN channels. Methods: We conducted whole-cell voltage and current clamp recordings from primary hippocampal cultures prepared from P0-P1 wild-type (WT) and PrP-null pups. Results: We found that the absence of PrP profoundly affected firing properties of hippocampal neurons. In the presence of synaptic blockers there was an increase in the number of action potentials (APs) and a decrease in the spike threshold (p < 0.001). However, no measurable differences in resting membrane potential, AP amplitude, AP duration at 50% amplitude, maximum rate of depolarization and threshold were found. In contrast, membrane impedance was greater in PrP-null neurons (p < 0.05), and this difference was abolished by the HCN blocker ZD7288. PrP-null neurons exhibited a decrease in Ih peak current, as well as in the amplitude of voltage sag (p < 0.05). The time course of Ih activation became significantly slower in PrP-null neurons over a wide range of command voltages. Conclusion: These data suggest that the absence of PrP down-regulates activity of HCN channels, which in turn reduces membrane impedance to potentiate neuronal excitability in the hippocampus.

2-B-23 Kainate receptor mediated regulation of chloride homeostasis

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The potassium-chloride cotransporter KCC2 plays a critical role in inhibitory neurotransmission through its ability to maintain a low intracellular chloride level in the neuron. Surprisingly, KCC2 has also recently been found to interact with proteins involved in excitatory neurotransmission, including the kainate receptor subunit GluK2. It is known that independent of kainate receptor activity, the physical interaction between KCC2 and GluK2 is important for KCC2 surface expression and oligomerization. However, it is unknown whether the activity of kainate receptors can directly influence KCC2 function. In this study we hypothesized that the activation of GluK2-containing kainate receptors would increase KCC2s ability to extrude chloride from the cell. We tested this hypothesis by performing slice electrophysiology experiments in the CA3 region of the hippocampus, recording EGABA as a measure of KCC2 function. Our findings demonstrate that activation of the kainate receptor is able to regulate KCC2 function, revealing a novel mechanism for excitatory: inhibitory balance.

2-B-24 Selective viral manipulation of neostriatal matrix compartment

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Neostriatum is known to have a large neuronal network of high acetylcholine esterase activity (AChE) that configure the matrix compartment and a smaller set of neurons poorly stained for AChE that form the striosome compartment. In spite of much knowledge accumulated about these two compartments how they interact has been a matter of speculation. Since adeno-associate virus serotype rh10 (AAV10) expresses in striatal neurons located in matrix compartment sparing most neurons in striosomes and all striatal interneuron subtypes, selective manipulation of neurons in the matrix has become possible. Neuronal syntheses of channel rhodopsin 2 (ChR2) carried by AAVrh10 allowed us to evoke synaptic events by photo stimulation or glutamate puff (1mM/20psi/50ms) and perform whole cell patch-clamp recordings. We report that striatal and matrix compartments do not talk to each other via direct monosynaptic connections. Alternative ways of communication between striatal

compartments such as longer axonal influences or volume transmission must take place.

2-B-25 Modulation of GABAA receptors by a novel associated protein

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Gamma-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the adult mammalian central nervous system (CNS). The fast inhibitory actions of GABA are mediated by GABA type A receptors (GABAARs). Deficits in GABAAR-mediated transmission contribute to the etiology of epilepsy, anxiety disorders, and mood disorders. In contrast with numerous studies of glutamate receptor associated proteins and their involvement in the modulation of excitatory synapses, much less is known about GABAergic synapses. Using a novel combined transgenic, tandem affinity purification, and proteomic approach, we did a sequential purification from tagged GABAAR gamma2 subunit transgenic mice followed by mass spectrometry, and several associated proteins were identified including GARP1 (GABAA Receptor associated Protein 1). GARP1 is a novel trans-membrane protein, and no specific function has been reported. We found that overexpression of GARP1 dramatically decreases GABAAR-mediated currents by reducing surface expression of GABAARs in HEK293 cells. In rat hippocampal cultured neurons, overexpression of GARP1 reduces inhibitory synaptic transmission without altering excitatory synaptic transmission. Furthermore, shRNA-mediated knockdown of GARP1 selectively increases inhibitory but not excitatory synaptic transmission, and promotes synaptic surface expression of GABAARs, which can be rescued by a shRNA-resistant GARP1. These results indicate GARP1 is a novel associated protein which modulates function and trafficking of GABAARs.

2-B-26 Age-Dependent Odor Preference: Neuronal Properties and Responsiveness to Norepinephrine in the Anterior Piriform Cortex of the mouse

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Early odor preference learning in rodents occur within a critical period (≤ postnatal day 10-12), during which pups show heightened ability to form odor preference when the odor is paired with a tactile stimulation (e.g. stroking the pup's body with a brush). Norepinephrine (NE) release from the locus coeruleus (LC) during stroking acts as an unconditioned stimulus to mediate this learning. However, in older pups, stroking losses its ability to induce learning when paired with an odor. This sensitive period for odor preference learning has been shown to be caused by changes in the expression of adrenergic autoreceptors in the LC during maturation. However, there is also evidence suggesting changes in adrenoceptor responsiveness to NE in the olfactory bulb (OB) neurons of older pups. We have previously demonstrated that both OB and anterior piriform cortex (aPC) are critically involved in early odor preference learning. Activating β-adrenoceptors in either structure is sufficient to induce learning when paired with an odor, while blocking β-adrenoceptors abolishes odor preference learning induced by pairing an odor with stroking. Here we test whether there is developmental change in neuronal excitability and NE responsiveness in the aPC in neonatal mice. We show that odor paired with stroking induced odor preference in postnatal day 8-10 pups but not in postnatal day 14-16. We are currently investigating intrinsic neuronal excitability, synaptic properties and NE responsiveness of pyramidal cells in the aPC in different age groups.

2-B-27 Semaphorin 5A in Synapse Pruning and Autism Spectrum Disorders

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Autism Spectrum Disorders (ASDs) are a complex group of neurodevelopmental disorders characterized by impairments in communication and social behaviour. Although there is a limited understanding of the neuropathology of ASDs, previous work has shown decreased connectivity between brain regions and an overabundance of synaptic connections within specific regions of the brain, leading to imbalanced neural networks. This increased connectivity is believed to occur through disruption of normal synaptic pruning during development. Surprisingly, our knowledge about the

molecular and cellular processes regulating synapse elimination is still in its infancy. Here we demonstrate that semaphorin 5A (Sema5A) plays a central role in synapse elimination and that neuronal activity regulates synapse formation and elimination by changing the subcellular localization of Sema5A. This is of particular interest as Sema5A is genetically linked to ASDs and down-regulated in people with autism. Together, we suggest that Sema5A is essential for activity-dependent pruning of synaptic connections through regulation of its subcellular localization and that down-regulation of Sema5A may account for the exuberant synaptic connectivity observed in autistic patients.

2-B-28 Regulation of the transient receptor potential vanilloid 1(TRPV1) channel by heat shock protein 70 (HSC70)

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The transient receptor potential vanilloid 1 (TRPV1) channel is a non-selective cation channel that plays a pivotal role in inflammatory pain signaling. TRPV1 is activated by chemical and physical noxious stimuli and various inflammatory mediators. Lately its involvement in neuronal apoptosis and cell survival has come into focus. HSC70 is a constitutively and ubiquitously expressed chaperone protein that was classically described as having a central role in cell protection from damage caused by physical and chemical hazards. Here we set out to examine whether HSC70 was involved in regulating the activity and/or expression of TRPV1. Our results show that TRPV1 and HSC70 form a signaling complex and there is a significant decrease in the capsaicin-induced TRPV1 current after heat shock (1hour at 42⁰C) in both transfected HEK cells and dissociated Dorsal Root Ganglion neurons. Furthermore, Bioluminescence Resonance Energy Transfer (BRET) assay showed a decrease in TRPV1 subunit association after heat shock. HSC-mediated inhibition of the channel was strongly dependent on the ATP/ADP ratio and triggered by Rho-associated kinase (ROCK) inhibition. Altogether, we are showing for the first time a role for HSC70 in regulating TRPV1 channel activity during conditions of cell stress and that this effect involves ROCK and ATPase activity. This regulation may represent a survival mechanism ensuring neuronal protection or preventing TRPV1-mediated neurogenic inflammation in particular setting of cellular stress such as observed during ischemic conditions.

2-B-29 Nitric oxide modulation of phosphodiesterase activity and cAMP levels in astrocytes

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Astrocytic cAMP signaling plays an important role in several crucial physiological processes in the brain, including metabolic coupling to neurons. However, the regulation of cAMP levels by phosphodiesterases (PDEs) in astrocytes has not been determined. Recent transcriptome and protein analyses have confirmed that astrocytes express the PDE isoform PDE3, which is inhibited by cGMP. Here we investigate the hypothesis that PDE3 is an important regulator of astrocytic cAMP levels in the brain. To examine the role of PDE3 in modifying cAMP levels, we performed ELISAs in both acute hippocampal brain slices and primary astrocyte cultures. Our preliminary data indicate that the PDE3 inhibitor, cilostamide (10μM), enhances forskolin-induced cAMP increase. In addition, application of the nitric oxide (NO) donor, SNAP (100μM), also potentiated the cAMP increase, consistent with PDE3 sensitivity to cGMP. To further confirm that NO-induced cGMP is modulating cAMP levels through PDE3 inhibition, the experiment will be repeated in the presence of the soluble guanylyl cyclase inhibitor, ODQ. We are also examining the downstream consequences of increasing astrocytic cAMP by NO modulation. Previous studies have shown that cAMP modulates connexin43 (Cx43) expression and gap junction assembly in hepatoma and mammary tumor cells. We therefore predict that NO can alter Cx43 levels and subsequent gap junction coupling of astrocytes. Thus we will perform immunoblotting and dye flux imaging to assess the level of plasma membrane Cx43 and astrocyte coupling, respectively.

2-B-30 Synaptic mechanisms gating the cortico-hippocampal information flow through activation of the CA1 disinhibitory circuit

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Information processing in cortical circuits requires a delicate balance between excitation and inhibition.

In addition to inhibitory interneurons that control the activation of pyramidal cells, there are interneuron specific (IS) interneurons that coordinate the level of network inhibition by innervating interneurons. Here, we focused on the hippocampal CA1 disinhibitory circuit composed of the type 3 IS (IS3) interneurons. These cells innervate the somatostatin-expressing interneurons, which in turn control the integration of cortical input by distal dendrites of pyramidal cells. To study the mechanisms of the temporoammonic (TA) pathway integration by the IS3 cells, we used whole-cell patch clamp recordings in combination with two-photon Ca2+-imaging in hippocampal slices from VIP-GFP mice. Our data showed that EPSCs evoked in IS3 cells by the TA pathway stimulation had a small amplitude (15.3 ± 3.6 pA) and slow rise time (3.1 ± 0.6 ms), consistent with a distal location of TA synapses. Moreover, TA-EPSCs had two components: fast and slow mediated by the activation of AMPA and NMDA receptors, respectively. In addition, TA-EPSCs evoked at different frequencies of stimulation (5-40 Hz) demonstrated the paired-pulse and multiple-pulse facilitation, pointing to the increased recruitment of IS3 cells during physiologically relevant patterns of cortical activity. These results indicate that IS3 interneurons are well suited to integrate the consecutive excitation of TA afferents and provide disinhibition necessary for the selective gating of cortico-hippocampal information.

2-B-31 Protein Tyrosine Phosphatase Alpha-mediated Akt activation is required for oligodendrocyte differentiation and myelination

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The brain-enriched tyrosine phosphatase PTPα regulates many cellular processes, including the differentiation of oligodendrocyte progenitor cells (OPCs) to oligodendrocytes (OLs) that is required for CNS myelination. PTPα-/- (ΚΟ) OPCs have impaired differentiation and brains of KO mice are hypomyelinated. In our current study, we recapitulated this myelination defect in neuron/OPC co-cultures. WT and KO OPCs were grown on neurite beds formed by dorsal root ganglion neurons for 14 days and immunostained for MBP (OLs) and NFH (axons). MBP expression and MBP/NFH co-localization were reduced in ΚΟ OPC compared to WT OPC co-cultures, indicating impaired differentiation and myelination. We also observed these defects in organotypic cerebellar slices from neonatal WT and KO mice. Slices were grown for 10 and 20 DIV and immunostained as above, revealing reduced MBP/NFH co-localization in KO slices as compared to WT controls. Moreover, immunostaining for paranodal Caspr revealed impairment in myelin-directed organization of axonal domains in KO slices, confirming suboptimal myelination. We are investigating PTPα-dependent signaling in OPC differentiation. We show that Akt is activated in differentiating WT OPCs, but not KO OPCs, as indicated by increased phosphorylation of AktSer473 and Akt substrates. Future studies will elucidate how PTPα regulates Akt activity to promote OL differentiation. Improved knowledge of molecular pathways orchestrating OL development will provide insights to developing effective and specific treatments to promote myelin repair in demyelinating diseases.

2-B-32 T-type-mediated calcium spikes in dendrites of CA3 pyramidal neurons couple to Kv4 channels and mGluR1 receptors

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T-type voltage gated calcium channels are densely expressed in the hippocampus, although their functional characteristics and interactions with postsynaptic receptors are not well understood and low threshold calcium spikes (LTS) have not been reported. Using whole-cell electrophysiology, we demonstrate that LTS in CA3 pyramidal neurons can be evoked by somatic current injection. LTS were only evoked when 4-aminopyridine (4AP) was applied to depress A-type potassium channels. Evoked LTS occurred independently from somatic sodium action potentials and high voltage activated calcium channels, and were associated with fast-rising calcium transients in somatic and dendritic compartments. Using two-photon imaging of LTS-induced calcium transients, we mapped the distribution of T-type channels in CA3 pyramidal cells. We observed substantial calcium influx in dendrites with greater signals in the proximal versus distal (>50μm) dendrites. In contrast, in CA1 pyramidal neurons, LTS-associated calcium signals were restricted to the somatic region and not observed in dendritic arbors. We further demonstrate that mGluR1 activation inhibits LTS via

a phospholipase C-dependent pathway. Our data identify a new T-type mediated calcium signalling pathway in CA3 pyramidal cell dendrites that is unlocked by A-type potassium channel blockade and inhibited by mGluR1 receptors.

2-B-33 Activity Dependent Changes to Resting Astrocyte Ca2+

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Astrocytes play an important role in the maintenance and regulation of synaptic function, and are also capable of affecting brain blood vessel tone. These functions are dependent on changes in intracellular astrocyte calcium. While much research has been aimed at studying the effects of rapid and large increases in astrocyte calcium, the investigation of the role of resting calcium levels in astrocytes remains untouched. We sought to address this deficiency by using two-photon microscopy and examined changes to astrocyte resting calcium in response to specific patterns of synaptic activity. By applying in situ theta burst stimulation it was shown that astrocyte resting calcium levels decreased following stimulation and were reset at a new, lower baseline. This effect was successively compounded by subsequent stimulations. Application of a glutamate receptor blocker cocktail, designed to target metabotropic glutamate receptors (mGluRs), N-methyl-D-aspartate receptors (NMDARs), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), suppressed the effect suggesting a glutamatergic mechanism. These results may have functional consequences to the control of blood vessel tone by astrocytes, as well as their effects at the synapse, and may provide further insight into the means by which astrocytes perform their vascular and synaptic regulatory roles.

2-B-34 Ultrastructural analysis of blood-brain barrier breakdown in the peri-infarct zone of young adult and aged mice

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Following ischemia the blood-brain barrier (BBB) is compromised in the peri-infarct zone, leading to secondary injury and dysfunction that can limit stroke recovery. There is considerable uncertainty regarding what structural changes could account for BBB breakdown, particularly in aged animals. Here we employed electron microscopy to analyze early and late changes (3 vs. 72 hours) in the BBB in young and aged mice (3-5 vs. 18 month old) subjected to photothrombotic stroke. At both time points and ages, BBB permeability was accompanied by swollen endothelial cells packed with small transcytotic vesicles (~60 nm diameter) and vacuoles (>100 nm diameter). In a small fraction of microvessel tight junctions, we observed a fluid-filled space at the junction suggesting that partial disruption can occur. Of note, ischemia in young adult mice led to an increase in pericyte area and coverage of endothelial cells, whereas in older mice, pericyte area and coverage was significantly reduced. In both age groups, the basement membrane was expanded, especially at 3 hours. Peri-vascular astrocytes and their mitochondria were severely swollen (~2-3 fold increase in area) at both times points and ages. At 3 days, astrocytes were replete with glycogen deposits suggesting a change in their energy metabolism and storage. Our results indicate that upregulation of endothelial transcytosis and vacuolation, rather than breakdown of endothelial tight junctions, mediates BBB permeability in the peri-infarct cortex. Further, our data suggest that pericyte responses to ischemia are affected by aging.

2-B-35 Sensory-evoked dendritic activity and somatic firing instruct morphogenesis in the awake brain

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Sensory activity is essential during normal development of neural circuits. However, the role that patterned sensory activity plays in the detailed structural reorganization of the dendritic arbor remains unknown. Using a custom made random-access microscope that allows rapid simultaneous activity sampling throughout the entire 3D dendritic arbor in awake Xenopus Laevis tadpoles, in combination with targeted single cell electroporation to label single neurons with selected receptive field properties, we characterized the learning rules that neurons follow to modify their detailed morphology in response to sensory activity. We found that neurons' somatic firing and plasticity patterns were associated with the patterns of dendritic growth and

pruning. The observed motility was determined by the combination of evoked calcium transients at filopodial tips and adjacent shafts in response to sensory stimulation. Our data show that sensory stimulation evoked activity passing through dendrites guides the specific filopodial growth and retraction patterns, shaping how the developing brain processes the environment and how individual neurons integrate into functional networks.

2-B-36 Thalamic modulation of the cortical slow oscillation

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The slow oscillation present during non-REM sleep and under different types of anesthesia has a cortical origin however a growing number of evidence suggest that the thalamus plays a significant role in the regulation of this rhythm, but also of faster frequencies. The slow wave power, which includes the slow oscillation and delta waves, is one of the main quantifiable parameters characterizing the quality of sleep. We hypothesize that the first-order (specific) thalamic nuclei provide a control of slow waves in primary cortical areas, while high-order (non-specific) thalamic nuclei may synchronize the slow wave activities across wide cortical regions. We analyzed local field potentials (LFP) from different cortical areas of mice while a thalamic nucleus was inactivated by either the sodium channel blocker QX-314 or the GABA-agonist muscimol. Microinjection of these substances blocked or significantly decreased neuronal firing in the corresponding thalamic nuclei. Inactivation of specific thalamic nuclei significantly decreased the power of slow/delta and gamma waves in the cortex. Inactivation of specific thalamic nuclei (VL, VPM) mainly affected primary cortical areas. The inactivation of non-specific nucleus (CL) affected all cortical areas. Generally, the use of QX-314 affected larger cortical territories as compared to muscimol, likely because it affected not only local neurons but also passing fibers. We conclude that the non-specific thalamic nuclei play a major role in the regulation of the global cortical slow wave activity.

2-B-37 Optogenetic quantification of glutamate clearance following synaptic release in health and disease

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Upon synaptic release, glutamate is cleared from the extracellular space by diffusion as well as uptake through glutamate transporters on neurons and astrocytes. The rate of glutamate clearance can critically affect neuronal health as excessive glutamate receptor activation can cause neuronal death. Here, we used widefield imaging of the recombinant glutamate sensor, iGluSnFR, in mouse brain slices to quantify glutamate clearance in the striatum following synaptic release. Electrical stimulation of striatal afferents resulted in robust increases in iGluSnFR fluorescence intensity, and the decay of the signal was significantly slowed by partial blockade of the glutamate transporter GLT-1. Pharmacological experiments suggest that GLT-1 is responsible for maintaining a low, steady-state level of extracellular striatal glutamate whereas non-GLT-1 transporters are chiefly responsible for the rapid clearance of glutamate following its evoked release. We are currently expressing iGluSnFR under different promoters to understand whether the time-course of glutamate sensed by astrocytes differs from that of neurons. Finally, we use this sensor to show that in Huntington disease (HD), a neurodegenerative disease in which striatal neurons are particularly vulnerable, glutamate uptake is intact. This finding contrasts with traditional biochemical uptake assays and provides important insight into the mechanisms of neurodegeneration in HD. In all, our data demonstrates that iGluSnFR is a powerful tool that can increase our understanding of glutamate clearance in both health and disease.

2-B-38 Synaptic Plasticity in the Globus Pallidus

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Plasticity-based therapies using electrical stimulation of brain circuits offer new therapeutic approaches in Parkinson's disease (PD). Field evoked potentials (fEPs) have previously been used to demonstrate synaptic plasticity in the basal ganglia (BG) of patients with PD that is dependent on exogenous dopamine levels. Establishing how these findings translate to the healthy brain is essential in giving us a baseline of "normal" synaptic plasticity in the BG.

Here, we explore the effects of high frequency stimulation (HFS) on synaptic plasticity induction in a healthy non-human primate in the globus pallidus (GP), a major output structure of the BG. Four independent electrodes recorded neuronal activity and stimulation-induced fEPs from sites in the GPe and GPi. At a given site, baseline fEP amplitudes and slope were monitored from 3 recording electrodes for 1 minute using 0.25 Hz test pulses from a 4th stimulating electrode, then HFS (four 2s trains, 100 Hz, 100uA) was delivered through the stimulating electrode. Post HFS, 0.25 Hz test pulses were resumed and fEP amplitudes and slopes were monitored for 10 minutes to measure changes in synaptic efficacy resulting from HFS. Following HFS, fEP amplitudes significantly increased above baseline in the GPe and GPi at 10 minutes, indicating changes in synaptic plasticity are present in the BG of healthy subjects. The magnitude of change is intermediate to previous findings comparing PD patients ON and OFF medication, indicating that the "normal" response to these stimulation paradigms is not the same as the PD ON response.

2-B-39 All-or-none axonal Ca2+ dynamics in recurrent circuits of the hippocampus

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An action potential (AP) is assumed to travel actively and reliably along axons and trigger Ca2+ entry into nerve terminals without fail, leaving all-or-none regulation of release probability (Pr) in the hands of downstream SNARE proteins. Using viral delivery in mature hippocampal slice cultures and two-photon imaging of fast genetically-encoded Ca2+ sensor, GCaMP6f, we explored the dynamics of axonal Ca2+ transients (CaTs) evoked by single AP stimulation of CA3 pyramidal neurons in organotypic slices studied at 30°C. CaT amplitudes followed a right-skewed distribution (>700 boutons). Strikingly, 1AP CaTs imaged along single axons failed intermittently on repeated (30) trials at neighbor boutons. Conservative histogram analysis of CaT amplitude revealed a bimodal distribution with clear separation of CaT successes and failures. CaT failures rarely spread across an entire branch, suggesting that they were not due to conduction failure. Notably, prolonging AP duration with the K+ channel blocker 4-aminopyridine (100 μM) reduced CaT failure rate and increased both their amplitude and duration. Finally, a subset of axons exhibited functionally silent boutons that were unresponsive to single trial AP stimulation but responded to a stronger 50 AP train pattern. In these silent boutons, a visible CaT signal evolved within 5-10 APs, possibly due to recruitment of Ca2+ channels or build-up of residual Ca2+. Altogether, our finding uncover large all-or-none variations in axonal CaT dynamics and leave ample room for Pr regulation, upstream of SNAREs, via Ca2+ channel modulation.

2-B-40 The cellular mechanisms of neuronal swelling underlying cytotoxic edema

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Cytotoxic brain edema is the principal cause of mortality following brain trauma and cerebral infarct yet the mechanisms underlying neuronal swelling are poorly understood. Here we show that neuronal swelling and death was triggered by activating either voltage-gated sodium channels or NMDA receptors and was independent of Ca2+ entry but required Cl- entry via an unknown pathway. During swelling, two photon fluorescence lifetime imaging demonstrated that increases in intracellular sodium concentration ([Na+]i) were followed by a secondary Cl- influx leading to volume increases. 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 LNP delivery systems. Surprisingly, knockdown of a previously unidentified neuronal chloride channel attenuated both neuronal swelling and a Cl- current that was activated when cortical neurons were depolarized to membrane potentials > -20mV. We conclude that cytotoxic brain edema occurs when sufficient Na+ influx depolarizes neurons to activate Cl- entry, thereby causing subsequent neuronal swelling leading to neuronal death. The identification of a unique Cl- channel that is activated by depolarization as a significant Cl- entry pathway during pathological swelling triggered after Na+ entry suggests that new strategies could be developed towards reducing brain edema.

2-B-41 The X-linked Intellectual Disability Gene, DHHC9, in Neurite Outgrowth and Synapse Formation

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Palmitoylation is a reversible post-translational modification involving the attachment of palmitate to cysteine residues on substrate proteins. This process is catalyzed by a family of palmitoyl acyltransferase (PAT) enzymes that contain a conserved DHHC motif. Palmitoylation enhances the recruitment of proteins to the membrane and the palmitoylation of a number of synaptic proteins is believed to regulate synapse organization, function and plasticity. Although this is a relatively new field of research, disruption of DHHC function has been implicated in a number of neurodegenerative and neurodevelopmental disorders, underscoring their importance for proper brain development and function. DHHC9 loss of function mutations have been identified in patients with intellectual disability, however its role in the development and function of neural circuits is still unknown. Here we demonstrate that DHHC9 is localized to both excitatory and inhibitory neurons where it plays an important role in promoting dendritic outgrowth and arborisation and constraining exuberant synapse formation. Preliminary data suggest that this is mediated through the palmitoylation of the DHHC9 substrate, Ras. Taken together, this work suggests that palmitoylation-dependent regulation of Ras by DHHC9 modifies neural complexity by regulating neuronal growth and synaptic density.

2-B-42 Diacylglycerol and inositol triphosphate modulate a protein kinase C-dependent change in Aplysia bag cell neuron excitability

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In response to cholinergic stimulation, the bag cell neurons of Aplysia californica undergo a long-term change in excitability, leading to an afterdischarge and the secretion of egg-laying hormone to initiate reproductive behaviour. A key pathway activated at the onset of the afterdischarge is the phospholipase C (PLC)-mediated breakdown of PIP2 into inositol triphosphate (IP3) and diacylglycerol (DAG). We examined the effects of OAG, a membrane-permeable analogue of DAG, as well as IP3, on cultured bag cell neurons. OAG activated a prolonged, Ca2 -permeable inward current in a dose-dependent manner. Gd3 inhibited the current, but traditional cation channel blockers, MDL-12330A and flufenamic acid, did not. In addition, pretreatment with the protein kinase C (PKC) inhibitor, H7, prevented the current. However, in an independent bioassay, unlike the phorbol ester PMA, OAG did not activate PKC in bag cell neurons. Inclusion of IP3 in the internal saline enhanced the OAG current. OAG depolarized cultured bag cell neurons, increased excitability, and, in some cases, caused afterdischarge-like action potential firing. Neurons pretreated with H7 exhibited attenuated depolarization, with the majority of neurons not firing in response to OAG. OAG-induced afterdischarge-like behaviour appears to require PKC permitting lipid-gating of the channel. This suggests an interaction between the PLC pathway and PKC to control the strength and duration of the afterdischarge.

2-B-43 Paradoxical excitation by primary afferent depolarization requires dual changes in GABAergic signalling and neuronal excitability

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The central terminals of primary afferent fibers experience depolarization upon activation of GABAA receptors (GABAAR) due of their high intracellular Cl- concentration. Primary afferent depolarization (PAD) is normally inhibitory because of Na channel inactivation and shunting, but PAD can evoke spikes under certain pathological conditions. Using computer simulations and dynamic clamp experiments, we sought to identify which biophysical changes are required for PAD to become paradoxically excitatory and whether inhibitory effects of PAD are retained. Computational modeling predicted that GABAAR-induced spiking requires both a depolarizing shift in GABA reversal potential and increased intrinsic excitability such as that produced by reduced K channel function. We tested our predictions experimentally by using dynamic clamp to insert virtual GABAAR conductances with

different reversal potentials and kinetics into dorsal root ganglion neurons. Neurons from naïve rats were compared before and after pharmacological reduction of Kv1-type current, and against neurons from nerve-inured rats. Results confirmed that both predicted changes are indeed necessary to yield GABA-induced spiking. However, even when PAD can elicit spiking, sustained GABAAR input tends to block spontaneous spike initiation and the propagation of spikes through the depolarized region, arguing that PAD retains its inhibitory effects. Unlike postsynaptic inhibition, which is readily disrupted by Cl- dysregulation, these findings argue that presynaptic inhibition via PAD is resilient to pathological disruption.

2-B-44 Modelling morphology and integration in developing dendrites

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This poster will present modelling techniques and results of dendritic integration that are both informed and informing experiments in vivo of xenopus tadpoles during development.

2-B-45 D-Serine influences retinotectal synapse maturation and axonal refinement in the developing visual system

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The gliotransmitter D-serine is a co-agonist for synaptic N-methyl-D-aspartate receptors (NMDAR). Here we examined the role D-serine plays in regulating synaptic transmission and axonal remodeling in the developing visual system of the tadpole. We find acute D-serine (100 µM) wash-on enhances NMDAR currents of optic tectal neurons, whereas degradation of D-serine by RgDAAO reduces NMDAR currents, indicating that endogenous D-serine is normally present below saturating levels. To investigate the pathways involved in modulating D-serine release we used D-serine amperometric biosensors and find that AMPAR activation results in an increase in D-serine release in vivo. We next tested whether chronically elevating D-serine levels could influence the maturation of glutamatergic synapses. We find that tadpoles raised in D-serine (100 µM) for 2 days have higher frequencies of miniature excitatory postsynaptic AMPAR currents and higher retinotectal synaptic AMPA/NMDA ratios compared to control animals. To examine the effects of D-serine on axonal development, images were collected daily, over 4 days to assess growth and branch elaboration and at shorter (10 min) intervals to assess branch stabilization. We find that increasing available D-serine results in the hyperstabilization of retinal axon branches. Axonal arbors become less complex compared to control axons over 4 days of treatment with D-serine. These results suggest that D-serine levels are modulated by glutamatergic neurotransmission in vivo and can influence the maturation of retinotectal synapses and circuit refinement.

2-B-46 Glycine-mediated fast inhibitory synaptic transmission in the hypothalamic paraventricular nucleus

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Glycine is a major inhibitory neurotransmitter in the spinal cord/brainstem but in the forebrain it is largely thought to be restriced to modulatory actions either on extrasynaptic glycinergic receptors or as a co-agonist to NMDA. Here we present functional electrophysiological data from neurons in the paraventricular hypothalamic nucleus (PVN) of mice demonstrating its role as a bona fide fast inhibitory synaptic mediator. We performed whole cell recordings on slices from CRH:Cre:TdTomato mice. Inhibitors of glutamate and GABA-A receptors (CNQX 10 uM, D-AP5 50 uM, Bic 20 uM) were constantly present in the perfusion medium. In response to focal application of glycine (puff, 0.1-3 mM) we observed a large inward current that was inhibited by 0.6 uM strychnine in >90% of the PVN neurons tested. Electrical stimulation of the neuropil resulted in fast synaptic currents (-109±33 pA) that was inhibited by 0.6 uM strychnine. These synaptic events were observed in a fraction of cells in PVN (21%, 26 out of 126 cells), while being completely absent in CRH-positive cells (n=19). We also noted sporadic spontaneous synaptic release of glycine (sIPSCs frequency: 0.16±0.06 Hz, amplitude: -85±17 pA). Occasionally we observed bursts of high frequency sIPSCs, suggesting there may be

conditions under which the glycinergic system can potently impact electrical activity in postsynaptic neurons. Here we show the first evidence for the fast glycine mediated synaptic transmission in the hypothalamus. Experiments designed to understand the physiological role of this signaling system are ongoing.

2-B-47 Role of palmitoylation in NMDA receptor trafficking and function in corticostriatal co-culture

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Previous studies show a critical role of NMDA receptor (NMDAR) localization and subunit composition in determining its excitotoxicity in Huntington's disease (HD). Our lab has found in an HD mouse model that GluN2B-NMDAR at extrasynaptic (Ex) sites are increased in striatum at an early stage and lead to impaired striatal neuronal survival signaling; however, the mechanism underlying altered NMDAR trafficking in HD remains unknown. Others have shown that posttranslational modification of GluN2A and GluN2B subunits by palmitoylation, the addition of palmitate to cysteines in two C-terminal domain clusters, regulates surface expression and synaptic targeting of NMDAR. Notably, two palmitoyl acyl transferase (PAT) enzymes, DHHC17 (HIP14) and DHHC13 (HIP14L), also interact with huntingtin, the protein mutated in HD, and both HIP14- and HIP14L-deficient mice develop features resembling HD. We have examined, for the first time, the role for palmitoylation and HIP14 in regulating GluN2A-/GluN2B-NMDAR trafficking and NMDAR functioning in corticostriatal co-culture. We found that reduced GluN2B-NMDAR palmitoylation may contribute to increased Ex-NMDAR in striatal neurons, but that decreased HIP14 function is not involved in either GluN2B- or GluN2A-NMDAR trafficking. Further, knock-down of HIP14 in co-culture has no effect on NMDAR function. These studies will grant us deeper understandings on the dynamics between synaptic/Ex NMDAR trafficking and provide novel therapeutic targets for pharmacological interventions in HD. Supported by CIHR funding.

2-B-48 A new signaling modality for NMDA receptors in excitotoxic cell death and ischemic stroke

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Over-activation of neuronal N-methyl-D-aspartate receptors (NMDAR)s causes excitotoxicity and is critical for neuronal death. In the classical view, these ligand-gated Ca2+ permeable ionotropic receptors require co-agonists and membrane depolarization for activation. We report that NMDARs signal during ligand binding without activation of their ion conduction pore. Pharmacological pore block with MK-801 or physiological pore block with Mg2+ prevented NMDAR currents, but failed to block excitotoxic membrane currents and dendritic blebbing induced by NMDA. Recruitment of pannexin-1 (Panx1) channels was critical in this excitotoxic response. Indeed, Panx1 opening mediated the bulk of excitotoxic Ca2+ influx during NMDA receptor excitotoxicity. In contrast to MK-801, competitive antagonists that prevent ligand binding to the NMDAR prevented Panx1 opening, membrane currents and blebbing. NMDARs, Src family kinases and Panx1 were found in a signaling complex and activation of Panx1 involved phosphorylation at Y308. Block of this NMDAR-Src-Panx1 signalsome in vitro, in situ, or in vivo by administration of a Y308 interfering peptide either before or 2 hours after ischemia/stroke was neuroprotective. Our observations provide key insights into a novel signaling modality of NMDARs that has broad reaching implications for brain physiology and pathology.

2-B-49 Class 5 semaphorins mediate synaptic elimination and activity-dependent plasticity in rodent hippocampal neurons

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Autism spectrum disorders (ASD) is a highly heritable developmental brain disorder caused by altered synaptic connectivity and function. In 2009, a genome-wide association study has identified Semaphorin 5A (Sema5A) as a novel autism

susceptibility gene. Sema5A is a member of the semaphorin family, a large family consisting of secreted and membrane-associated proteins that play numerous key roles in the development and function of the nervous system. Compared with other semaphorins, Sema5A's function in brain development is largely unknown. Here, we provide evidence that Sema5A regulates synaptic elimination in hippocampal neurons. Both of the overexpression of Sema5A as well as the bath application of secreted Sema5A protein caused excitatory synaptic elimination in hippocampal neurons without affecting inhibitory synapses. In contrast the knockdown of Sema5A significantly increased excitatory synaptic density. Further, we investigated the relationship between class five semaphorins (Sema5A and Sema5B) and long-term potentiation (LTP), one of the most widely studied cellular models of synaptic plasticity. We demonstrated that the overexpression of class 5 semaphorins was capable of attenuating the increase of synaptic density caused by LTP, implicating the involvement of Sema5A and Sema5B in activity-dependent plasticity. Accompanied with our previous findings of Sema5B, these data reveal a new role for semaphorins in mediating synapse maintenance and morphology and therefore provide insights into the role that these guidance molecules may play in the developing brain.

2-B-50 Persistent firing and its transition to ictal-like response in hippocampal CA1 pyramidal neurons

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The hippocampal CA1 region is crucial for temporal association tasks that require short-term (< 30s) retention of memory. These tasks also require an intact cholinergic system. However, it remains unclear what cellular mechanisms support short-term memory retention in CA1. Here, using in vitro recordings from rats, we show that brief stimulation (100pA, 2s) can initiate persistent firing in CA1 pyramidal cells in the presence of cholinergic agonist carbachol (1-10 μM). This persistent firing lasted more than 30 s and its frequency was around 10 Hz. Our observations suggest that the canonical transient receptor potential channels (TRPC4/5) support persistent firing. These data suggest that persistent firing supported by a heightened cholinergic tone may contribute to information retention during temporal association tasks. On the other hand, cholinergic system is also involved in mesial temporal lobe epilepsy. In a relatively high concentration of carbachol (20 μM), the same brief stimulation often drove a response similar to ictal depolarizations, which accompanied a depolarization block. We further demonstrate that TRPC4/5 channels contribute to, and the SK and M-type potassium channels suppress this response. Our results indicate that individual neurons may exhibit persistent firing and epileptiform responses depending on the level of the cholinergic tone. Downstream mechanisms involve the TRPC4/5 channels and the AHP currents. These modulations of single cell properties might underlie contrasting in vivo neural activity during memory tasks and epilepsy.

2-B-51 Regulation of endosome fusion by Cav2.2

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Voltage-dependent calcium channels (including Cav2.2) are a group of voltage-gated ion channels found in the membrane of excitable cells with permeability to the calcium ion Ca2+. However, the intracellular functions of these channels have been largely unexplored. In this study, we have investigated the role of Cav2.2 in endocytic transport. In the presence of bafilomycin A1 which inhibits the fusion of late endosomes and lysosomes, the GFP tagged Cav2.2 channels expressed in tsA-201 cells resided in the membrane of late endosomes and co-localized with Rab7 (a late endosome/lysosome marker). These results then suggested that Cav2.2 channels may affect late endosome function. Indeed, Cav2.2 significantly enhanced the degradation of peptides expressed in late endosomes, indicating that Cav2.2 may play a general role in regulating endosomal membrane trafficking by increasing the fusion of late endosomes and lysosomes. Overall, we propose a new role of Cav2.2 in regulating endocytic transport.

C - Disorders of the Nervous System

2-C-52 Glutamatergic transmission is enhanced in the amygdala in Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is often associated with co-morbidities like neuropsychiatric and cognitive impairments, affecting around 50% of the patients. We investigated these abnormalities in an animal model of MS, called experimental autoimmune encephalomyelitis (EAE) during the early onset of the disease. We have shown that at this presymptomatic stage, the EAE mice show emotional and cognitive deficits in absence of motor deficit. Herein, we investigated the potential synaptic changes in the amygdala associated with inflammation, a brain region involved in emotional behaviour. EAE was induced in C57/BL/6 mice with MOG35-55 /CFA and pertussis toxin (PTX). CFA and PTX only mice were controls. Dendritic spines were counted after Golgi staining. Whole-cell recording was carried out in the principal neurons of basolateral amygdala (BLA). Glutamatergic currents were recorded in the presence of GABA receptor blocker picrotoxin. Investigation into glutamatergic synaptic transmission revealed increased frequency of the mini-excitatory postsynaptic currents (mEPSC) without any changes in amplitude compared to the controls; this is indicative of increased glutamate release. This was associated an increase in the synaptic spines in the principal neurons of the BLA and alteration in the AMPA receptor subunit composition, with an increase in GluA2 subunit. In conclusion, emotional and cognitive deficits observed in EAE (and possibly MS) were associated with an increased dendrite spines, enhanced glutamatergic transmission and changes in AMPA receptor composition in the BLA.

2-C-53 PDGFRa-positive progenitor cells form myelinating oligodendrocytes and Schwann cells following contusion spinal cord injury

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Contusive spinal cord injury (SCI) results in considerable demyelination of spared axons, which impairs signal transduction and may leave axons vulnerable to degeneration. NG2 cells, characterized by the near ubiquitous co-expression of platelet derived growth factor receptor α (PDGFRα) in the uninjured central nervous system (CNS), are oligodendrocyte progenitors (OP)s which may serve as a source of new OLs following SCI. PDGFRα-CreERT mice were crossed with Rosa26-YFP mice and administered tamoxifen to label OPs two weeks prior to contusive thoracic spinal cord injury. In the uninjured spinal cord we found that YFP was expressed in NG2τ OPs at very high efficiency, as well as αSMAτ pericytes and fibronectinτ fibrocytic cells in the spinal roots. Following injury, many recombined cells continue to express the PDGFRατ, Olig2 and NG2, indicative they have remained as OPs, but substantial differentiation into new oligodendrocytes (CC1τ) was observed, responsible for de novo ensheathment of >30% of the myelinated axons by three months. Strikingly, the majority of P0τ Schwann cells in the spinal cord expressed YFP, suggesting they originated from central nervous system PDGFRατ OPs. However, further work is required to characterize if other YFPτ populations like αSMAτ pericytes or the peripheral fibrocytic-like cells can contribute to the formation of myelinating Schwann cells or OL's in the injured CNS. Overall, this work reveals phenotypic plasticity of PDGFRα precursors following SCI as a source of the new remyelinating Schwann cells and oligodendrocytes in the injured cord.

2-C-54 Neuronal sodium elevation and COX-2 activation in post-traumatic epileptogenesis in vitro

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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 organotypic hippocampal slice cultures from wild-type C57BL/6J mice, imaged with the Na+-sensitive dye SBFI. Immediately post-trauma, neurons had significantly higher [Na+]i than has been reported in undamaged

neurons, particularly near the cut surface of the slice. After a briefly recovery period, [Na+]i again rose to high levels and remained elevated for weeks. [Na+]i was found to depend on the activity of Na+/K+ ATPases, cation/Cl- cotransporters, and Na+/Ca2+ exchangers in order to support high rates of transmembrane Na+ flux during epileptogenesis. Wash-in of the dye fluorescein indicated that traumatized neuronal membranes may also allow Na+ to leak into damaged neurons, resulting high [Na+]i, propidium iodide-positive status, and cell swelling. Inhibition of COX-2 and related inflammation pathways both significantly lowered [Na+]i and drastically reduced seizure activity in epileptic slices. Overall, elevated [Na+]i is a promising new biomarker for neuronal compromise, as it precedes many traditional indicators of epileptic activity and ictal cell death.

2-C-55 Development of a primate model of Alzheimer's Disease I. Characterization of molecular pathology

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder, and therapeutics have proven difficult to translate from mouse models to human clinical trials. An intermediary primate model would greatly advance our understanding of mechanisms involved in AD pathogenesis and could be used to vet promising therapeutics. Here, we describe the molecular characterization of a non-human primate model of AD generated in macaque monkeys by icv injections of amyloid-β oligomers(AβOs). Soluble AβOs accumulate in the brains of AD patients and correlate with disease-associated cognitive dysfunction. In our first set of experiments molecular pathology was examined by immunohistochemistry on brains perfused several weeks after AβO injection. AβOs diffused into the brain and accumulated in several regions associated with memory and cognitive functions. Cardinal features of AD pathology, including synapse loss, tau hyperphosphorylation, astrocyte and microglial activation, were observed in regions of the macaque brain where AβOs were abundantly detected. Most importantly, AβO injections in macaques induced AD-type neurofibrillary tangle formation, unlike most rodent models. In ongoing experiments, Aβ, total tau and phosphor-tau in cerebrospinal fluid, as well as blood chemistry and endocrinology panels, are being examined before and several time points after icv injection of AβOs. Behavioural phenotype is now being characterized via activity tracking, and performance on learning and memory tasks using touchscreen CANTAB and saccadic eye movements (see Wither et al, this meeting).

2-C-56 Experimental traumatic brain injury: Bad to the bone?

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Traumatic brain injury (TBI) has been associated with accelerated bone fracture healing, however pathophysiological mechanisms involved in TBI have the potential to be detrimental to bone. The current study assessed the effect of experimental TBI in rats on the quantity and quality of mineralized bone within the femur. Rats were randomly assigned into either sham or lateral fluid percussion injury (FPI) groups. Open-field testing assessed locomotion at 1, 4, and 12 weeks post-injury, with the rats killed at 1 and 12 weeks post-injury. Bones were analysed using peripheral quantitative computed tomography (pQCT), histomorphometric analysis and three-point bending. pQCT analysis revealed that at 1 and 12 weeks post-injury, the distal metaphyseal region of femora from FPI rats had reduced cortical content (10% decrease at 1 week, 8% decrease at 12 weeks; p < 0.01) and cortical thickness (10% decrease at 1 week, 11% decrease at 12 weeks p < 0.001). There was also a 23% reduction in trabecular bone volume ratio of this region at 1 week post-injury and a 27% reduction in FPI rats at 12 weeks post-injury (p < 0.001). Despite the changes at the femoral distal metaphysis we observed no changes in bone quantity and quality at the diaphysis. Overall, our results indicated that experimental TBI induced region-specific bone loss. Importantly, there were no differences in locomotor outcomes, which suggested that post-TBI changes in bone were not due to

immobility. These findings indicate that the pathological systemic effects of TBI also have a negative effect on bone.

2-C-57 High density lipoproteins benefit function and reduce inflammation in human brain microvascular endothelial cells.

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Increasing evidence suggests that cerebrovascular dysfunction has a major role in Alzheimer's disease (AD) as amyloid is found within the cerebrovasculature, integrity of the blood-brain barrier is reduced, and cerebral blood flow becomes dysregulated. Substantial research has demonstrated that high density lipoproteins (HDL) have profound vasoprotective properties in peripheral vessels. However, whether these benefits extend to the CNS have yet to be confirmed. Here we show that plasma-derived HDL increases nitric oxide (NO) production and decreases the inflammatory status of human brain microvascular endothelial cells (hBMECs). Plasma HDL was isolated from healthy human volunteers by density gradient ultracentrifugation and exposed to hBMECs to assess endothelial function and inflammation. NO production was measured using 4,5-diaminofluorescein (1 μM) in cells after incubation with HDL (100ug/ml, 15 min). HDL (50 ug/ml, 16 hr) suppression of tumour necrosis factor α (TNFα)-induced inflammation (1 ng/mL, 4 hr) was assessed by ELISA of human IL-6 in cell media and western blotting of vascular cell adhesion molecule in cellular protein extracts. Production of NO in hBMECs is significantly increased and TNFα stimulated inflammation is significantly reduced with HDL treatment. Our results suggest that plasma HDL influences cerebrovascular endothelial cell health. As the cerebrovasculature system is emerging as one link between whole body health and the brain, capitalizing on the vasoprotective functions of HDL may provide novel therapeutic opportunities for AD.

2-C-58 Pregabalin Alters Cortical Spreading Depression and Synaptic Function in a Model of Familial Hemiplegic Migraine Type-1

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Cortical Spreading Depression (CSD) is believed to be the pathophysiological trigger mechanism in migraine headaches. Familial Hemiplegic Migraine Type-1 (FHM-1) patients suffer from migraine, sometimes including ataxia and seizures, resulting from gain-of-function mutations in the Cav2.1 (P/Q-type) calcium channel. The gabapentinoid neuropathic pain drug, Pregabalin (Lyrica) binds to the alpha-2-delta calcium channel ancillary subunit and is thought to inhibit Cav2.1 currents. Therefore, we examined its potential for suppressing CSD in mice containing FHM-1 mutations, which display a reduced threshold for CSD. We utilized intrinsic optical signalling to examine the rates of propagation of CSD in vitro using acute brain slices from wild-type and FHM-1 mice. In addition, we have correlated these findings with electrophysiological analysis of synaptic activity using whole-cell patch clamp in acute brain slices and exogenously expressed Cav2.1 channels. Furthermore, we performed 7 Telsa diffusion-weighted, magnetic resonance imaging in FHM-1 knock-in mice to visualize the speed and threshold of CSD in vivo at high spatial and time resolution. This has allowed us to identity the specific brain regions affected by FHM-1 mutations during CSD and our findings indicate that both CSD speed and threshold are differentially affected by action of the pregabalin in wild-type versus FHM-1 mutant mice.

2-C-59 A mouse model of SNCA multiplication

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The identification of several mutations causing familial forms of Parkinson's disease (PD) has led to the development of multiple mouse models reproducing similar genetic alterations. Mutations and multiplications in the alpha synuclein (SNCA) gene have been linked to familial forms of PD. SNCA is ubiquitously expressed in neurons and intraneuronal accumulations of aSyn are the pathological hallmark of the disease. SNCA overexpression in mice reproduced many features of sporadic PD, including motor activity alteration,

alphasynuclein pathology, deficits in nonmotor functions and biochemical and molecular changes similar to those observed in PD. The present study investigates the consequences of human SNCA overexpression in mice, resembling pathogenic multiplication, through a P1-derived artificial chromosome (PAC) vector. Striata from WT and TG mice were processed for WB and probed for aSyn in both the supernatant and pellet fractions. An established set of behavioral paradigms has been used, including open field, novel object location, novel object recognition and cylinder test. Open field measures locomotor activity and anxiety, novel object location and novel object recognition tests assess spatial and recognition memory respectively, and lastly, cylinder test analyzes spontaneous, vertical motor activity. All tests have been performed on two separate cohorts of animals, at 3 and 6 months of age, revealing progressive hyperactivity and anxiety.

The preliminary results support a key role for SNCA in synaptic function and revealed a behavioral phenotype in hSNCA OE mice.

2-C-60 A model of epilepsy based on optogenetic kindling

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To study circuit changes associated with epileptogenesis, we developed a novel optogenetic animal model of epilepsy. We hypothesized that repeated high-frequency stimulation (kindling) pathologically rewires local circuits by excessive recruitment of Hebbian plasticity. We targeted Channelrhodopsin-2 (ChR2) to primary motor cortex (M1) of male C57BL/6J mice by stereotactic delivery of AAV-CaMKIIa-hChR2-E123T/T159C-p2A-EYFP. After 21 days of recovery, we stimulated M1 of awake behaving animals with a 445-nm laser while recording EEG and video. Seizures were not elicited in early stimulation sessions, but gradually emerged in 6 out of 6 animals after ~15 sessions. These seizures were defined as periods longer than 3 seconds where EEG power exceeded a noise threshold. We quantified seizure duration by EEG, and their severity by a modified Racine scale. In a representative animal, we found that the duration (r=0.75, p<0.001) and severity (r=0.77, p<0.001) of seizures increased with session. Seizure onset threshold was also reduced (r=-0.69, p<0.001), and the average number of seizures increased over session (r=0.83, p<0.001). We find that repeated optogenetic stimulation eventually elicits seizures in awake behaving animals. This may occur through repeated recruitment of Hebbian plasticity by driving excessive neuronal activity. Our optogenetic approach is highly cell-specific and has no appreciable inflammation or injury. Our model thus allows the identification of directly activated cells, so that the role of specific cell populations in epileptogenesis can be investigated.

2-C-61 Chronic stress induces anxiety via an amygdalar intracellular cascade that impairs endocannabinoid signaling: identification of a common therapeutic target for metabolic and anxiety disorders

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Collapse of endocannabinoid (eCB) signaling in the amygdala contributes to stress-induced anxiety, but the mechanisms of this effect remain unclear. eCB production is tied to the function of the glutamate receptor mGluR5, itself dependent on tyrosine phosphorylation. Herein, we identify a novel pathway linking eCB regulation of anxiety through phosphorylation of mGluR5. Mice lacking LMO4, an endogenous inhibitor of the tyrosine phosphatase PTP1B, display reduced mGluR5 phosphorylation, eCB signaling and display profound anxiety that is reversed by genetic or pharmacological suppression of amygdalar PTP1B. Chronically stressed mice exhibited elevated plasma corticosterone, decreased LMO4 palmitoylation, elevated PTP1B activity, reduced amygdalar eCB levels and displayed anxiety behaviours that were restored by PTP1B inhibition or by glucocorticoid receptor antagonism. Consistently, corticosterone decreased palmitoylation of LMO4 and its inhibition of PTP1B in neuronal cells. Collectively, these data reveal a stress-responsive

corticosterone-LMO4-PTP1B-mGluR5 cascade that impairs amygdalar eCB signaling and contributes to the development of anxiety. PTP1B is also known to affect metabolic disorders, including obesity and type II diabetes (T2D). Elevated PTP1B activity interferes with leptin and insulin signaling. People with T2D have a higher prevalence of anxiety. Together with our findings, PTP1B is an attractive target for the treatment of metabolic and anxiety disorders.

2-C-62 Neuropathology in APP/PS1 Mice is Exacerbated after CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration)-Induced Traumatic Brain Injury

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Objectives

Traumatic brain injury (TBI) may increase Alzheimer Disease (AD) risk up to 10 folds. Mild TBI, which comprises over 75% of all cases, upon repetitive exposure may lead to long-term development of AD-like neuropathologies. This study investigates if mild repetitive TBI exacerbates neuropathologies in an AD mouse model. Methods: The novel experimental rodent TBI model recently developed by our group - Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) is used to induce closed-head impact-acceleration TBI. We subjected 5-mo male APP/PS1 mice to two mild TBI spaced 24 hours apart. The mice were sacrificed at 2 days post-TBI. Behavioral, histological and biochemical tests were conducted to assess post-TBI outcomes. Results: Immediately after TBI, APP/PS1 mice suffered a prolonged loss of righting reflex compared to sham-operated APP/PS1 mice. By 2 days post-TBI, they showed increased neurological deficits (neurological severity score) and poorer motor coordination (Rotarod) compared to sham-operated APP/PS1 mice. Immunohistochemical staining revealed increased 6E10%2Bve amyloid deposits, microglial activation (Iba-1), argyrophilic fibre (silver staining) and axonal bulb-like structures (phospho-neurofilaments). TBI also induced brain A-beta level. Cerebrovascular changes are being assessed by cell adhesion molecules and tight junction proteins.

Conclusion: These findings suggest that mild repetitive TBI acutely exacerbates neuropathology in APP/PS1 mice. The long term consequence in the trajectory of Alzheimer pathology is being studied.

2-C-63 Chronic Minocycline Treatment Rescues Social Interaction Deficit in Fmr1 KO Mice

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Fragile X syndrome (FXS) is the most common form of inherited intellectual disability. It is caused by a mutation in the Fmr1 gene which leads to silencing of the gene and loss of its gene product, Fragile X Mental Retardation Protein. Minocycline is a tetracycline antibiotic with anti-inflammatory, neurotrophic and neuroprotective properties and has been used as an effective treatment in clinical trials of FXS patients. Using the Fmr1 knockout mice, we examined if chronic minocycline treatment could rescue the behavioral deficit in social interaction in FXS. Minocycline was administered in drinking water to the newborn mice until they reached two months of age, followed by sociability and social preference tests using the three-chamber apparatus. Test mice were introduced to a restrained stimulus mouse (S1) for 10 min to assess sociability, followed by introduction of a second restrained stimulus mouse (S2) for 10 min to assess social preference. Neither Fmr1 knockout nor minocycline treatment affects performance in sociability test. However, Fmr1 knockout significantly decreased social preference to S2 mice as indicated by decreased exploration ratio to S2 mice. Conversely, minocycline treatment effectively increased exploration ratio to S2 mice for Fmr1 knockout mice, without affecting performance in their wild-type littermates. The findings suggest that minocycline may be a promising treatment for improving behavioural deficits in social interaction in FXS.

2-C-64 Stimulatory effects of nACh-R agonist activation on open field locomotor behaviour in a Rett syndrome mouse model.

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Rett syndrome (RTT) results from loss of function of the X-linked transcription factor MECP2. Several studies indicate that cholinergic hypofunction is one of the consequences of MeCP2 loss of function. Acute nicotine (NIC) decreases open field movement (OFM) of wild-type (WT) male and female mice in a dose-dependent manner with reduced movement evident at 1mg/kg and immobility at 2mg/kg. Compared to saline injection, NIC injected symptomatic male RTT mice showed increased OFM injection at 0.5 mg/kg, no difference at 1mg/kg and normal suppression at 2mg/kg. Early symptomatic female het mice showed no significant change in activity following 0.5 or 1 mg/kg with normal immobility at 2mg/kg. QRT-PCR of midbrain tissue of male mice (incl. VTA and S. nigra) showed a 4-fold reduction in α-4 & α-6 nAChR expression. We therefore tested the effect of the selective α-4/6 nAChR agonist TC-2559. TC-2559 suppresses WT-OFM above 1mg/kg with maximal effect at 2.5 mg/kg. Unlike NIC complete locomotor suppression was not produced by doses up to 10 mg/kg. Locomotor paths taken by WT in the test chamber were significantly changed by 2.5 mg/kg TC-2559. They walked more slowly, stopped and turned more often and spent significantly less time in the centre, away from the walls. In contrast RTT mice showed a doubling of average velocity during the 5 min. post injection test period, without changes in locomotor pattern. We conclude reduced nAChR expression in RTT mice results in a reduced inhibitory effect of receptor activation on locomotion revealing a net excitatory effect at submaximal doses.

2-C-65 Extracellular Vesicles from Amyotrophic Lateral Sclerosis Tissue have Misfolded SOD1 Cargo and Are Implicated in Propagation of Protein Misfolding

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons. ALS disease pathology is known to spread spatiotemporally through the neuroaxis from one contiguous area to the next, reminiscent of a prion-like mechanism of propagation. Mutations in the Cu/Zn superoxide dismutase (SOD1) gene are linked to inherited cases of ALS, and misfolded SOD1 is found in neuronal tissues of ALS patients. We have previously shown that cell-to-cell transmission of SOD1 misfolding can occur via the uptake of nanometer sized vesicles collected from misfolded SOD1-expressing cells. However, the mechanism by which misfolded SOD1 is propagated in vivo remains ambiguous. We hypothesized that vesicles found in the extracellular spaces of ALS neuronal tissues would bear misfolded SOD1 and participate in propagation of SOD1. We isolated and characterized extracellular vesicles (EVs) from frozen neuronal tissues of ALS mouse models and human ALS patients. Immunoprecipitation using conformation-specific antibodies that detect misfolded SOD1 showed the enrichment of misfolded SOD1 on ALS tissue-derived EVs compared to control. When the secreted EV-containing fraction from mutant-SOD1 expressing cells was applied onto wild-type cells in culture we observed an induction of SOD1 misfolding, a phenomenon that was abolished by heat-denaturation of the EV-containing fraction. Our results suggest that EVs bearing misfolded SOD1 are competent to induce misfolding of wild-type SOD1, implicating EV dissemination in the propagation of SOD1 misfolding seen in ALS.

2-C-66 Neonatal Odor Learning Impairments Following Prenatal Ethanol Exposure

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Fetal Alcohol Spectrum Disorders, which result when a mother consumes alcohol during pregnancy, are the leading cause of mental disability in North America. These disorders are characterized by a wide array of behavioral abnormalities in executive, motor and sensory function. Simple sensory systems, such as the olfactory system, have been shown to be structurally and functionally damaged by prenatal and early postnatal ethanol exposure (PNEE) and thus may serve as a unique diagnostic tool. Recent work from our laboratory has indicated a relationship between hippocampal synaptic plasticity and reduced levels of the endogenous antioxidant glutathione (GSH). GSH acts as a redox modulator in the brain, and can be depleted by alcohol-induced oxidative stress. The goal of this study was to examine whether olfactory learning in PNEE rodent offspring was impaired early in life and whether these behavioral impairments are paralleled by

alterations in GSH in brain areas responsible for this learning. Offspring exposed to ethanol via a liquid diet, an isocaloric liquid diet or regular chow throughout gestation. We have found that PNEE offspring do not show conditioned odor preferences as observed in offspring of dams fed an isocaloric liquid diet or regular chow. Biochemical analyses were performed on key olfactory structures and found altered GSH levels in these areas. These data indicate a possible relationship in the olfactory system of PNEE offspring with GSH.

2-C-67 CD8+ T cells increase the encephalitogenic potential of CD4+ T cells in a novel mouse model of multiple sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system in which T cells cross the blood-brain barrier, recognize myelin self antigen and mediate an attack against oligodendrocytes resulting in neuronal dysfunction. Approximately 85% of MS patients display a relapsing-remitting (RR) to secondary phase (SP) disease course in which successive relapse and recovery cycles are ultimately followed by a phase of chronically worsening disease. Recent findings suggest that the frequency of cytolytic CD8%2B T cells greatly exceeds that of CD4%2B T cells in acute MS lesions. Experimental autoimmune encephalomyelitis (EAE) is a murine model that recapitulates the immunopathogenesis of MS, and EAE induced in the non-obese diabetic (NOD) strain follows a RR to SP disease course. Our group exploits T cell receptor transgenic 1C6 mice on the NOD background which possesses CD4%2B and CD8%2B T cells that has specificity for the encephalitogenic peptide MOG[35-55]. Here we describe a new model in which 1C6 CD4%2B and CD8%2B T cells, or 1C6 CD4%2B T cells alone, are stimulated in vitro and are adoptively transferred to lymphocyte deficient NODscid mice. We found that transfer of 1C6 Th1 alone induced EAE with lesser severity in NODscid mice as compared to the co-transfer of Th1 and Tc1. The presence of CD8%2B T cells influenced the pathogenicity of CD4%2B T cells as evidenced by their increased production of pro inflammatory cytokines IFNγ, TNFα and IL-2. Thus, this model will help us to better understand the contribution of CD8%2B T cells to CNS autoimmunity.

2-C-68 Can five weeks of arm cycling training improve walking and interlimb coordination in chronic stroke?

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Strong interactions between the upper and lower body are well established across species and recent work has highlighted the implications of these connections for rehabilitation in humans. During walking, interlimb spinal networks regulate gait in healthy individuals and remain at least partially intact after stroke. What remains to be investigated is whether training the arms after stroke will facilitate these networks and result in improved walking. Chronic stroke participants (at least 6 months post infarct) were recruited to a 5 week long (30 min x 3 / wk) arm cycling intervention with a within-subject multiple baseline control. Muscle activation during treadmill walking was assessed via surface electromyography (EMG) and functional walking parameters were assessed through standard clinical tests. Strength in the legs was evaluated via maximum isometric plantarflexion and dorsiflexion. Preliminary results suggest a "normalization" of locomotor EMG in the arms and legs after training. This effect is accompanied by increased strength in the ankle musculature of both legs, particularly the more affected side. Clinical outcomes show improvements in walking speed and an increase in distance walked in the six minute walk test. Taken together, these preliminary results suggest that locomotor training with the arms may have a beneficial effect on walking ability after stroke.

2-C-69 Seizures during brain circuit formation

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The immature brain is exceptionally susceptible to abnormal seizure activity, and in the majority of individuals who develop epilepsy, the onset of spontaneous seizures occurs during childhood. However, it remains unclear whether early-life

seizures affect critical processes on synaptogenesis and how neurons affected by seizures can self-regulate and maintain homeostasis. Using in vivo two-photon time-lapse imaging of growing neurons and calcium imaging of network activity within intact and awake embryonic Xenopus brain, we demonstrate that PTZ-induced seizures lead to destabilization of existing dendritic processes and hyperstabilization of new extensions. These growth changes are persistent, leading to stunted dendritic arbors in mature neurons. We hypothesize that these events are mediated through aberrant activation of intrinsic plasticity mechanisms, not through excitotoxicity.

2-C-70 Arm and leg cycling training improves neurological function and walking ability after stroke

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Rhythmic arm and leg (A&L) movements found in walking, cycling, and stepping share elements of central neural control. Despite these shared connections, the extent to which A&L cycling can lead to training adaptations which transfer to improved walking function remains untested. The purpose of this study was to test the efficacy of A&L cycling training as a modality to improve locomotor function after stroke. Chronic stroke participants (<6 months) were recruited and performed 30 minutes of A&L cycling training three times a week for 5 weeks. Changes in walking function were assessed with: 1) gait analysis (e.g. joint range of motion, muscle activity patterns, etc); 2) neurophysiological measures (e.g. reflex excitability); and 3) clinical tests (e.g timed up and go, 10 m and 6 min walking tests). To test the strength of arm and leg coupling, interlimb cutaneous reflexes (evoked by simultaneous electrical stimulation (5x1.0ms trains@300Hz) to cutaneous nerves innervating the foot and hand) were tested during treadmill walking. Strength during dorsiflexion and plantarflexion was also tested. A multiple baseline (3 pre-tests) within-subject control design was used. Preliminary data show that A&L cycling improves neurological function and walking ability as inferred by changes in gait characteristics, reflex function, ankle strength, and increased distance walked in the six minute walk test. These results suggest that arm and leg cycling could be used as an additional modality to improve interlimb coupling and walking ability after stroke.

2-C-71 Burst-Predicting Neurons Survive an in vitro Excitotoxic Injury Model of Cerebral Ischemia

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Network bursts, defined as transients of increased multi-unit activity (MUA) flanked by silence, are related to the propagation of signals. Recent work suggests that a subset of neurons - termed leaders - are predictive of upcoming network bursts, and may promote information transmission. The loss of leaders as a result of insult may affect the function of the neuronal network. Here, we examined if leaders were more likely than other neurons to survive and remain active after being subjected to an in vitro excitotoxic model of cerebral ischemia, applied for two different durations to cause 'low' and 'high' levels of toxicity, as evaluated in live vs. dead assays or vehicle (control). Dissociated cortical neurons were plated on multi-electrode arrays and, spontaneous electrical activity was first recorded for 20 minutes at 17 days in vitro (DIV); followed by exposure to the insult at DIV 18, and a post-insult recording at DIV 21. Leaders were identified in 17 DIV recordings as electrodes that were active in a 50 ms time window prior to network bursts. MUA rates for leaders did not differ between pre- (17 DIV) and post- (21 DIV) insult recordings (Fig). In contrast, non-leaders showed large positive changes in vehicle and low-kill cultures, suggesting preservation of most neurons, but negative shifts were observed in high-kill cultures, indicating greater numbers of dead neurons unable to contribute to activity (Fig). Thus, leader neurons display heightened resilience to an excitotoxic insult, possibly resulting from pre- and/or post-synaptic signal transduction properties.

2-C-72 Cortical spreading depression induces a transient disruption of the blood-brain barrier prevented by rho-kinase inhibition and associated with increased transcytosis

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The blood-brain barrier (BBB) is a specific property of brain microvessels, which allows maintenance of brain homeostasis. Cerebral endothelial cells form the BBB by virtue of interendothelial tight junctions and restricted transendothelial vesicular trafficking (transcytosis). Cortical spreading depression (CSD) is the electrophysiological correlate of migraine aura but has also been associated with traumatic head injury and stroke. CSD is suspected to disrupt the BBB. Here, we aimed at assessing the integrity of the BBB following CSD, and at exploring the underlying mechanisms in relation to rho-associated kinase (ROCK) which inhibition leads to stroke protection. A total of six CSDs were evoked over an hour by topical application of 300 mM KCl over the right hemisphere in anesthetized mice. BBB disruption was assessed by Evans Blue extravasation in piriform cortices at 6h, 12h and 24h after CSD. To assess BBB morphology, endothelial cell ultrastructure was examined by electron microscopy. Mice were treated with vehicle, isoform non-selective ROCK inhibitor fasudil (10mg/kg), or ROCK2-selective inhibitor KD025 (200 mg/kg). EB leakage was detectable within 6h, and increased over 24h. Both fasudil and KD025 suppressed EB leakage. Transcytosis was enhanced in the ipsilateral hemisphere at 6h, reached a peak around 12h, and returned to baseline levels at 48h. Interendothelial tight junctions appeared unaffected by CSD. These data suggest that CSD-induced BBB transient disruption can be prevented by ROCK inhibition and that transcytosis is the primary mechanism.

2-C-73 A graph theoretical approach to altered resting state fMRI cortical networks in Multiple Sclerosis

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Prior work has emphasized the association between specific lesions seen in multiple sclerosis (MS) and symptoms. However, recent studies suggested widespread disruption of cortical networks in MS, often remote from specific lesions. Here we examined resting state fMRI networks in MS using graph theory concepts. Ten MS patients (9 females, age 38.0±6.9) and 10 normal controls (NC) (5 females, age 31.1±4.75) were recruited. T1-weighted image and 8-minute fMRI scan (TR 2 sec, 240 volumes) were performed. Image preprocessing was performed with in-house scripts including SPM and FSL functions. Cortical parcellation was performed with Freesurfer and 38 cognition-association ROIs were chosen as nodes. Binary connectivity matrices were derived using both partial correlation and simple correlation approaches. With partial correlation, NCs had significantly higher betweenness and node degree than MS subjects in the right fusiform gyrus (p<0.005 and 0.002, respectively). On the other hand, MS subjects demonstrated higher node degree in left postcentral gyrus (p<0.002). With simple correlation, similar to the partial correlation results, control subjects demonstrated higher node degree only in the fusiform gyrus (p<0.0003). Betweenness represents how well a region integrates information from networks, which is an indicator of a "rich hub", and node degree measures the connections passing through the region. Our results imply that both decreased and increased cortical connectivity can be seen in MS, with the increased connectivity likely representing compensatory changes.

2-C-75 A comprehensive database of cell-type specific marker genes for the mammalian brain

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Large-scale gene expression analyses have improved our understanding of the pathophysiology of complex psychiatric and neurodegenerative disorders like schizophrenia and Huntington's. Nevertheless, interpreting changes in gene expression in bulk tissue from human post-mortem brains can be misleading, since such changes may stem from alterations in cellular composition often observed in neuropsychiatric disorders (e.g., loss of dopaminergic cells in Parkinson's). Such changes could potentially be inferred from the expression of cell-type specific marker genes, however, reliable marker genes do not currently exist for the majority of brain cell-types. Here, we develop and apply a computational approach to identify changes in cellular populations based on expression of newly-established cell-type marker genes. We identified

novel marker genes by compiling a brain-wide database of expression datasets from ~35 cell-types from mouse. Using this database, we created lists of marker genes for each of the available cell types. Specifically, we identified previously unknown marker genes (such as Cox6a2 as a marker for cortical fast-spiking basket cells) and show that some of the currently used marker genes are not cell-type specific and/or might not be present in all the cells from the targeted population (like NeuN). By linking these novel mouse marker genes to their human homologs, we show that these genes can further be used to inform cellular proportions in diseased and healthy human brains.

2-C-76 Exercise modulates neural stem cell proliferation in a mouse model of Fragile-X syndrome

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Fragile-X syndrome (FXS) is the most common type of heritable intellectual disability, which affects 1 in 4000 male and 1 in 6000 female Canadians, and is caused by a mutation in the Fmr1 gene encoding Fragile-X-Mental Retardation Protein (FMRP). This transcriptional regulator is involved in mediating neuronal development by modulating progenitor cell proliferation. In its absence, adult hippocampal neurogenesis, a process long known to be crucial for learning and memory function, is significantly reduced. This may contribute to intellectual deficits associated with FXS. Neurogenesis is subject to modulation by many extrinsic factors. Exercise is known to enhance the proliferation of new neurons in the hippocampus, and to improve associated learning and memory function. In order to determine whether exercise can rescue neurogenesis in FXS, we investigated how wheel running affects the proliferation of neural stem cells in the subgranular layer in a mouse model of FXS. After one or four weeks of wheel running, P49 FMRP knockout (KO), true wild type (tWT) and littermate wild type (lWT) were injected twice with BrdU 12 hours apart. They were sacrificed 24 hours later, and proliferating cells were examined using IHC staining of BrdU and proliferation marker Ki67. We found that one week of exercise improved proliferation in tWT, lWT, and KO mice, while four weeks of exercise reduced proliferation in each group. We conclude that shorter duration of exercise reduced proliferation, while longer duration may be detrimental, perhaps due to sustained increase in metabolic load.

2-C-77 Using eye movements to identify early biomarkers of disease progression in Parkinson's patients with and without LRRK2 gene mutations

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In some patients with Parkinson's disease (PD), variations of the Leucine-rich repeat kinase 2 (LRRK2) gene has been associated with the development of the disease. Patients with PD exhibit specific deficits when completing anti-saccades, an inhibitory process that requires looking in the opposite direction of a peripheral visual stimulus. These deficits include longer reaction times, more directional errors (erroneously looking at a stimulus), and hypometric saccades. We employed an interleaved pro- and anti-saccade task to age-matched controls, idiopathic PD patients, and LRRK2 mutation carriers either before (non-manifesting) or after they manifest PD (manifesting carriers). The pro-saccade task (look at the peripheral stimulus) assesses the basic sensory-motor processing of eye movements via automatic tendencies to look at salient visual stimuli, whereas the anti-saccade task assesses the inhibitory control of this automatic response and generation of a voluntary command to look in the opposite direction. We hypothesize that carriers of pathogenic LRRK2 genetic mutations, who have not yet developed parkinsonism, will have anti-saccade deficits similar to PD patients. Preliminary data has revealed that non-manifesting carriers resemble PD patients in terms of performance: they make more direction errors in the anti-saccade task, faster eye movements in the pro-saccade task, and hypometric saccades. Follow up of these results will be important to identify pre-symptomatic behavioural biomarkers of PD that accurately predict disease leading to an earlier detection of PD.

2-C-78 A variant of the presenilin-1 protein protects against an aggressive familial Azlheimer-related mutation

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Alzheimer's disease (AD) is identified by overt symptomatology, at a stage when there is evidence of extensive aggregation (as plaques) of the â-amyloid (Aâ) peptide, a hallmark of AD neuropathology. However, this late-stage in disease progression precludes effective disease management and underscores the crucial need to identify AD in earlier stages. Risk of AD is influenced by factors such as advancing age, sex and genetics. Mutations in presenilin-1 (PS-1) associated with the early-onset/familial form of AD cleave the Amyloid Protein Precursor (APP) to yield the toxic and hydrophobic Aâ42 fragment that is thought to cause AD. During a screen of control and AD brain samples, we identified a splice variant of PS-1 that appears to protect against AD-related amyloid pathology. The relative proportion of the wildtype and splice variant forms of PS-1 can be used as markers for diagnosis of AD. We are in preclinical development of antibodies designed to differentiate these forms of PS-1. Surface plasmon resonance reveals the high-affinity and specificity of these antibodies for their immunizing peptides. Specificity is confirmed by western blot in human brain samples. We hope to validate these antibodies as diagnostic tools for the earliest stages of AD, when treatment options would still provide benefit.

2-C-79 Age-dependent vulnerability to nicotine self-administration in mice correlates with expression of a4* nicotinic receptors

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Greater than 75% of smokers begin before age 20, a developmental period known for susceptibility to substance use disorders. However, it is unknown if the molecular composition of the adolescent brain contributes to their heightened susceptibility to nicotine addiction. Nicotine is the pharmacological component of tobacco and binds to nicotinic acetylcholine receptors (nAChRs) in the brain, the most prevalent being the α4β2 subtype. Different age groups of mice, postnatal day (PND) 40-86 days old, were exposed to a two bottle-choice oral nicotine self-administration paradigm for five days. We found age-dependent changes in the daily dose of nicotine that mice self-administered through oral consumption. Nicotine self-administration was elevated in the PND 44 group, peaked at PND 54-60 and then was drastically lower in the PND 65 through PND 86 groups. Furthermore, a three day abstinence period precipitated a significant surge in nicotine consumption in the PND54-60 mice. We also quantified α4 nAChR expression via confocal imaging of brain slices from α4YFP knock-in mice, in which the α4 nAChR subunit is tagged with a yellow fluorescent protein. Quantitative fluorescence revealed age-specific α4 expression in dopaminergic and GABAergic neurons of the ventral tegmental area that showed a strong positive correlation with daily nicotine dose. These results suggest that α4 nAChR expression levels are age-specific and may contribute to the propensity of individuals to self-administer nicotine.

2-C-80 Determining the Efficacy of Endogenous Stem Cell Based Therapy as a Means to Promote Cognitive Recovery Post-Stroke in Adult Mice

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Current treatments for stroke, a leading cause of death and chronic disability worldwide, are limited in their function and availability. Direct activation of adult neural stem and progenitor cells [collectively termed precursors (NPCs)] using small molecules in the adult mammalian brain may provide a novel self-repair avenue for treating stroke. Our group has previously shown that Cyclosporine A (CsA) has direct pro-survival effects on NPCs in vivo, resulting in a significant increase in the size of NPC pool. Upon systemic CsA administration post-stroke, NPCs migrate to the injury site, promote tissue regeneration and ameliorate motor impairments in two distinct models of sensorimotor stroke. Importantly, CsA administration starting at the time of stroke, as well as 4 days post-stroke, led to functional recovery. Herein, for the first time, we investigated the efficacy of this endogenous cell-based therapy to promote recovery of post-stroke cognitive impairments, particularly executive dysfunctions, which heavily impact rehabilitation outcome and quality of life. In the cognitive stroke model, adult mice received focal, bilateral endothelin-1 induced ischemia in the medial prefrontal cortex (mPFC). Expansion of NPC

population was observed at 4 and 7 days after stroke, similar to observations following sensorimotor stroke. Most importantly, mPFC lesions produced measurable cognitive deficits in the Puzzle Box task, which persisted up to 45 days after stroke. We predict that CsA treatment will repair the injured brain following stroke and contribute to cognitive recovery.

2-C-81 Functional Impairment among children with neurodevelopmental disorders in Abeokuta, Nigeria

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BACKGROUND: Beyond symptomatology, functional impairment has been shown to be an additional dimension in neurodevelopmental disorders which influences the outcome for children with these disorders. Very little is known about such functional impairment among African children. METHODS: Children with neurodevelopmental disorders receiving treatment at the Child and Adolescent Unit of the Neuropsychiatric Hospital, Aro Abeokuta, Nigeria, were assessed with a socio-demographic questionnaire, the Children's Global Assessment Scale and the World Health Organisation Disability Assessment Schedule. RESULTS: Results are presented as frequencies, with descriptive and inferential statistics as appropriate. CONCLUSION: relevant conclusions are drawn from the data on the prevalence and factors associated with functional impairment among children with neurodevelopmental disorders.

2-C-82 Evidence of motor neuron specific misfolded SOD1 in wild type mice

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ALS is a fatal neurodegenerative disease caused by the loss of motor neurons, leading to progressive paralysis and muscle atrophy. Familial cases of ALS (FALS) account for 10% of all cases, defining the remaining 90% as sporadic (SALS). Mutations in superoxide dismutase 1 (SOD1), occurring in 2% of all cases of ALS, cause the protein to misfold resulting in a cytotoxic gain-of-function. Although the exact mechanism is unknown, the cytotoxic misfolding of SOD1 is remarkably specific for motor neurons, despite SOD1 being ubiquitously expressed in many cell types. Under oxidizing conditions, wild type SOD1 misfolds and acquires the same cytotoxic properties as mutant SOD1. Previous work in our laboratory has shown that misfolded SOD1 is immunoprecipitable from spinal cord homogenates from FALS (w/ SOD1 mutations) and from SALS. The phenomenon of wild type SOD1 misfolding in cases of SALS prompts the question of what is happening with the normal SOD1 protein before the protein becomes misfolded and pathogenic. To examine this, misfolded SOD1 was examined in young non-transgenic mice. Fresh frozen spinal cord tissues were analyzed for misfolded SOD1 with different cell-type specific markers. The results show that there is a small but distinct amount of misfolded SOD1 detectable in the dendrites of normal mouse spinal cords. The specificity of the signal to motor neuron dendrites is revealing, given the selective vulnerability of motor neurons in ALS, and is consistent with a role for misfolded SOD1 in the "dying back" of motor neurons in the disease progression of ALS.

2-C-83 Early cell death in oligodendrocytes measured by spectral changes of the fluorescent nuclear dye acridine orange

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Cell death is a feature of all central nervous system trauma and disease. To study cell death, it is possible to model the normal central nervous system cytoarchitecture with culturing tissue slices or short-term maintenance of ex vivo samples. However, the thickness of tissue slices and ex vivo samples preclude the use of common antibody-based approaches to measure cell death in living samples. Deep penetrating cell-impermeant nuclear dyes such as propidium iodide offer one strategy to label dead cells, but these dyes label a very late stage of apoptosis/necrosis and are thus not sensitive to detect early stages of injury. In the hopes of detecting early apoptotic events we measured the spectral changes of a nuclear dye, acridine orange, under normal and pathological conditions in cell culture. A human oligodendroglial cell line and

mouse oligodendrocytes were cultured with or without apoptosis and necrosis inducing agents and stained with acridine orange. Acridine orange emits in the green spectrum when bound to double-stranded DNA and in the red spectrum when bound to RNA or single-stranded DNA. We found that cell death induces a spectral change in cells, with less nuclear red emission. Importantly, the spectral change of acridine orange preceded the entry of a cell impermeant dye, propidium iodide, in agreement with our hypothesis that acridine orange is a sensitive dye for early cellular damage. Imaging of acridine orange could provide a novel strategy to measure cell death in live samples allowing additional mechanistic interrogation of early cell injury.

2-C-84 Blocking the propagation of misfolded SOD1 using small molecules as a potential treatment for ALS

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Amyotrophic lateral sclerosis (ALS) is an incurable motor neuron disease that is associated with the mutation and misfolding of the Cu/Zn superoxide dismutase (SOD1) protein.

We previously reported that mutant forms of SOD1 can induce the misfolding of endogenous wild-type (wt) SOD1 in human neuroblastoma and mesenchymal cells. Misfolded wtSOD1 is secreted extracellularly where it can be transferred to fresh cell cultures to induce further wtSOD1 misfolding. Strikingly, the induction of misfolding by a mutant SOD1 template is restricted by a single tryptophan (Trp) residue at position 32, indicating a possible point of contact between the converting and converted protein species. X-ray crystallography has identified small molecules that bind SOD1 at or near the critical amino acid Trp32. Given the importance of the Trp32 residue to propagated SOD1 misfolding, we hypothesized that small molecules binding at or near the Trp32 site will block this process and mitigate the spread of pathological SOD1 misfolding. Treatment of conditioned media containing misfolded wtSOD1 seed with 25 μM of uridine appears to completely block the induction of SOD1 misfolding in the recipient cells, while 5 μM uridine reduces induction by over 70%. Other structurally similar molecules also reduce the propagation of SOD1 misfolding. We believe that the spread of SOD1 misfolding throughout the neuro-axis plays a central role in the progression of ALS. The inhibition of intercellular transmission and propagation of SOD1 misfolding by these small molecules may represent novel treatments for ALS.

2-C-85 Modulation of ARNT2, a neuroprotective transcription factor, as a regulator of neurodegenerative processes in models of multiple sclerosis

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Multiple sclerosis (MS) is characterized by immune mediated damage, excitotoxicity and oxidative stress. ARNT2 (aryl-hydrocarbon receptor nuclear translocator 2) is a transcription factor that influences neuronal survival/protection. Our objective is to characterize the role of ARNT2 expression and associated changes in neuronal viability/function in MS models. E16-18 cortical cultures were treated with KCl, glutamate or H2O2 to examine their influence on ARNT2 expression. Cell viability was assessed by morphological analysis/lactate dehydrogenase (LDH) release. By 8h, 100mM KCl reduced ARNT2 expression by 90% compared to control, associated with cell swelling/cytotoxicity with LDH release. Glutamate exposure had little effect at low doses, but by 1h of 500µM glutamate exposure, ARNT2 levels decreased by 30%, concurrent with axonal/dendritic retraction. ARNT2 declined to 50% of controls at 24h, associated with toxicity/cell death. At 25, 50, 100, and 300µM H2O2 to model oxidative stress, ARNT2 levels increased up to 30% within 30min exposure compared to control suggesting a protective response to oxidative stress. At 100µM and 300µM, ARNT2 levels declined over time, preceding increases in LDH release and cell death by 24h. This work suggests that increases in ARNT2 expression may contribute to protective responses under certain stresses, while declining expression is associated with loss in cell integrity and viability. This work will characterize the influence of pathological mediators involved in MS on ARNT2, improving current understanding of endogenous neuroprotection.

2-C-86 The comparative predictive value of early treatment response in antipsychotic-naïve patients

with first-episode psychosis: haloperidol versus olanzapine

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Background: It has been proposed that early response to antipsychotic treatment can predict longer term outcome for psychotic patients. However, it is not known to what extent this relationship might apply to antipsychotic-naïve patients, or to affective symptomatology that often accompanies psychosis. Methods: We examined this issue in a cohort of 125 consecutive antipsychotic-naïve, first-episode psychosis inpatients randomized to treatment with haloperidol or olanzapine. All patients were assessed at baseline and three times weekly thereafter using the Brief Psychiatric Rating Scale (BPRS), Young Mania Rating Scale (YMRS), Hamilton Depression Rating Scale (HAM-D), and Hamilton Anxiety Rating Scale (HAM-A). Regression analyses were used to determine whether improvements on these measures at two weeks predicted improvements at discharge. Results: While length of hospital stay and improvement at discharge were similar between treatment groups, the predictive value of early treatment response differed between cohorts. In the haloperidol group, week two improvement was associated with improvement at discharge for BPRS (p=.001), YMRS (p=.003) and HAM-D (p<.001) scores, though not HAM-A scores. In the olanzapine group, week two improvement was not predictive of improvement at discharge for any measure. Conclusions: Early response predicted eventual recovery with haloperidol, but not olanzapine, in first-episode psychotic patients. This information has important implications for physicians deciding whether to switch antipsychotic medications early in treatment.

2-C-87 Longitudinal Magnetic Resonance Spectroscopy Changes in Premanifest Huntington's Disease

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Huntington's Disease (HD) is a fatal disease causeing cognitive, psychiatric and motor dysfunction. Premanifest HD (pre-HD) subjects have a positive genetic test but have not progressed to clinically defined HD. Magnetic resonance spectroscopy was used to investigate brain metabolite concentration changes in pre-HD over 6 years. MR spectra (3T) were acquired of the left putamen in 15 controls and 12 pre-HD subjects annually over 6 years. LCModel was used to estimate metabolite concentrations. A linear mixed effects model for each metabolite including age at baseline and gender as factors was used to compare groups over time. At baseline, mean [tNAA] was lower in pre-HD than in controls (-0.40mM, p=0.04). Mean control [tNAA] and [mI] decreased over time; -0.06mM/yr, p=0.03 and -0.13mM/yr, p=0.004 respectively. Pre-HD subjects experienced an additional decrease over controls of -0.11mM/yr (p=0.007) for [tCr], an increase of 0.02mM/yr (p=0.04) for [tCho], and an increase of 0.16mM/yr (p=0.01) for [mI]. Age and gender had no significant effect on the models except for [Glx], where men displayed higher [Glx] (p=0.004). Although progressive brain metabolic changes have been found in symptomatic HD, this work shows significant differences in putaminal metabolite concentrations with age between pre-HD subjects and controls for the first time. We propose [tNAA], [tCr], and [mI] as biomarkers for progression from pre-HD to manifest HD. In addition, subtle changes in metabolite concentrations over time in controls may provide insight into aging effects in brain metabolism.

2-C-88 Loss of the Huntington disease-associated palmitoylacyltransferase HIP14 in adulthood leads to sudden unexplained death, motor and psychiatric disturbances, and astrogliosis and microglial activation

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Huntington disease (HD) is caused by a CAG expansion in HTT and is characterized by striatal atrophy and motor, cognitive, and psychiatric symptoms. The interaction between mutant HTT and HIP14 (zDHHC17), a palmitoyl acyltransferase for HTT, is disturbed, resulting in reduced palmitoylation of HTT and other HIP14 substrates. Hip14 deficient mice recapitulate many features of HD, including striatal atrophy and motor deficits. However, the

phenotype is developmental, unlike in HD mice and patients. A model of post-developmental loss of HIP14 was generated to examine the role of HIP14 in neurological deficits and neurodegeneration (Hip14F/F;Cre-ERT2). This mouse model allows for Hip14 deletion upon tamoxifen administration at 6-weeks of age. This resulted in a >90% reduction in HIP14 protein and mRNA in Hip14F/F;Cre-ERT2 tamoxifen treated mice (iHip14Δ/Δ). iHip14Δ/Δ mice show dramatically reduced survival due to sudden unexplained death in epilepsy ~10 weeks after loss of Hip14 and at 3 months (~7 weeks after loss of Hip14) show motor deficits, anhedonia, and increased escape response. Electrophysiological analysis suggests that iHip14Δ/Δ mice have striatal dysfunction and an imbalance between excitatory and inhibitory synapses in the hippocampus. iHip14Δ/Δ mice have increased cortical volume, likely due to astrogliosis and microglial activation, and decreased corpus callosum volume. This indicates that loss of Hip14 from conception allows for developmental compensation that cannot take place if Hip14 deficiency occurs in the adult.

2-C-89 Does multi-trauma worsen the outcome of traumatic brain injury?

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Multi-trauma is an international medical problem and commonly involves traumatic brain injury (TBI) and bone fracture. Despite the high incidence of combined TBI and fracture, pre-clinical TBI research commonly employs independent injury models that fail to incorporate the pathophysiological interactions occurring in multi-trauma. Therefore, here we developed a novel mouse model of multi-trauma, and assessed whether bone fracture worsened TBI outcomes. Male mice were assigned into four groups: sham-TBI and sham-fracture (sham); sham-TBI and fracture (FX); TBI and sham-fracture (TBI); and TBI and fracture (MULTI). The injury methods included a weight-drop TBI model and a closed tibial fracture. After a 35-day recovery, mice underwent behavioral testing and MRI. MULTI mice displayed abnormal behaviors in the open field compared to all other groups, had significant motor impairments on the rotarod compared to the sham and TBI groups, and had enlarged ventricles and diffusion abnormalities in compared to all other groups. These changes occurred in the presence of heightened neuroinflammation in MULTI mice at both 24 h and 35 days post-injury. Together these findings indicate that a tibial fracture worsens TBI outcomes, and that an exacerbated inflammatory response may be an important factor contributing to these effects and warrants further investigation.

2-C-90 Improving molecular diagnostic predictions in infantile epileptic encephalopathies using structural modelling of SCN1A

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Epilepsy is a heterogeneous group of neurological disorders characterized by recurrent and unprovoked seizures impacting ~65 million people worldwide. The clinical spectrum ranges from extreme seizures and cognitive delay (Dravet Syndrome) to the more moderate Genetic Epilepsy with Febrile Seizures Plus (GEFS). Genetic testing has revealed >600 variants in SCN1A, the gene encoding the voltage-gated sodium channel Nav1.1. The majority of SCN1A variants are de novo and not previously reported in the pediatric population. Currently, variants are classified as pathogenic using bioinformatic tools, but these reveal little correlation between disease severity and genotype. This drastically limits the application of SCN1A genetic testing in clinical decision making. We hypothesize that clinical severity is related to the altered structural stability of the channel during its transition from the deactivated (closed) to active (open) state, impacting channel biophysics. Homology models of the human Nav1.1 protein in the open and closed conformations were generated using bacterial sodium channel crystal structures as templates. In silico mutagenesis quantified the relative change in energy caused by SCN1A disease-causing amino acid mutations in each state. Mutations were ranked to establish the extent of inherent energetic instability in each state. These energy profiles when integrated with known functional data and clinical phenotypes will improve gene test interpretation, aiding diagnosis, prognosis, and therapeutic intervention.

2-C-91 Synaptic scaling in cultured neurons from the YAC128 mouse model of Huntington disease

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Huntington disease (HD) is a neurodegenerative disorder caused by a polyglutamine expansion in the huntingtin protein, producing mutant huntingtin (mHtt). Many pre- and postsynaptic proteins interact with mHtt, and the function of at least some of these proteins is affected by the disease-causing mutation. One of the consequences of these changes in protein function is alterations in synaptic signaling and plasticity. Particularly affected are the cortico-striatal synapses, especially those between cortical neurons and striatal spiny projection neurons (SPNs). We set out to examine changes in synaptic scaling, a form of homeostatic plasticity in which the strength of synapses in a network is increased or decreased in response to the overall level of activity in the network, in excitatory synapses onto cortical pyramidal neurons and striatal SPNs from the YAC128 HD mouse model. We used immunocytochemical analyses and electrophysiological measurements to determine the effect of 48 hours of treatment with either tetrodotoxin, bicuculline or vehicle (water) on synaptic strength and organization in striatal-cortical cocultures and cortical monocultures from embryonic wild-type (WT) and YAC128 mice. Specifically, we analysed spine density, miniature excitatory postsynaptic current amplitude and frequency, and the distribution of several synaptic proteins. We compare and contrast the responses of our two genotypes in both types of culture to determine whether a deficit in synaptic scaling exists at this early time point in a Huntington disease model.

2-C-92 Modifying lipid rafts promotes regeneration and functional recovery

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Therapies aimed at regeneration of the CNS should promote both neuronal survival and axon growth. Neutralization of the Repulsive guidance molecule a (RGMa) after CNS injuries promotes regeneration. However, its receptor, Neogenin, is a dependence receptor and causes apoptosis in the absence of RGMa, counteracting any positive outcomes. Here, we explore strategies to inhibit Neogenin, thus simultaneously enhancing neuronal survival and axonal regeneration. We show that the recruitment of Neogenin into lipid rafts is mediated through RGMa and bone morphogenic proteins (BMP). We have mapped the domains of RGMa and Neogenin involved in the recruitment into rafts and have shown that this process can be prevented through RGMa/Neogenin peptides, Noggin, or reduction of membrane cholesterol. Blocking Neogenin recruitment into rafts promotes neuronal survival and axonal regeneration in the injured adult optic nerve and restores locomotor function after spinal cord injury. These data reveal a unified strategy to promote both survival and regeneration in the CNS.

2-C-93 Optogenetic stimulation of thalamocortical projections to promote structural plasticity and recovery of function after somatosensory cortex stroke

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Most stroke survivors live with chronic disability, often affecting the upper limbs. Improved use of the stroke-affected limb is generally accompanied by neuroplasticity in intact brain areas surrounding the stroke. Modulating this innate plasticity should promote further gains in recovery. Unpublished data from our lab has shown that while peri-infarct thalamocortical axons are relatively resilient to the effects of ischemia, they lose a significant number of terminaux and en passant boutons in the first few weeks after stroke. Here, we ask whether optogenetic stimulation of peri-infarct thalamocortical projections promotes the re-wiring of axonal boutons and improves behavioural recovery after somatosensory cortex stroke. Thalamocortical axons from the VPL nucleus of the thalamus were transfected with AAV2.CamKII.hChR2(E123A) and a chronic cranial window was implanted over the sensorimotor cortex to allow for longitudinal in vivo two-photon imaging of axon terminals before and after photothrombotic stroke. Optogenetic stimulation was driven by a blue LED magnetically attached to the cranial window and was initiated 3 days after stroke. Optical stimulation or control procedures were administered in awake mice for 1 hour per day, 5 days per week for 6 weeks after stroke. Behavioural performance was assessed once weekly. Changes in axonal branching patterns,

length, and the number of axonal varicosities were analysed. Preliminary data suggest that post-stroke optogenetic stimulation of thalamocortical projections improves stroke-related deficits in tactile sensory function.

2-C-94 Dynamic changes in dendritic spine number in an animal model of Multiple Sclerosis

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Multiple sclerosis, a demyelinating disease with sensory and motor deficits, is associated with behavioural co-morbidities such as neuropsychiatric and cognitive impairments. Inflammation occurring even in the early stages of the disease can alter brain structure and function, leading to these abnormalities, but the mechanism is poorly understood. We investigated if there were changes in dendritic spines using an animal model of MS, called experimental autoimmune encephalomyelitis (EAE). Neurons were labeled using Golgi staining at two timepoints: day (d7), when behavioural changes can be seen, and at d17, where motor deficits are evident. Neurons were imaged with confocal microscopy and analyzed using Imaris software to examine dendritic spines, which form the postsynaptic structure of the synapse. Spine number was increased by 22% (p<0.05) in the principal neurons of the basolateral amygdala at d7, a stage when the animals do not show any sign of paralysis but have increased levels of cytokines in the brain. At d17, the difference in the spine count between the EAE and controls disappeared, indicating there was most likely increased spine loss in EAE. We did not observe changes in the spine density in CA1 pyramidal neurons of the hippocampus at d7, and d17 brains are currently being analyzed. There appears to be a temporal and regional alteration in spine density in the brains of EAE mice. Further analysis, such as electrophysiology, and further characterization of spines will shed light on synaptic function and circuit dynamics that lead to these behavioural changes.

2-C-95 5-HT6 serotonin receptor is a new therapeutic target in Neurofibromatosis type 1: the first GPCR regulated by neurofibromin

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The serotonin 6 receptor (5-HT6R) is almost exclusively localized in the CNS, predominantly in regions involved in the regulation of cognitive processes. Using proteomic approaches, we identified novel signaling proteins associated with the receptor. In this study, we characterize the interaction of the 5-HT6R with Neurofibromin (Nf1), the protein responsible of the most common genetic diseases, type 1 neurofibromatosis (NF1). The phenotype of NF1 is highly variable, with benign (neurofibromas) or malignant peripheral nerve sheath tumors, CNS tumors (gliomas, astrocytomas) and cognitive deficits in 40% of NF1 patients. We first characterized R5-HT6/Nf1 interaction using co-immunoprecipitation: three domains of Nf1 are capable of interacting with the receptor. Then, using Bioluminescence Resonance Energy Transfer (BRET), we demonstrated that PH (Plextrin Homology) domain of Nf1 interacts directly with the R5-HT6 and with the strongest affinity compared to the other domains. In order to explore the role of Nf1 on 5-HT6 receptor-operated signaling, we studied the consequence of down-regulating Nf1 expression on Gs-adenylyl cyclase pathway. Silencing Nf1 expression in primary striatal neurons resulted in reduction of the constitutive activation of the 5-HT6 receptor: the basal level of cAMP induced by 5-HT6 receptor expression was reduced by at least 60% in Nf1 depleted cells. In conclusion, 5-HT6R, which is known to control synaptic plasticity, learning and memory formation and identified as a new partner of Nf1 may constitute an interesting therapeutic target in NF1.

2-C-96 Studying the neuronal activity changes in the motor cortex after deep brain stimulation at the subthalamic nucleus of Parkinson?s disease model rat

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Parkinson's disease (PD) is a neurodegenerative disease of central nervous system, and PD patients generally have many motor problems.

High-frequency deep brain stimulation (DBS) of subthalamic nucleus (STN) is an effective and the most common surgical procedure to sever PD patients. However, the exact mechanism of DBS is still unknown. In this research, we used rats as the model animals to study the mechanisms of STN-DBS. First, we used c-Fos as marker to know which brain areas were activated after STN-DBS, and we observed a group of large and dark c-Fos positive cells (giant c-Fos positive cells) in the layer Vb of the ipsilateral motor cortex after ipsilateral STN-DBS in PD rats, but in the contralateral side of PD rats or normal rats. Second, we used NeuN, GABA and SMI32 to double stain these giant c-Fos positive cells, and we found that these giant c-Fos positive cells were almost pyramidal tract type (PT-type) neurons which sent the signals from layer Vb of the motor cortex to basal ganglia including STN. Resulted from these data we proposed that STN-DBS antidromically excited afferent axons which originated from the PT-type neurons and the therapeutic effects of STN-DBS might be due to, at least partly, by the activation of these afferent axons. Last, we injected Fast blue between the bipolar electrode tips in the STN of PD rats to retrograde label and check the neurons. And then, we observed that the afferent axons we stimulate in STN are originated from the giant c-Fos positive cells we find in layer Vb of the motor cortex.

2-C-98 Glycine rescues impaired hippocampal synaptic plasticity in Female Fmr1 heterozygous knockout mice

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Fragile X Syndrome (FXS), the most common form of inherited intellectual disability,

is caused by over expansion of cytosine-guaine-guanine (CGG) trinucleotide in Fmr1 gene, preventing production of fragile X mental retardation protein (FMRP). FXS affects approximately 1 in 4000 males and 1 in 8000 females. We have previously reported that NMADR co-agonist glycine or D-serine rescued N-methly-D-aspartate receptor (NMDAR)-dependent synaptic plasticity in the dentate gyrus (DG) of Fmr1 knockout male mice. In the current study, we report that female heterzygoze Fmr1 knock out mice displays a DG-specific decrease in long-term potentiation, that can also be rescued by glycine. Whole cell patch clamping showed significant decreases in á-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and NMDAR-mediated currents as well as decreased AMPA/NMDA ratio in the knockout mice when compared to their wildtype littermates. Glycine potentiated NMDAR-mediated currents in both wildtype and knockout mice. The knocout mie displayed learning deficit in a DG-specific catogorial spatial processing task, but did not show learnining deficit in temporal order task and depressive behaviors in forced swim test and tail suspension test. Our findings indicate that impairment in synaptic plasticity in the DG of female FX mice can be rescued by modulating NMDAR activity using its co-agonist glycine, suggesting a potential treatment for improving cognitive impairment in FX patients.

2-C-99 Identification of a Novel Modulator of Apolipoprotein E in Astrocytes

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Apolipoprotien E (apoE) is the most abundant apolipoprotein in the brain, where it is predominantly synthesized by astrocytes and mediates cholesterol transport in the central nervous system. As APOE is the most highly associated susceptibility locus for Alzheimer's disease, modulating apoE levels or function is of therapeutic interest. To identify compounds that increase apoE secretion, we performed a high throughput screen using a molecule library of 104,000 compounds in human CCF-STTG1 astrocytoma cells. A hit compound, CD82, belonging to the pyrethroid ester class of insecticides, was confirmed to increase both expressed and secreted apoE up to 7-fold or greater starting at a concentration of 10μM. Upregulation of apoE was concomitant with an increase in other liver x receptor (LXR) target genes including LXR-α itself, and a robust upregulation of the lipid transporter ABCA1 (9-fold at 30μm). As apoE is transcriptionally regulated though the LXR pathway, we evaluated the requirement of LXR function for the observed effect using mouse embryonic fibroblast deficient in LXR-β or both LXR-

α and -β. Induction of both ABCA1 and apoE mRNA expression by CD82 required LXR activity. Importantly, CD82 shows minimal upregulation of LXR target genes in HepG2 liver carcinoma cells. By contrast, a known LXR agonist, GW3965, induced a clear increase in LXR target genes, including the undesirable upregulation of the transcription factor SREBP1c in HepG2 cells. Ongoing work is focused on evaluating the mechanisms by which CD82 mediates apoE upregulation and the functional outcomes.

2-C-100 Downregulation of MIF by NFκB signaling under hypoxia accelerated neuronal loss during stroke

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Neuronal apoptosis is one of the major causes of post-stroke neurological deficits. Inflammation during the acute phase of stroke results in NFκB undergoing nuclear translocation in affected cells in the infarct area. Macrophage migration inhibitory factor (MIF) promotes cardiomyocyte survival in mice following heart ischemia. However, the role of MIF during stroke remains limited. In this study, we showed that MIF expression is downregulated in the infarct area in the mouse brains. Two functional cis-acting NFκB response elements were identified in the human MIF promoter. Dual activation of hypoxia and NFκB signaling resulted in significant reduction of MIF promoter activity to 0.86±0.01 fold of the control. Furthermore, MIF reduced caspase-3 activation and protected neurons from oxidative stress- and in vitro ischemia/reperfusion-induced apoptosis. H2O2 significantly induced cell death with 12.81±0.58 fold increase of TUNEL-positive cells and overexpression of MIF blocked the H2O2-induced cell death. Disruption of the MIF gene in MIF-knockout mice resulted in caspase-3 activation, neuronal loss and accelerated infarct development during stroke. Our study demonstrates that MIF exerts a neuronal protective effect and downregulation of MIF by NFκB-mediated signaling under hypoxia accelerates neuronal loss during stroke. Our results suggest that MIF is an important molecule for preserving a longer time window for stroke treatment, and strategies to control MIF expression at a therapeutic level could have beneficial effects for stroke patients.

D - Sensory and Motor Systems

2-D-101 Electrophysiological investigation of TMC9 in mechanotransduction processes

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Mechanosensitive ion channels (MSCs) are membrane proteins that conduct ionic currents in response to mechanical stimuli. They are thought to be present in most cell types and underlie processes such as touch, hearing, and vascular homeostasis, but very few MSCs have been cloned. Previous experiments in the lab suggest that TMC9 is a novel non-selective cationic MSC. Here, we investigated the role of TMC9 in mechanotransduction in nociceptors (pain-sensing neurons). These cells are dorsal root ganglion neurons specialized to transduce noxious mechanical forces into electric currents. We tested the hypothesis that TMC9 is involved in this process by recording mechanosensitive currents in dissociated nociceptors. To record those currents, we applied pulses of negative pressure through the recording electrode while recording in the cell-attached patch clamp configuration. Neurons transfected with TMC9 siRNA had significantly reduced mechanosensitive currents compared to mock-transfected neurons, indicating that TMC9 contributes to mechanosensitive currents in nociceptors. To conclude, the present study identified a MSC involved in mechanical pain transduction and, given the ubiquitous expression of TMC9, provides a candidate channel for other mechanotransduction processes where the molecular players are unknown.

2-D-102 Relationship between neck muscle neural control and biomechanics

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The relationship between the biomechanics and neural control of human neck muscles remains unclear. Here we hypothesized that the preferred

activation direction (neural control) and the electrically stimulated moment direction (biomechanics) of neck muscles would align. Eight male subjects sat with their torso constrained and their head rigidly fixed to a 6-axis load cell. Indwelling electrodes were inserted in the right sternocleidomastoid, splenius capitis, and semispinalis capitis. The preferred activation directions were calculated from each muscle's spatial tuning curve, which was estimated using 7.5% of maximum voluntary isometric contractions (MVC) in 26 directions, including the principal directions of flexion, extension, lateral bending, and axial rotation and various combinations. Electrical muscle stimulation was bipolar and used the same indwelling electrodes to deliver currents that generated moment magnitudes similar to those recorded during the voluntary activation. The preferred activation direction and muscle stimulation direction did not align for any of the three muscles (p<0.05). Variability was consistently higher for the preferred activation direction than for the muscle stimulation direction. Our findings show that individual neck muscle biomechanics cannot be directly used to predict neural control. The work also suggests that despite each subject having similar actuators (i.e. neck muscles) available to control head position, their central nervous system uses these actuators in different ways.

2-D-103 Differential Patterns of Projections to the Posterior Auditory Field in Early- and Late-Deaf Cats

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When one sensory modality is absent, compensatory advantages are often observed in remaining modalities. These advantages are thought to result from recruitment of cortical areas that typically process stimuli from the missing modality. For example, evidence from both human and animal studies suggests that auditory areas contribute to enhancements in the visual domain following deafness. Moreover, these changes have been shown to take place predominantly in areas upstream from primary sensory cortices. Indeed, visually-evoked activity has been observed in the posterior auditory field (PAF) in the cat cortex using functional MRI. This current study sought to examine how patterns of thalamo-cortical and cortico-cortical projections to PAF are altered following deafness to determine the anatomical basis of these functional changes. A retrograde neuronal tracer (BDA) was deposited in the PAF of hearing and ototoxically-deafened cats. Coronal sections were taken and neurons showing a positive retrograde labeling were counted and assigned to cortical and thalamic areas according to published criteria. The proportion of labelled neurons in each area was determined in relation to the total number of labeled neurons in the entire brain. Group level analyses of the proportion of labels arising from auditory and non-auditory fields will be discussed to illustrate the effect of hearing loss. Additionally, these changes will be compared to changes in a primary field (A1) to illustrate differences in the capacity for reorganization between primary and non-primary auditory cortex.

2-D-104 In vivo, mesoscale voltage imaging of cortical dynamics as a platform for investigating mouse models of neurodegenerative and psychiatric disease

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Increasingly, functional neuroimaging, principally using fMRI, is being applied to the investigation of psychiatric and neurodegenerative disease to reveal underlying pathologies in brain function and the potential discovery of novel biomarkers. We propose that parallel neuroimaging approaches, using modalities that more directly reflect neuronal activity, in mouse models of psychiatric and neurodegenerative disease presents a complimentary approach that can reveal much about disease pathology not possible in human imaging paradigms alone and may provide a platform for testing therapeutic interventions. We used high-speed (150 Hz), wide-field (8.5x8.5 mm), voltage-sensitive dye (RH1692) imaging to examine spontaneous and sensory-evoked networks of cortical activity in awake and anesthetized C57Bl6 mice. We demonstrate that infraslow (<0.1 Hz) and slow (0.5-6.0 Hz) spontaneous activity can recapitulate analogs of human resting-state networks. In particular, we describe a mouse analog of the Default Mode Network, involving medial cortical structures centered on retrosplenial cortices,

alterations to which, are linked with abnormal brain function. More significantly, our approach also allows for analyses of cortical dynamics such as altered sensory processing, impaired plasticity, and excitation/inhibition imbalance that are common features of disturbed brain function. These approaches along with chronic imaging and the advent of genetically-encoded reporters of neuronal activity hold great promise in furthering our understanding of these complex pathologies.

2-D-105 A combined optogenetic and fMRI approach for the study of cerebellum-to-cerebrum connections

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Existing anatomical and functional evidence suggests both motor and non-motor areas of the cerebrum are influenced by multi-synaptic cerebellar input; however, these correlative pieces of evidence fail to address the functional impact of this cerebellar input. To directly examine this, we combined whole-brain functional imaging with precise manipulation of cerebellar output via in vivo optogenetics. Optic fibers were unilaterally implanted into the cerebella of adult transgenic mice expressing the light-activated outward proton pump Archaerhodopsin (Arch) in Purkinje neurons (PNs), and Blood-Oxygen Level Dependent functional magnetic resonance imaging (BOLD-fMRI) signals were collected using a Bruker 7 Tesla MRI system. Laser-mediated activation of Arch in the cerebellar cortex markedly reduced the intensity of the BOLD-fMRI signal over the area adjacent to the site of fiber implantation (n=3 mice), and these decreases were absent in control mice not expressing Arch. Furthermore, concomitant increases in the BOLD-fMRI signal were detected in both the ipsilateral and the contralateral cerebellar nuclei containing neurons under constant PN inhibition. These results suggest that optogenetic inhibition of PNs induces robust and reliable decreases in BOLD-fMRI signals in the cerebellar cortex as well as triggers disinhibition-mediated signal increases in the cerebellar nuclei. Future experiments will involve a systematic brain-wide inspection and analysis of BOLD-fMRI signal triggered by optogenetic stimulation of the cerebellum.

2-D-106 Neural coding strategies used by the vestibular system are matched to the statistics of natural stimuli

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Efficient processing of incoming sensory input is essential for an organism's survival. A growing body of evidence suggests that sensory systems have developed coding strategies that are constrained by the statistics of the natural environment. Consequently, it is necessary to first characterize neural responses to natural stimuli in order to uncover the coding strategies used by a given sensory system. Here we report for the first time the statistics of vestibular rotational and translational stimuli experienced by rhesus monkeys during natural (e.g. walking, grooming) behaviors. We find that these stimuli can reach intensities as high as 1500 deg/s and 8 G. Recordings from afferents during naturalistic rotational and linear motion further revealed strongly nonlinear responses in the form of rectification and saturation which could not be accurately predicted by traditional linear models of vestibular processing. Accordingly, we used linear-nonlinear cascade models and found that these could accurately predict responses to naturalistic stimuli. Finally, we tested whether the statistics of natural vestibular signals constrain the neural coding strategies used by peripheral afferents. We found that both irregular otolith and semicircular canal afferents, because of their higher sensitivities, were more optimized for processing natural vestibular stimuli as compared to their regular counterparts. Our results thus provide the first evidence that the neural coding strategies used by the vestibular system are matched to the statistics of natural stimuli.

2-D-107 Prolonged cognitive-motor impairments in children with a history of concussion

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We have previously shown impaired cognitive-motor integration (CMI) in asymptomatic adult athletes following concussion. Here we investigate whether the same is seen for still-developing children. Asymptomatic children with concussion history

(n=23, 0.25-40 months post, mean 10; mean age 13.17 yr) and age-matched no-history controls (n=23, mean age 12.30 yr) performed two tasks using a dual-touchscreen laptop in which they had to slide a cursor from a central to a peripheral target using their finger. There was one direct-interaction task where target location and motor action were aligned, and a CMI task where targets were in a different plane from hand motion, and visual feedback was reversed. We observed a significant impairment in both movement timing and trajectory formation with concussion history, and an interaction effect with CMI. Importantly, we observed a significant regression whereby those with a history of concussion did not perform the CMI task at the non-concussed baseline level until 18 months following their concussion. We previously observed similar timing but not trajectory deficits in varsity athletes with concussion history. We suggest that these performance deficits are due to concussion-induced disruptions in the fronto-parietal networks responsible for rule-based movement guidance, networks that are likely vulnerable in the developing brain. The observed prolonged deficits in CMI suggest that current return to sport/school/work assessments that do not test this ability, crucial in sport, are not fully capturing functional abilities post-concussion.

2-D-108 Suppression of vestibulocollic reflexes during head movements

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A key feature of the vestibular system is its ability to distinguish between externally imposed (i.e. passive) and self-generated (i.e. active) movements. During the latter, vestibular signals are suppressed at the earliest stage of vestibular processing. Our understanding of how vestibular suppression affects vestibulocollic reflexes (VCRs) during self-generated movements remains sparse. In this study, we examined the electrically-evoked VCR in humans to assess vestibular processing during externally imposed and self-generated head movements. Subjects actively moved their heads to match an oscillating target at a frequency of 0.5 Hz in pitch, yaw and roll directions against a constant load. The self-generated head movements were recorded and then externally imposed on the subjects using a head motion robot that allowed for both the measurement and application of loads while controlling angular position in three degrees of freedom. We found that the VCR decreased during externally imposed and self-generated movements relative to isometric conditions. For some subjects, the VCR decreased during self-generated relative to externally imposed movements but only when the movement involved activity of homologous muscle pairs, e.g., the VCR in the sternocleidomastoid muscles decreased only during self-generated movements in pitch. These results demonstrate that the VCR is mainly attenuated as a result of head movement, while self-generated head movement has a limited effect.

2-D-109 Switch in the type of t-SNARE protein during trafficking of the transient receptor potential vanilloid 1 (TRPV1) in a model of inflammatory pain

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The transient receptor potential vanilloid 1 (TRPV1) plays an important role in inflammatory pain. The axonal trafficking of this receptor from the dorsal root ganglia (DRGs) to the periphery plays an important role in its availability in the periphery and accordingly pain. In this work, I used male Sprague Dawley rats and gave them intraplantar injection of 5µM of dmPGE2 and then isolated the DRGs, the sciatic nerve and the skin from these rats after 3 and 6 days of injection to extract proteins and do western blot using antibodies for different t-SNAREs. Generally, the availability of a receptor on the surface of the membrane through synaptic vesicles requires one á helix from the v-SNARE and 4 á helices from t-SNAREs. My results show that there is switch in the type of t-SNARE protein during dmPGE2 induced trafficking of TRPV1 from the DRGs to the periphery. In more details, SNAP25 was the t-SNARE involved in the availability of TRPV1 in DRG neurons without involvement on syntaxin-1. In contrast, syntaxin-1, but not SNAP25, is the t-SNARE involved in the availability of TRPV1 in the periphery (sciatic nerve and skin). Accordingly, these results indicate that there is switch in the type of t-SNARE during trafficking of TRPV1. Studying in more details the interaction between TRPV1 and syntaxin-1 (in

dmGE2 injected rats), the role of interaction in pain and inhibiting this interaction can have therapeutic value in finding analgesics in the future.

2-D-110Mere Expectation of Haptic Feedback Facilitates Shift from Pantomimed to Natural Grasp

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Skilled sensorimotor acts like grasping are normally directed at goal objects, programmed using real-time visual input, and entail tactile, proprioceptive, and kinaesthetic sources of haptic feedback. Studies of visual form agnosic patient DF and of healthy participants suggest that 'natural' and pantomimed (simulated) grasps rely on different cortical structures. Work in our laboratory (and others) has shown that denying a tangible object (or a proxy) at the end of the reach (haptic feedback) induces a shift in the response away from 'natural' grasps towards pantomimed ones in blocked trial orders. Interestingly, randomly interleaving trials with and without haptic feedback ameliorates these differences. However, because the trial orders were always either blocked or randomized, one cannot know whether 1) predictive knowledge of haptic feedback on the upcoming trial or 2) the trial history of haptic feedback (i.e. consistency) accounts for these differences. Here, we remedied this confound by organizing trials with and without haptic feedback into blocked, alternating, and two randomized trial orders - one with and one without predictive knowledge of the availability of haptic feedback. Replicating our earlier work, we found that removing haptic feedback induced a shift from natural to pantomimed grasps. Critically, predictive knowledge of the availability of haptic feedback on the upcoming trial potentiated this effect. These findings indicate that the mere expectation of haptic feedback can determine whether a grasp is controlled by dorsal or ventral stream processing.

2-D-111 Cumulative activation effect predicts faster reaction times compared to startle only related activity

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In a simple reaction time (RT) paradigm, a response is known in advance thus allowing for response selection and preparation processes to occur prior to the imperative "go" signal (IS). These processes can be described using a neural activation model, in which neural activity increases until an "ignition" threshold is reached. This model can be probed using a startling acoustic stimulus (SAS), which is known to elicit pre-programmed responses at a shorter latency. The mechanisms of this phenomenon are currently debated; however, a recent study suggested that a cumulative effect of both voluntary and startle-related initiation processes following the IS may be responsible. The purpose of this experiment was to further probe this cumulative effect by presenting a SAS at specific time points following the IS. It is hypothesized that a SAS presented at a short latency following the IS will result in faster RTs than a SAS presented concurrent with the IS, which can only make use of startle-related initiation processes. Participants performed 5 blocks of 20 trials involving a ballistic wrist extension movement. In 20% of trials, a white noise SAS (120 dB) was randomly presented at 0, 12, 24, 36 or 48 ms following the IS (82 dB). Preliminary results have indicated that premotor RTs for control trials were 187 ms, and premotor RTs for SAS trials were 92, 89, 93, 111 and 126 ms respectively. The faster RTs seen at 12 ms compared to at 0 ms lend further support to the cumulative model and demonstrate that the additional activation provided by the SAS increases the rate of activation.

2-D-112 The influence of somatosensory feedback on visuomotor adaptation

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Subjects quickly adapt their movements when aiming in a virtual reality environment in which the visual representation of their hand is rotated relative to their actual hand motion. Specifically, if a visual cursor representing the hand position is rotated 30° clockwise relative to actual hand motion, subjects compensate for the visual distortion and aim counter-clockwise of the target. Importantly, subjects continue to reach with adapted movements (i.e. exhibit aftereffects) in the absence of visual feedback. In the current study we looked to determine if providing additional somatosensory information during reaches would lead to benefits in

reach adaptation. Two groups of participants aimed to targets when (1) the cursor accurately indicated hand position and (2) the cursor was rotated 30 degrees clockwise from the actual hand position. To determine the benefit of enhanced somatosensory information on reaching, one group of subjects was provided with additional haptic feedback at the end of their reaching movements, such that the robot handle they were reaching with vibrated with a frequency of 5 Hz for 1500 milliseconds. All subjects adapted their reaches after training with a rotated cursor. Moreover, adaptation levels were similar across both groups of subjects, suggesting that additional somatosensory information did not benefit reach adaptation. These findings suggest that the CNS may rely more on visual feedback and ignore somatosensory information during visuomotor learning paradigms.

2-D-113 Optogenetic Silencing of Mouse Primary Visual Cortex Affects Orientation Adaptation

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Information processing in the visual system is shaped by recent stimulus history, such that prolonged viewing of an adaptor stimulus can alter the perception of subsequently presented stimuli and modify the visual response properties of neurons in multiple brain areas. In the tilt-aftereffect (TAE), 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 TAE has been described in cat and macaque primary visual cortex (V1), where adaptation produces repulsive shifts in the orientation tuning curves of V1 neurons. We studied orientation adaptation in mice with the hope of using the genetic tools available in this species to shed light on mechanisms underlying these tuning curve shifts. Adaptation in wildtype mice caused orientation tuning curves to shift like those in cats and primates, but also produced a large decrease in responsivity not seen in other species. When the same adaptation paradigm was repeated in transgenic mice that express channelrhodopsin-2 (ChR2) in GABAergic neurons, optogenetic cortical silencing of V1 during adaptation strongly attenuated the decrease in responsivity. This preliminary data suggests that adaptation induced changes in response gain are spike-rate dependent, whereas shifts in orientation preference rely on other mechanisms.

2-D-114 Real-time in vivo measurement of corticostriatal afferent activity during skill learning

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Dynamic changes in cortico-basal ganglia circuits underlie action learning. How changes in discrete, connectivity-specified circuits manifest in vivo during such learning remains unknown. Using in vivo fiber photometry, we assessed real-time activity and plasticity of distinct cortical inputs to the striatum during motor skill learning. Emx1Cre mice expressing Cre recombinase in excitatory cortical neurons were injected with AAVs encoding Cre-dependent GCaMP6s, a calcium indicator, into the motor cortex (M1) or medial prefrontal cortex (mPFC). An optical fiber was implanted into the dorsolateral striatum of M1-injected mice to target sensorimotor corticostriatal inputs, and the medial striatum of mPFC-injected mice to target associative corticostriatal inputs. Activity-dependent fluorescent calcium dynamics were assessed in presynaptic elements of these inputs as a proxy for projection activity as mice trained on the accelerating rotarod. Preliminary work indicates that sensorimotor inputs were engaged by performance on the rotarod, and their activity scaled flexibly with rotarod velocity. Velocity-correlated activity persisted across training, despite a progressive decrease in overall engagement of this pathway. Associative projection activity was also engaged in a velocity-correlated manner during early rotarod performance, but was markedly reduced with extended training. Our work describes a novel approach to observe real-time activity dynamics in discrete corticostriatal inputs, and reveals projection-specific plasticity that likely underlies motor skill learning.

2-D-115 Chloride dysregulation causes disproportionate disinhibition of excitatory interneurons in spinal dorsal horn: implications for neuropathic pain

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Neuropathic pain is a debilitating condition whose symptoms arise from abnormal processing of

somatosensory input. Many features of neuropathic pain are attributable to reduction of synaptic inhibition in the spinal dorsal horn. Disinhibition can arise from reduced GABAergic or glycinergic transmission or from chloride dysregulation caused by hypofunction of the potassium-chloride co-transporter KCC2. The contribution of each disinhibitory mechanism and the effects on network-level processing remain poorly understood. Here we show, based on the differential susceptibility of each disinhibitory mechanism to treatment by acetazolamide, that chloride dysregulation contributes significantly to neuropathic pain. We also show that both excitatory and inhibitory interneurons experience chloride dysregulation but that excitatory interneurons are disproportionately affected because they normally receive more inhibitory input than inhibitory interneurons. Overall, reduction of surround inhibition and the unmasking of subliminal excitatory input fundamentally change how low threshold inputs are processed at the network level, resulting in mechanical allodynia. This work was supported by NIH grant R21 NS074146. SAP is also a Canadian Institutes of Health Research New Investigator and the 53rd Mallinckrodt Scholar.

2-D-116 Subsaccadic FEF microstimulation induces pupil dilation

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The orienting response is an organism's reaction to changes in its environment in order to heighten perception and prepare for action; this can include changes in gaze, attention, and pupil dilation. The frontal eye fields (FEF) are a part of the oculomotor system known to be involved in the generation of voluntary saccadic eye movements and covert shifts in visuo-spatial attention. While microstimulation of the FEF can evoke saccadic gaze shifts, lower levels of stimulation current can modulate components of the orienting response, such as covert attentional shifts, without evoking saccades. Based on recent results showing that pupil dilation can be evoked by subsaccadic stimulation of the superior colliculus in primates, we investigated the effects of subsaccadic FEF microstimulation on pupil dilation. Two non-human primates performed a fixation task in which we stimulated the right FEF with biphasic pulses. In a total of 101 sites, we parametrically varied stimulation currents (5- 60 µA), frequency (50-300 Hz), and duration (30-200 ms). Site-specific vectors ranged from 5 to 20°. In 44% of the sites, we found a significant increase in pupil diameter after subsaccadic stimulation (2-way-ANOVA, p<0.05). The level of the induced response was positively correlated with increasing stimulation factors. Our results imply a contribution of FEF to pupil dilation, presumably mediated through the superior colliculus. They provide an important link for how high-level processes may influence pupil diameter, which could serve as a biomarker for sub-threshold oculo-motor processes.

2-D-117 Postural Threat Influences Vestibular-Evoked Muscular Responses

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While vestibulo-ocular gain has been known to be modulated by arousal and vigilance, the effect of such threat-related factors on vestibulospinal reflexes and related balance responses are less clear. Recent studies using stochastic (Horlsen et al. 2014) and square-wave (Osler et al. 2013) galvanic-vestibular stimulation to examine height-induced effects on vestibular-evoked responses provide conflicting results based on kinetic and kinematic data. The current study used stochastic vestibular stimulation to examine the effect of postural threat on vestibular-evoked muscle responses. 25 subjects stood under 2 conditions. In the 'No-Threat' condition, subjects stood quietly on a stable surface. In the 'Threat' condition, subjects' balance was perturbed by a series of rapid, unpredictable medial-lateral surface tilts. Pre-perturbation periods of quiet stance (5 sec) were concatenated (total=115 sec) and compared to an equivalent time in the No-Threat condition. Surface EMG was recorded from bilateral leg, hip, and trunk muscles. Leg and hip muscles exhibited significant increases in the amplitude of vestibular-evoked muscle responses in the Threat compared to No-Threat condition, and these changes correlated with changes in reported fear of falling and arousal. In contrast, paraspinals revealed decreased peaks in the Threat condition and external obliques showed no change. These findings show a relationship between postural threat and the vestibular-evoked muscle responses and

may provide some insight to muscle specific changes of balance responses in threatening situations.

2-D-118 Is reduced cutaneous sensitivity predictive of weakened synaptic coupling between skin and muscle in the elderly?

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One mechanism for age-related balance impairment could be decreased coupling between cutaneous receptors and postural muscles. A decline in cutaneous sensitivity with age may lead to decreased cutaneous drive to spinal reflex circuits. We explored whether cutaneous reflex coupling is degraded in the elderly, and tested the hypothesis that coupling strength is correlated with sensitivity. We obtained monofilament, and vibratory thresholds at 30 and 250 Hz bilaterally on the medial forefoot. We also measured electromyography (EMG) from Tibialis Anterior (TA) during a low-level contraction while vibratory stimulation at 30 or 250 Hz was applied. To analyze cutaneo-motor coupling, we performed spectral analysis in the frequency (coherence) and time (cumulant density) domain using the probe displacement and EMG waveforms. Elderly adults had elevated pressure and vibratory thresholds, bi-laterally, compared to young adults. With 30 or 250 Hz vibration, we observed corresponding oscillations in the time cumulant, which we argue is the result of synaptic coupling between vibration-sensitive skin receptors and motor neurons innervating TA. At 30 Hz, the elderly adults appear to have both lower coherence, and lower peak-to-peak amplitude in the cumulant density. At 250 Hz, coherence and cumulant density peaks failed to reach significance. While preliminary, our results suggest that reduced sensitivity is accompanied by decreased coupling between cutaneous receptors and primary motor neurons with age.

2-D-119 Asymmetrical medical geniculate body volume in people with one eye

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We have previously shown that people who have lost one eye early in life have enhanced sound localization (Hoover et al., 2011), lack visual dominance (Moro & Steeves, 2011) and integrate auditory and visual information optimally (Moro et al., 2013) compared to binocular and eye-patched viewing controls. Structurally, people with one eye have decreased lateral geniculate nuclei volume (LGN; thalamic visual relay station). However, this decrease is less severe in the LGN contralateral to the remaining eye, indicating altered structural development (Kelly, et al., 2013). The medial geniculate body (MGB; thalamic auditory relay station) plays a central role in auditory processing with both efferent and afferent tracts to primary auditory cortex (Schönwiesner et al., 2007). Given the existing audiovisual processing differences and LGN changes in people with one eye, we investigated whether structural MGB changes are also present. MGB volumes were measured in adults who had undergone early unilateral eye enucleation and were compared to binocularly intact controls using the current gold standard methodology for anatomical localization of the MGB (Devlin, 2006). Unlike controls, people with one eye had a significant asymmetry with a larger MGB volume in the left compared to the right hemisphere, independent of eye of enucleation. The volume asymmetry in the MGB in people with one eye may represent increased interaction between the left MGB and primary auditory cortex as compensation for the loss of one half of the visual inputs early in life.

2-D-120 RAGE-dependent sensitization of sensory neurons innervating airway submucosal glands: possible role in airway hypersecretion

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Neurogenic inflammation contributes to mucus and fluid hypersecretion by submucosal glands (SMGs) in several airway diseases. "Sensory-efferent" pathways, arising from thoracic dorsal root ganglia (DRG), stimulate SMGs and can trigger hypersecretion by neurogenic inflammation. However the mechanisms underlying neurogenic inflammation in the upper airways are poorly understood. The receptor for advanced glycation end-products (RAGE) plays a central role in inflammation in both, sensitization of pain fibers and

allergic airway responses. Therefore, we hypothesize that RAGE contributes to neurogenic inflammation and airway hypersecretion. To test this idea we used DRG primary cultures from wild type (WT) and RAGE knock-out (RAGE KO) mice. To activate inflammatory signaling, cells were exposed to lipopolysaccharide (LPS; 24h, 1ug/mL). Next, we used whole-cell patch-clamp to study capsaicin(CAP)-evoked currents (1µM, 1s) and action potentials (APs) evoked by injection of depolarizing current (100pA, 500 ms). Our data indicates that LPS induced a significant increase in CAP-induced currents (amplitude, density and charge) in DRGs from WT mice. Furthermore, LPS caused a significant increase in the number of APs triggered by 200pA. Remarkably, LPS did not induce any changes on CAP currents or excitability in RAGE KO cells. Taken together our data suggest that RAGE signalling mediates the sensitization of DRG neurons during inflammation, which may play a role on neurogenic hypersecretion in chronic airway disease such as asthma, cystic fibrosis or COPD.

2-D-121 Activation of glutamate receptors in rat dural blood vessels mediates vasodilation

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Background: Glutamate is an excitatory neurotransmitter involved in pain transmission. Blood plasma glutamate concentration is elevated in migraineurs, but how this contributes to their headache is unknown. We hypothesized that glutamate receptors (GluRs) are expressed by afferent fibres that innervate dural blood vessels and that their activation causes vasodilation. Methods: Four male Sprague-Dawley rats were used for histology. Animals were perfused with paraformaldehyde and the dura was extracted. Antibodies were used to assess the expression of GluRs; NMDA (NR2B subunit), AMPA (iGluR1 subunit), kainate (iGluR5-7), and group 1 metabotropic (mGluR5) receptor, in dural afferent fibres and blood vessels. The expression of amino acid transporters 1-3 (EAAT1-3) was also assessed. Tissue samples were visualized with a confocal microscope. Laser Doppler flowmetry was utilized to measure potential changes in dural blood flow evoked by systemic administration of 50mg/kg monosodium glutamate (MSG) in 5 male Sprague-Dawley rats. Results: NMDA, AMPA, kainate, and mGluR5 receptors were expressed in 30.0%, 33.3%, 45.0%, and 13.3% of dural blood vessels, respectively. EAAT2 was the only transporter expressed in the dura. MSG evoked a mean increase of 24.5% in dural blood flow, and this increase was reproducible one hour later with a mean increase of 23.4% following a second administration of MSG. Conclusion: Dural vasodilation evoked by MSG suggests that activation of peripheral GluRs expressed in the dura mater may play a role in mediating headache pain.

2-D-122 Does plasticity in muscle afferent reflex pathways accompany cross-education of the wrist flexors?

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Cross-education refers to bilateral increases in strength induced by training only one side of the body. The adaptations accompanying cross-education have largely been attributed to cortical mechanisms; however, spinal circuitry may also adapt. The purpose of this study was to examine bilateral spinally-mediated adaptations in muscle afferent reflex pathways in wrist flexors after unilateral handgrip training. Eleven neurologically intact, right-handed participants completed 6 weeks of unilateral handgrip training using the dominant hand across 3 sessions of 5 sets of 5 maximal voluntary contractions (MVC) each week. Handgrip MVC force, electromyography (EMG) of wrist flexor and extensor muscles and H-reflex recruitment curves (unconditioned and cutaneous[superficial radial nerve]-conditioned to modulate Group Ia presynaptic inhibition) were assessed in both arms before and after training. Handgrip force increased bilaterally after training providing evidence for effective cross-education. Preliminary analysis revealed that cutaneous-conditioned maximal H-reflexes (Hmax) were greater than unconditioned Hmax, indicating a reduction in presynaptic inhibition during the conditioned response. After training, there was little modulation of conditioned or unconditioned Hmax amplitudes of both the trained and untrained wrist flexors. Since plasticity associated with training may be unevenly distributed to motor unit subtype and excitability, further analysis of possible adaptations at smaller reflex amplitudes, particularly threshold responses, is warranted

2-D-123 a5GABAA receptors mediate tonic inhibition and central sensitization in the dorsal horn of the spinal cord

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The transmission of nociceptive information in the dorsal horn (DH) of the spinal cord is constrained by persistent inhibitory control through the activation of GABAA receptors. The loss of GABAergic inhibition can increase excitability of DH neurons and produce hyperalgesia. Tonic activation of extrasynaptic α5 subunit-containing GABAA (α5GABAA) receptors is known to regulate neuronal excitability in different brain regions. We explored the role of tonic inhibition mediated by α5GABAA receptors in the processing of pain. Immunohistochemical analyses showed that the expression of α5GABAA receptors in the dorsal horn follows a laminar pattern. Whole-cell recordings were obtained from DH neurons located in laminae I and II from α5 subunit null mutant (Gabra5−/−) and wild type (WT) mice. We found no change in either frequency or amplitude of miniature inhibitory postsynaptic currents (mIPSCs), consistent with the extrasynaptic nature of these receptors. Tonic current as measured by the inhibition of tonic GABAergic activity with bicuculline, was decreased only in cells from lamina II of Gabra5−/− mice. In behavioural tests, Gabra5−/− mice exhibited increased responses in the late phase of the formalin test, associated with central sensitization in the DH. However, no difference was found between Gabra5−/− and WT mice in acute or thermal sensitivity, or in mechanical hyperalgesia produced by intraplantar administration of capsaicin. Our data suggest that α5GABAA receptors contribute to central sensitization in the dorsal horn of the spinal cord in a modality specific manner.

2-D-124 Spatial transformations of the vestibular control of standing balance

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The vestibular control of standing balance is tightly coupled with head orientation suggesting a reliance on sensorimotor information regarding head-on-body posture. However, during sustained head-turned postures in which the face is directed over the shoulder, the vestibular response is proposed to be influenced by conscious perception of head orientation. Here, we attempted to determine whether conscious perception influences spatial transformation of the vestibular control of balance during self and externally maintained head-turned postures. Volunteers stood on a force plate with the head turned for a prolonged period and were exposed to bouts of stochastic vestibular stimuli (SVS; 0-20 Hz, duration = 90 s). Vision was occluded for segments of the trial and maintenance of head posture was achieved through an external support or self-generated neck muscle activity. When the head was externally supported, perception of head orientation shifted in the same direction as the vestibular balance response. However, visual recalibration of the head on body position did not affect the balance response direction. In self-maintained postures, perception of head orientation shifted minimally towards a neutral posture but the balance response remained aligned with head position. We propose that sensorimotor signals associated with self and externally maintained head postures predominate the spatial transformations underlying the vestibular control of balance, while perception appears to be a secondary outcome of these factors.

2-D-125 Role of the Cav3-Kv4 complex in mediating synaptic learning in cerebellar granule cells

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Based on sensory information carried by mossy fibre (MF) inputs and related cerebellar outputs, a compartmentalization of function can be found in cerebellar lobules I-X. The granule cell (GrC) layer is the primary input layer of the cerebellum in direct receipt of MF inputs. We know that the subunits responsible for a Cav3-Kv4 interaction are differentially expressed across the lobules, with most influence on GrC excitability in lobule 9. As a result, the functional ability of MF inputs to activate GrCs is lobule-specific. The high input transmission (1 kHz)

at the MF-GrC synaptic relay in cerebellum can induce long term changes in synaptic efficacy through pre- and postsynaptic mechanisms that shape the response to subsequent inputs. We used in vitro slices of rat cerebellum to test the hypothesis that LTP of MF EPSCs can differentially modify Kv4 A-type channel (IA) properties in GrCs across the cerebellar lobules. We found that LTP of MF input induced by pairing theta burst stimulation with postsynaptic depolarization invoked a select leftward shift of IA voltage for inactivation (Vh) only in lobule 9. Moreover, a late long-lasting EPSC present in control conditions in lobule 2 cells was unmasked in lobule 9 cells after blocking the Cav3-Kv4 complex or following LTP of MF input. These results indicate that MF repetitive synaptic input dynamically regulates the biophysical properties of IA in posterior lobule 9 GrCs. Funded through grants from the Canadian Institutes of Health Research (RWT, GWZ).

2-D-126 Natural scene movie responses are more precise in synchronized than desynchronized cat V1

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We recorded spiking responses simultaneously from dozens of single units across all layers of isoflurane-anesthetized cat V1 using silicon polytrodes. Unlike responses to more artificial visual stimuli, responses to short repeated natural scene movie clips consisted of remarkably sparse, temporally precise, reliable events. Each unit had a distinct temporal pattern of such response events, some precise to within as little as 20 ms. Cortical state was quantified by the power ratio of low and high frequency bands of deep-layer local field potential. Cortical state spontaneously switched between synchronized (1/f distribution) and desynchronized (broadband). Contrary to reports in anesthetized rodent cortex [Goard2009,Marguet2011,Zagha2013,Pachitariu2015], responses were more precise and reliable during the synchronized than desynchronized state. This is surprising, because the synchronized state under anesthesia is thought to correspond to quiet wakefulness in awake animals, and the desynchronized state to alert attending periods. Neural responses are known to be more precise and reliable to attended than unattended stimuli. Our results therefore question the analogy between cortical states in anesthetized and awake animals. One possible reason for this conflicting result may be the greater columnar organization of stimulus features in cat V1 than in rodent V1. Travelling waves of activation (UP phases) in the synchronized state may interact differently with incoming stimuli in the two species. This explanation predicts a similar result in anesthetized ferret and primate V1.

2-D-127 The functional organization of local neural networks providing input to single cortical neurons

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Introduction: Many cortical neurons respond selectively to specific cues. For instance, in visual cortex, many neurons respond to specific directions of visual motion. How the response selectivity of a single cortical neuron relates to the selectivity of its local presynaptic partners remains unclear. Methods: Here, we performed single cell initiated, monosynaptically restricted, retrograde transsynaptic tracing with rabies viruses expressing GCaMP6s to image, in vivo, visual motion evoked activity of individual layer 2/3 pyramidal neurons and their presynaptic neuronal networks in primary visual cortex. Results: Using this technique, we were able to visualize > 200 presynaptic cells connected to individual layer 2/3 pyramidal cells of primary visual cortex. The largest subset of presynaptic neurons was in primary visual cortex, and spanned across all cortical layers. Longer range connections could also be consistently found, including inputs from the lateral geniculate nucleus. In addition to mapping the location of presynaptic neurons, we used in vivo calcium imaging to characterize the response properties, to moving visual stimuli, of the electroporated cell (the postsynaptic cell) as well as the response properties of its local presynaptic partners in primary visual cortex. We found the existence of different functional organization principles for networks of presynaptic neurons providing input to individual layer 2/3 pyramidal neurons. Conclusion: Our results indicate the existence of multiple presynaptic network organization principles of layer 2/3 pyramidal neurons.

E - Homeostatic and Neuroendocrine Systems

2-E-128 cFos expression in newborn chicks: relationship to sleep and waking

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cFos is a marker of neuronal activation that has been extensively used to identify sleep- and wake-active areas in mammalian brains. Birds, including chickens, show similar sleep and waking states as mammals (Lesku & Rattenborg 2014). To determine the extent of the similarity in cFos expression patterns between avian and mammalian brain states, cFos expression was examined in sleeping (S), sleep-deprived (SD), and sleep-recovery (SR) newborn chicks using immunohistochemistry. cFos staining was much higher in SD compared to S and SR states in analogous areas to those of awake rats, including the pallium, hippocampus, amygdala, thalamus, septum, hypothalamus, and brainstem areas (Pompeiano et al. 1994). This suggests that the waking state in birds is characterized by a similar pattern of cFos activation as seen in mammals. In order to characterize the role of particular neuronal systems involved in arousal during waking, we next examined cFos expression in orexinergic, noradrenergic locus coeruleus, and serotonergic dorsal raphe neurons using fluorescent double-immunostaining techniques. We found that these populations showed a much higher cFos expression in SD than in S or SR chicks, similar to what has previously been described in mammals (Modirrousta et al. 2005; Maloney et al. 1999). This suggests that the functional regulation of the waking state by these arousal systems may be very similar between birds and mammals. Continuing work is aimed at identifying and studying the cFos activation patterns of sleep-active neurons involved in sleep regulation of newborn chicks.

2-E-129 Hypothalamic CRH neurons are an entry point for a circuit that drives stress coping behavior

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Survival threats require immediate actions, and trigger a distinct repertoire of externally focused defensive behaviours and adjustments in hormonal state. In many species, acute stress is followed by stereotyped, internally focused, repetitive actions that contribute to coping such as grooming, but a cellular entry point into circuitry that links stressful experiences and internally focused behaviour is unknown. Corticotropin releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) launch the endocrine response to stress, but here we show they drive rapid, stereotyped, repetitive grooming behaviour and are also necessary for the expression of grooming observed during recovery from acute stress. This rapid behaviour is initiated through a glutamatergic connection from CRH neurons to hypothalamic perifornical neurons. These findings reveal a new role for these cells as drivers of a circuit for stereotyped behaviour following stress; manipulating this circuit could serve as a new model for understanding core behavioural phenotypes in psychiatric disorders.

2-E-130 The Role of Ghrelin in the Mediation of the Stress Response in Female Mice

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Ghrelin, an orexgenic gut hormone, plays an important role in the metabolic response to animal models under chronic stress.These data, however, has been obtained using males, in spite of multiple studies suggesting that there are sex differences in the stress response as well as in the behavioral responses to exogenous ghrelin administration. This study was conducted to determine if behavioral and metabolic alterations associated with the stress response are also affected in female mice and if ghrelin mediates the magnitude of these effects. We exposed female mice with a targeted deletion of the ghrelin receptor gene (GHSRKO) and their wildtype littermates (GHSRWT) to a chronic unpredictable stress paradigm. Our results show, regardless of genotype, all stressed animals showed greater stress induced depressive -like symptoms. Furthermore, stressed females ate less calories than controls during the stress paradigm, but maintained their body weight. In a second study, females were allowed to recover period following the stress paradigm. As in Experiment 1, stressed females showed significantly more signs of stress- induced depressive like behaviors. Interestingly, while there were no differences in food intake between the groups during the experimental time points, stressed

females ingested more calories during the recovery period and had elevated glucose levels.Taken together, these results suggest that chronic stress causes a number of behavioral and metabolic alterations in female mice, but the role of the ghrelin receptor in these processes is not as well defined.

2-E-131 Sex-specific consequences of neonatal stress on laryngeal chemoreflex stimulation in rat pups: Contribution of excitatory currents onto key brainstem regions

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In preterm infants and rat pups, the presence of liquids or solids in the airways stimulates the laryngeal chemoreflex (LCR). In an immature mammal, LCR stimulation results in prolonged apneas, O2 desaturation and bradycardia with potentially life threatening consequences. LCR afferences arrive at nucleus tractus solitaris (NTS) and go to motor vagus nucleus (NX). NX is involved in effect of LCR on heart rate in young pups. Previous experiments in our laboratory show that neonatal maternal separation (NMS) increases cardiorespiratory responses evolved by LCR. Because neonatal stress interferes with respiratory control development, we tested the hypothesis that NMS has an impact on synaptic inputs converging in these regions. Experiments were performed on 14 and 15 days old pups that were undisturbed (controls) or subjected to NMS (3h/day each day beginning at P3 to P13). Brainstem slices were obtained on anesthetized pups. Spontaneous EPSCs were recorded in NX region. We found that NMS increased amplitude and frequency of spontaneous EPSCs in NX. This research was supported by CIHR.

2-E-132 D2 autoreceptor function is intact after diet induced obesity

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Altered mesolimbic dopamine function is a well-researched adaptation associated with diet-induced obesity. Previous research has demonstrated decreased striatal D2 dopamine receptor (D2R) expression in obese rodents or humans. D2Rs are expressed throughout the striatum at both presynaptic and postsynaptic sites. D2Rs expressed on dopamine terminals act as autoreceptors to limit dopamine release. While diet induced obesity can decrease striatal D2Rs, it is unknown if this alters autoreceptor control of striatal dopamine. Therefore, we investigated whether obesity altered pre-synaptic D2R modulation of dopamine release. Adult Long-Evans rats were given 6-8 weeks of unrestricted access (extended access), 1 hour access (restricted access) or no access (chow-fed) to a cafeteria diet along with ad libitum standard laboratory rat chow. Extended access rats consumed significantly more calories and were heavier than restricted access or chow fed rats after 40 days of food intake. Rats with extended, but not restricted access to a cafeteria diet did not suppress their food intake in the presence of aversive cues. We measured evoked dopamine concentrations before and after quinpirole in striatal slices using fast scan cyclic voltammetry. Quinpirole-induced suppression of dopamine concentration in dorsolateral striatum or nucleus accumbens core was not significantly different amongst extended access, restricted access or chow-fed rats. Taken together, diet induced obesity does not alter autoreceptor control of dopamine release.

F - Cognition and Behavior

2-F-133 Probing procedural strategy with a spatial working memory task: A potential marker of intact frontal function

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When engaging in spatial working memory tasks, in which subjects generate sequences of responses to spatial arrays of stimuli, patients with frontal lobe damage often fail to implement procedural strategies to facilitate memory performance. Conversely, impaired performance among patients with temporal lobe damage is unrelated to strategy use. Assessing spatial working memory in a non-human primate (NHP) affords the ability to parse the contribution of higher executive functioning (e.g., strategy formation) on short-term memory. The present study aimed to examine the spatial working

memory performance of a healthy rhesus macaque across six weeks of task acquisition. The NHP was trained on the self-ordered spatial search (SOSS) task adapted from a human neuropsychological test battery (CANTAB, Cambridge Cognition, Ltd). This touch-screen task required the NHP to produce self-ordered sequences of responses to spatial arrays of 2, 3 or 4 targets. Delay periods (0.1 - 2s) between responses probed short-term memory. The NHP exhibited protracted learning to increases in task difficulty and reduced performance with additional targets and longer delay periods, demonstrating task sensitivity to short-term memory capacity. Analysis revealed evidence for a procedural strategy employed independent of delay duration, which predicted trial accuracy. These results suggest that the SOSS task is capable of modeling not only learning and memory but also higher cognition in an NHP, offering a means of investigating the cognitive systems that are differentially impaired in clinical populations.

2-F-134 Morphine withdrawal critically involves spinal P2X7 receptors

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Opioids, such as morphine, are among the most powerful and widely prescribed analgesics for managing pain. However, their repeated use can lead to opioid physical dependence, which manifests as a withdrawal syndrome upon discontinuing opioid use. Converging evidence suggests that opioid withdrawal is critically mediated by changes in the spinal dorsal horn, which is a primary site of action for opioid analgesia. The present study examines the importance of spinal ATP-gated P2X7 purinoceptors (P2X7R) in the development of morphine physical dependence. We treated rats using a 5-day escalating morphine dosing paradigm and assessed the role of spinal P2X7Rs in morphine physical dependence by intrathecal injections of the selective P2X7R antagonist A740003. On day 5, rats received a single injection of morphine and 2 hours later challenged with an injection of the opioid receptor antagonist, naloxone, to rapidly precipitate morphine withdrawal. We found that morphine treated animals exhibited a robust naloxone precipitated withdrawal syndrome characterized by autonomic and somatic hyperactivity. The severity of withdrawal correlated with increased spinal expression of ionized calcium-binding adaptor molecule (iba-1), a cellular marker of microglial activation, and an upregulation of c-Fos, a marker of neuronal activation. Intrathecal treatment with A740003 significantly attenuated morphine withdrawal, and prevented the increase in iba-1 and c-Fos expression. Collectively, our findings reveal a critical role of spinal P2X7Rs in the development of morphine physical dependence.

2-F-135 Variations in brain activity as a function of hand/target visual feedback availability

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In this study, brain potentials associated with target encoding and movement execution under varying visual conditions were examined using electroencephalography. Specifically, we were interested in examining whether event related potentials (ERP) would differ as a consequence of visual feedback during movement execution. The four visual conditions included vision of hand & target, hand only, target only, and no vision of hand & target. At the start of each trial, a target was previewed, followed by a delay period. Participants executed a movement towards the target location upon hearing an imperative auditory tone. Epochs for ERP analyses were obtained by time locking EEG segments to: 1) target preview, in order to explore if participants encoded the target differently when the upcoming visual condition was known, and 2) movement start, to examine if ERPs varied depending on visual feedback availability of the hand and/or target. While the findings indicate no differences during the preview and encoding of the target; potentials associated with movement execution first occur over motor areas, contralateral to the acting hand, and migrate to medio-frontal regions of the brain. In addition, activity in occipito-parietal regions occurs when vision of the hand (hand & target or hand only conditions) is made available to participants. Additionally, time frequency analyses were performed in order to decipher the frequency band at which brain activity was predominantly occurring and to investigate whether these potentials are related to error detection processes.

2-F-136 Disruption of AMPA receptor endocytosis blocks context-dependent behavioral sensitization to amphetamine

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Drug addiction is often characterized by a strong association between the experience of drug-reward and environmental cues which may be crucial to the persistence of craving and in cue-induced relapse. Behavioral sensitization is the progressive and long-lasting increase in locomotor response following repeated drug exposure and provides an animal model of craving. We previously demonstrated that Tat-GluA23Y, a peptide which disrupts regulated AMPAR endocytosis and LTD, blocked amphetamine-induced sensitization in a single context. Behavioral sensitization is context-dependent, therefore this study included a 2nd distinctive environment to explore the role of regulated AMPAR endocytosis in context-dependent sensitization. Context-dependence was confirmed in control groups, as drug challenge in a novel environment failed to produce sensitization. Importantly, Tat-GluA23Y eliminated the differential response between the conditioned environment and a novel context, indicating an absence of context-dependent sensitization when regulated AMPAR endocytosis is disrupted. Furthermore, when animals were conditioned in 2 different contexts, augmented locomotor responses were still observed but were significantly smaller than in a single context. When Tat-GluA23Y was paired with amphetamine in 1 of the 2 conditioning contexts, the magnitude of the sensitized response was significantly greater in the non-paired context. These results are consistent with 'occasion-setting', when rats are exposed to multiple contexts. This in turn may involve active inhibitory processes mediated by LTD.

2-F-137 Stress and personality interact to modulate the neural response to food cues

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Psychosocial stress is a contributor to weight gain in vulnerable individuals. Here we test the theory that psychosocial stress can lead to over-eating in vulnerable individuals, both by increasing the reward value of foods and interfering with self-control mechanisms. 22 non-obese subjects came in for two scanning sessions: one during a period of low academic stress and the other during the final examination period. We used the Behavioral Inhibition Scale (BIS) to assess individual sensitivity to stress. Participants underwent functional Magnetic Resonance Imaging (fMRI) while viewing food and scenery pictures. All the data were processed using FSL standard methods. Academic final exam period, resulted in higher perceived stress levels measured by Perceived Stress Scale (PSS) ($t(21)=2.27, p=0.03$). BIS scores correlated with the increases in the PSS ($R=0.63, p=0.002$) and with an increased response to food cues in regions that are thought to reflect stimulus value: the right OFC and vmPFC, in the exam minus no exam comparison. vmPFC showed decreased connectivity with inferior frontal gyrus (IFG) as a function of the BIS score. These regions have been implicated in self-control. Stress-vulnerable students (as indexed by the BIS) show increased activation response to food cues in vmPFC and decreased connectivity to IFG during academic stress. We interpret these results as showing enhanced reward value of food cues and reduced self-regulation during stress, which may account for these individuals' increased vulnerability to stress-related weight gain and obesity.

2-F-138 A novel procedure for establishing appetitive latent inhibition that is unaffected by disruption of regulated AMPA receptor endocytosis

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An attentional deficit observed in schizophrenia can be modeled in animals using a latent inhibition (LI) paradigm in which the pre-exposure to an auditory stimulus retards subsequent learning of the association between this conditioned stimulus (CS) and an unconditional stimulus (UCS). Most latent inhibition research is performed using an aversive UCS. In a related study, we observed an enhanced LI effect following administration of an interference peptide, Tat-GluA2-3Y which blocks alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor endocytosis, a critical step in the induction of long-term depression (LTD). The aim of the present study was first to develop a LI protocol using

an appetitive UCS and then assess the possible role of LTD in this form of learning. The active peptide was administered intravenously (2.25 nmol/g) 60 min. prior to the acquisition of a two-way approach response in which an auditory stimulus signaled the availability of food UCS in niches located in both end walls. Rats pre-exposed to the auditory CS required significantly more trials to learn this conditioned approach response than a group never exposed to the auditory CS. Tat-GluA2-3Y failed to disrupt appetitive LI, a finding that may reflect important procedural difference between an appetitive as distinct from an aversive LI paradigm. These data may also indicate that LTD does not play a crucial role in switching the salience of a pre-exposed auditory stimulus to an appetitive conditioned stimulus.

2-F-139 Neural networks in attention and reading

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The processing of printed stimuli is facilitated by the allocation of visuospatial attention and the importance of attentional networks in reading has recently been emphasized. Given this relationship between attentional orienting networks and networks involved with language processing when reading words, we were interested in looking at overlapping networks of activation between these two tasks. To address this we used fMRI to assess the attentional control networks during visual spatial-orienting and the reading networks during lexical (or whole-word) reading of exception words (e.g. yacht) in 16 participants. We employed two verbal tasks: 1) voluntary orienting to central arrow cues with a verbal discrimination task, and 2) reading aloud of exception words. A conjunction fMRI analysis across attentional orienting and exception word reading showed common eye-movement networks including frontal eye fields (FEF), supplementary eye fields (SEF), and intraparietal sulci (IPS), as well as areas in attentional orienting such as superior parietal lobule (SPL), and reading processing (mid-inferior temporal activation). Contrasts revealed that activation in the attention task was greater than activation in the reading task in the temporal parietal junction (TPJ) and a portion of the SPL. These areas are known to be involved in assessment of visual unattended space and control of orienting to objects in unattended space. The reading task showed greater activation in regions typically associated with visual word form and speech.

2-F-140 Hippocampal activity during contextual learning and virtual navigation in non-human primates

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Contextual learning in complex environments requires both exploration and context-dependent association between stimuli and the condition in which they were encountered. Non-human primates are particularly useful for the study of contextual learning, since they: 1) rapidly form associations between stimuli, 2) show contextual learning deficits after hippocampal lesion similar to those observed in humans, and 3) rely on vision when gathering sensory information about their environments. However, there are few studies of single-neuron activity and local field potentials (LFPs) recorded from the primate hippocampus during contextual learning. We used an open-source video game engine (Unreal Engine 3) to create a contextual learning task embedded into a virtual reality environment. Rhesus monkeys freely navigated through the virtual environment using a joystick while we recorded hippocampal activity. We show that 1) hippocampal LFPs show phase-alignment and increases in theta power after visual fixation; 2) hippocampal theta power is elevated prior to the presentation of rewarded objects in the contextual learning task; and 3) single-units form place-specific firing fields in the virtual environment. How these activity patterns evolve over the course of contextual learning continues to be investigated. These results will provide valuable insight in to the neural substrates that support context-dependent associative learning, which may form the basis of other known hippocampal functions like spatial mapping and episodic memory.

2-F-141 Locomotor effects of cocaine are enhanced by ghrelin delivered directly into the Nucleus accumbens

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Ghrelin is a gut hormone that acts in the ventral tegmental area (VTA), a region important for the reward seeking behaviors. Within the VTA, ghrelin increases dopamine (DA) cell activity and it releases into the nucleus accumbens (NAc), an event associated with increased feeding and motivation. Peripheral injections of ghrelin enhance the psychostimulant effects of cocaine, a drug that binds to DA transporters to block reuptake and enhances or prolongs the effects of DA. This suggests that ghrelin could act at presynaptic sites to alter the availability of DA transporters and enhance the effects of cocaine. If this was true, ghrelin delivered into the NAc would have effects similar to those of cocaine, and would enhance the effects of a low dose of cocaine. We examined this by conducting a study where Wistar male rats with a bilateral cannula in the NAc were assigned to one of four groups: GC (ghrelin; cocaine - n=9); GS (ghrelin; saline - n=8); SC (saline; cocaine - n=10) and SS (saline; saline - n=7). Animals were placed in locomotor activity boxes and then received a central injection of either saline or ghrelin (0.5µg/0.5µl). 30 min later, they received an i.p. injection of cocaine (1mg/kg) or saline and had their locomotor activity recorded for the following 90 min. Results show that ghrelin infusion into the NAc increased the number of beam breaks, p<0.05. This effect was potentiated in mice that received ghrelin and then cocaine. These data suggest that ghrelin may act presynaptically onto DA terminals in the NAc to enhance DA release via a decrease in DA reuptake.

2-F-142Remembering in quadrants: Non-linear representation of mnemonic space in the primate brain

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Single neurons in the primate dorsolateral prefrontal cortex (dlPFC) are thought to encode working memory (WM) representations of visual space via sustained firing after the removal of external input. Four decades of research tacitly assume that such spatial representations are homogenous; this assumption has never been quantitatively verified. It is also unknown how correlated variability between neurons affects the fidelity of WM representations. In order to investigate these issues, we used microelectrode arrays to record neural ensemble activity in dlPFC area 8a of two Macaca fasciculares while they performed an oculomotor delayed-response task, which required remembering one of 16 potential locations across the visual field. We found that spatial WM representations are biased in a quadrantic manner: WM representations of stimuli on the opposite side of a visual field meridian from a neuron's preferred WM location are substantially decreased relative to representations of stimuli on the same side of a meridian. This bias is also present in simultaneously-recorded populations, and in the structure of spike count correlations, a measure of correlated variability. Removing these correlations facilitates decoding the contents of WM. Finally, saccades to remembered locations repel away from meridians and attract towards quadrant centers. These results reveal the need to re-conceptualize behavioral and neurobiological models of WM to accommodate the observed non-linearities in mnemonic representations of visual space.

2-F-143 How to achieve and stay at the top percentile: practices pattern analysis from mobile computerized cognitive trainings

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Frequent training with computerized cognitive training games may lead to benefits in cognitive function; however, prior to designing clinical evaluation on how training may lead to transferrable cognitive benefits, we first need to understand the relationship between training frequency and performance within the training tasks. To do this, we analyzed the database from the mobile computerized cognitive training application "Fit Brains", containing 20,660,413 scores generated by 727,390 users from 20-79 years old from twelve cognitive tasks each targeting 1 of 5 categories: executive function, memory, concentration, visiospatial and speed. We evaluated training frequencies associated with attaining and

maintaining scores in the top (>90%), medium (60-80%), and standard (40-60%) level. We found that age is the most important factor for how frequent a user had to train on a particular task to attain and maintain score at the top percentile (90% or higher) in all cognitive tasks. The age effect is the largest for memory and visiospatial tasks, but smallest for executive function. Gender had a small effect on the performance of concentration tasks, but not other tasks. Performance decay occurred fastest for concentration tasks, and second fastest for visiospatial tasks. This study provides recommendations for training frequency specific to tasks and age group that may be effective at attaining and maintaining scores at certain levels. The results may be used to develop training frequencies and task combinations for clinical evaluations.

2-F-144 Sensory Afferents Activated by Gentle Touch Contribute to Self-Grooming and Social Behaviour

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The biological embedding of social experience requires the integration of social information from multiple sensory systems. Touch is a key component of social interactions in mammals, yet its contribution to social experience or animal behaviour is not well understood, owing in large part to the difficulties in manipulating touch sensation. In mice, social touch may be mediated by MrgprB4+ sensory afferents that respond to gentle touch. Here, we characterize the effects of MrgprB4+ afferent activation and ablation in mice that express channelrhodopsin (ChR2) or the diphtheria toxin receptor (DTR) in MrgprB4+ afferents, respectively. The peripheral activation of MrgprB4-ChR2 afferents produced mild behavioural responses that were not indicative of noxious sensation. We observed subtle changes in behaviour after ablation of MrgprB4-DTR afferents with diphtheria toxin (DT). DT-injected MrgprB4-DTR mice exhibited normal locomotor and anxiety behaviour in light/dark box and open-field assays, but reduced self-grooming of the body compared to DT-injected MrgprB4-Cre mice that are insensitive to DT. In a social approach assay, DT-injected MrgprB4-DTR mice spent significantly less time near a novel mouse compared to MrgprB4-Cre mice. These results provide the first evidence for a role of these putative "social touch" MrgprB4+ afferents in self-directed and social behaviour.

2-F-145 Feeling 'blue' and seeing 'red': Associations of emotion and colour in variants of the Stroop task

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In the Stroop task, emotion words like sad (in blue) and angry (in red) have demonstrated congruency effects with colour-naming (eg Sutton & Altarriba, 2008). Other research has not replicated these congruency effects with color-naming, but rather showed that the colour red facilitates categorizing angry emotion relative to grey or blue font color (eg Fetterman et al, 2012). To address these findings, we employed four tasks with mad and sad words in red or blue font color to fully evaluate the association between colour and emotion: 1) Stroop task of color naming; 2) Emotion categorization; 3) A modified Stroop task naming red as 'mad' and blue as 'sad'; 4) A modified emotion categorizing task naming mad as 'red' and sad as 'blue'. The colour-naming and emotion categorizing tasks evaluate any association between colors and emotions, and their direction. The modified tasks are expected to enhance any effects found in the original tasks by highlighting the irrelevant dimension. Our results showed consistent congruency effects in the emotion categorization tasks, and no consistent effects in the Stroop tasks. We also found congruency effects for both mad and sad words in the emotion categorization tasks, extending the link previously shown only between red colour and angry emotion. Overall, these results support a perceptual grounding of emotions in colour over and above semantic association of colour and emotion. FMRI findings using an emotion Stroop task in a patient with intractable depression with a deep brain stimulator placed in the ventral ACC will also be discussed.

2-F-146 A glycine receptor subunit homologue, AVR-14, alters short-term memory in an interstimulus interval-dependent manner in C. Elegans

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Habituation is a learned decrement in responding following repeated exposure to a stimulus. Despite its importance the mechanisms underlying habituation remain largely unknown. Repeated exposure to taps (non-localized mechanosensory stimulation) leads to habituation of a reversal withdrawal response in C. elegans that is dependent on glutamate transmission and postsynaptic AMPA receptors. Here we use high throughput behavioural analysis to characterize the role of AVR-14, an inhibitory glutamate gated chloride channel homologous to vertebrate glycine subunits. avr-14 loss of function mutants display a larger initial reversal duration in response to tap and faster habituation to tap stimuli than wild-type animals at a 10s interstimulus interval (ISI). At long ISIs (60s), avr-14 mutants habituated significantly less than wild-type animals. The stark contrast in phenotypes at short and long ISIs necessitated analysis of habituation across ISIs (10-60s ISIs). This revealed that mutations in avr-14 result in faster habituation at short ISIs, wild-type habituation at intermediate ISIs, and slower habituation and longer ISIs. Together these studies suggest mutations in avr-14 alter habituation in an ISI-dependent manner. Experiments using cell-specific knockdown, rescue, and stimulation will localize the memory functions of AVR-14 to elucidate how it modulates the tap habituation circuit. These studies will determine how the inhibitory functions of glutamate mediate short-term habituation in C. elegans, furthering our understanding of the processes underlying learning and memory.

2-F-147 CaMKII mediates input-specific early odor preference learning in rats

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The synaptic tagging hypothesis explains how input specificity is preserved in long-term potentiation of synapses. This phenomenon requires new proteins that are synthesized in the nuclei, to be shipped cell wide. One specific molecule called calmodulin Kinase II (CaMKII) plays a major role and has been postulated to function as a synaptic tag due to its unique properties such as staying phosphorylated in the absence of calcium. The behavioural relevance of synaptic tagging has not been exclusively studied. Using an early odor-preference learning model, which occurs in a week-old rat pup when a novel odor is paired with a reward, we tested the hypothesis that CaMKII activation is critical for long-term memory and input-specificity of the odor learning. Using behavioral pharmacology, we first tested whether blocking CaMKII with KN- 62 infusion in the olfactory bulb blocks short-term (tested at 3 hr) and long-term memories (tested at 24 hr). Both memories were blocked. To test the role of CaMKII in input specificity of long-term memory, we used a PKA agonist Sp-CAMP to induce 24 hr memory and tested whether blocking CaMKII in this model affects memories for the learned odor (peppermint) as well as a control odor (vanillin). Co-infusion of Sp-CAMP with KN-62 did not impair 24 hr memory for peppermint; however, the input specificity was lost since animals also showed preference to vanillin. These experiments help us understand the specific role of CaMKII in short and long-term odor memories, its role as a synaptic tag, and in memory specificity.

2-F-148 The synthetic tetrahydroprotoberberine d-Govadine facilitates extinction of conditioned place preference induced by d-amphetamine or food reward

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Tetrahydroprotoberberines (THPB) derived from traditional Chinese herbal medicines for the treatment of mental illnesses have high affinity for dopamine D1 and D2 receptors and have potential as novel treatments for drug addiction. This study assessed the effects of the THPB d-Govadine on the acquisition, expression and extinction of amphetamine-induced conditioned place preference (d-AMPH CPP). CPP was established in rats by pairing d-AMPH (1.5mg/kg, i.p.) or saline with a specific environmental context. In separate experiments, rats received d-Govadine (0, 0.5, or 1mg/kg, s.c.) or vehicle, a) 5 min prior to each d-AMPH injection during the conditioning phase or b) immediately before tests for expression of d-AMPH CPP. CPP was assessed as time spent in the d-AMPH- and saline-paired contexts. Although d-Govadine administered during the conditioning phase did not affect the acquisition of d-AMPH CPP, it did facilitate the extinction of amphetamine-induced CPP in a dose dependent manner. In contrast, the expression of d-AMPH CPP was not affected by d-Govadine administered on the test day. In a separate

experiment, administration of d-Govadine (1mg/kg, s.c.) prior to the induction of food CPP facilitated the extinction process. Collectively, these data suggest that d-Govadine affects a learning process that subserves extinction, rather than the addictive properties of d-AMPH itself. Investigations into the effect of d-Govadine on the reinstatement of d-AMPH CPP are ongoing.

2-F-149 Approach-avoidance processing: the role of nucleus accumbens shell D2 receptors in conflict resolution

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The nucleus accumbens (NAc) is importantly implicated in the processing of approach and avoidance signals evoked by emotionally valenced environmental stimuli. Currently, there is evidence to suggest that such opposing motivational processes are differentially mediated by subpopulations of NAc neurons expressing either dopaminergic D1- or D2-receptors (D1R, D2R). Further, it has been suggested that dysregulation of this system is implicated in disorders such as drug addiction, where the individual elicits aberrant processing of competing motivational signals. It is therefore important to further elucidate the mechanisms that mediate approach-avoidance processing in states of motivational conflict. The present study utilized a mix-valenced conditioning paradigm to examine the effects of NAc shell D2R antagonism on approach-avoidance behavior. Male Long Evans rats were trained in a three-arm radial maze to associate visuo-tactile cues with sucrose, shock, or neutral outcomes delivered within the arms in which the cues were presented. Following conditioning, rats were intracerebrally infused with D2R antagonist Sulpiride in the NAc shell. Exploration time was then assessed in a conflict test where rats freely explored two maze arms containing either a neutral cue or a superimposition of the appetitive and aversive cues under extinction conditions. Our results revealed that D2R antagonism enhanced preference for the mix-valenced arm. We conclude that NAc shell D2R is important for suppressing approach behaviors when the valence of the outcome is uncertain.

2-F-150 The effect of reduced neurogenesis on visuo-spatial learning and memory in the GFAP-TK rat.

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Hippocampal adult neurogenesis is associated with visuo-spatial learning and memory ability in rodents, but its exact role is not clear given the growing number of studies that fail to detect cognitive impairments following reductions in neurogenesis. Therefore, we assessed visuo-spatial learning and memory in a transgenic GFAP-thymidine kinase (TK) rat, in which adult neurogenesis was ablated with the drug valgancyclovir. Male TK and WT rats were trained on the Morris water maze (5 days, 10 trials/day) followed by a probe trial to assess memory 10 days later. TK rats performed worse than WT rats on day 1 of training, which was partially due to increased thigmotaxis. However, with further training (days 2-5) TK and WT rats performed similarly and did not differ in memory performance. Given that thigmotaxic behaviour is associated with increased stress/emotionality, we next tested if the impairment in TK animals was dependent on the aversiveness of the maze, by testing rats in a short training protocol (3 days, 4 trials/day) using either cold (16°C) or warm (25°C) water. TK rats again performed worse than WT rats during acquisition training, but learning performance was not dependent on water temperature. During the probe trial, however, there was a trend for impaired memory performance in TK rats in cold but not warm water. Overall these results suggest that reducing neurogenesis in TK rats impairs visuo-spatial learning during the early stages of acquisition, and that impairments in memory performance may be more pronounced when tested under conditions of higher stress.

2-F-151 Catching the same wave: Successful teamwork is linked to between-brain synchrony

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Behavioral research shows that two person teams can perform a visual search task more efficiently

than two individuals working independently (Brennan & Enns, 2014). Here we use EEG hyperscanning with the same behavioral method to measure within- and between-brain phase synchronization in the brains of individuals working as a team or alone. 22 pairs completed team and individual trial-blocks in counterbalanced order. We computed three measures of phase synchronization: Phase-locked-index (PLI; within-brain phase invariance at a single electrode across trials), within-brain phase-locking-value (w-PLV; degree of constancy in phase difference across trials between two electrodes in the same brain) and between-brain phase-locking-value (b-PLV; phase locking between two electrodes in different brains). Partial Least Square analyses (PLS) showed that PLI and b-PLV synchronization patterns were both stronger when individuals performed as a team versus when alone. Follow-up regression analysis on these brain-scores (index of how strongly a pair contributed to a PLS latent variable) showed that b-PLV is more strongly correlated with PLI in the team than in the individual condition. Visual inspection indicates that task-dependent differences occur in frontal and central sites at 2-6 Hz. A positive relationship was found between team success and b-PLV "brain scores", controlling for PLI and block order. These findings establish a functional link between synchronized brain activity and team performance on a visual search task.

2-F-152 Toluene,Hippocampus Structure and Recognition Memory: adult and adolescent rats

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Toluene and toluene-containing volatile substances are the most widely abused solvents with demonstrative addictive potential in humans. Clinical and experimental studies have demonstrated that the exposure to toluene vapor leads to diverse consequences at the level ranging from the cell to the whole organism. The present study has been undertaken to determine whether toluene chronic exposure provokes immediate and/or persistent effect on the structure of hippocampus, learning and memory in adolescent and adult rats. We exposed male Wistar rats at ages P 28-32 (adolescents) and P 150-160 (adults) to 2000 ppm inhaled toluene for 40 days. The immediate and persisting effects of toluene misuse (immediately after the end of toluene chronic inhalation and 90-day after the end of toluene chronic inhalation, correspondingly) on pyramidal cell loss in the CA1 and CA3 of the hippocampus and exploratory behavior and recognition memory in the open field were evaluated. The results reveal that toluene chronic exposure affects the structure of the hippocampus, exploratory activity and recognition memory in the open field in adolescent and adult rats. In all cases the effect is age-dependent. In particular: in adolescent rats the more significant structural and behavioral alterations were observed immediately after toluene chronic exposure, while in adult rats the most considerable was persisting effect (90 days after withdrawal). Such data indicate that character of alterations depends upon the postnatal age of testing of the animals.

2-F-153 Adult neurogenesis increases preference for future rewards

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Neural progenitor cells in the hippocampal dentate gyrus generate new neurons for the dentate gyrus' granule cell layer throughout life. These newborn neurons are known to be involved in memory and mood regulation. Patients with hippocampal atrophy show decreased value of imagined outcomes when they are less detailed. Similarly, depressive patients discount future rewards at a higher rate than healthy controls. Depression is characterized by decreased neurogenesis and reduced hippocampal volume. Thus, we wanted to investigate the effect of reduced hippocampal neurogenesis on cost/benefit decision-making in a delay-discounting task. Therefore, we used hGFAP-TK rats to deplete actively dividing neural progenitors by administering Valganciclovir and consequently stop the production of new neurons. Delay-discounting was assessed in a delay-based decision making task where rats could choose between a low/immediate reward (1 press = 1 pellet) and a high/delayed reward (1 press = 4 pellets), with the delay time increasing over 4 blocks of trials (0sec, 15sec, 30sec and 45sec). Rats with depleted neurogenesis showed a decreased preference for the high reward option with increasing delay times. Our findings indicate that levels of adult neurogenesis could determine the value of future rewards. These findings are

important in understanding future thinking and discounting of delayed rewards in several neurological disorders where neurogenesis is affected, such as depression, addiction or neurodegenerative diseases, where value of future rewards might be decreased.

2-F-154 ERPs differentially reflect automatic and deliberate processing of the functional manipulability of objects

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An object's motor-related properties can influence later memory, particularly when motor aspects of the object were not intentionally attended-to. To better understand this effect we recorded EEG while participants made judgments about images of objects that were either high or low in functional manipulability (e.g., violin vs. vase). Using a between-subjects design, participants judged whether they (a) could manipulate the object using their hand (Functionality [Func]; N=31) or (b) have seen the object in the past three days (Personal Experience [PExp]; N=30). We focused on the P300 and slow-wave ERP waveforms as indexes of attentional allocation. In both groups, we observed higher P300 and slow-wave amplitudes for low-manipulability at both CPz and Pz. At both electrodes, P300 was higher for the Func group. Slow-wave did not differ between groups. A more complex pattern was observed at Cz: In the Func group, high-manipulability elicited a larger P300, but low-manipulability evoked a larger slow-wave. In the PExp group, high- and low-manipulability did not differ in either ERP. As P300 is thought to index attentional recruitment, greater P300 for low-manipulability suggests that they may have received more attention when being processed, and these effects were greater in the Func group. This differential recruitment of attention may have also played an important role in effects of manipulability on memory. These provide neural evidence that effects of manipulability on stimulus processing are further mediated by automatic vs. deliberate motor-related processing.

2-F-155 Effects of an acute bout of moderate-intensity aerobic exercise on motor learning in a continuous tracking task.

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Learning skilled movements is necessary for the successful execution of human motor behaviors. A single bout of high-intensity aerobic exercise facilitates continuous motor skill learning in healthy young adults; yet, this effect has not been examined at lower intensities. In this preliminary report we investigated the effect of acute moderate-intensity exercise on the learning of a continuous tracking task (CTT). Eleven healthy adults (mean±SD: age = 25.8±3.7 yr; height = 177.5±8.7 cm; mass = 70.4±8.7 kg) completed 10 min of CTT practice after 30 min of either seated rest or continuous cycling, at 60% peak aerobic capacity. CTT practice was followed by a 24-hr retention test. Rest and exercise conditions were separated by a ≥2 wk washout period. The CTT contained repeated and random sequences, to evaluate learning versus general improvement in motor skill. Performance was separated into temporal precision and spatial accuracy. A Condition×Sequence×Time rm-ANOVA revealed a significant main effect of Sequence (p=0.035), as well as a significant Condition×Sequence interaction (p=0.004) for temporal precision. Under the rest condition there was reduced temporal error on the random sequence, at both practice and retention. We also discovered a significant main effect of Time (p<0.001) and a significant Sequence×Time interaction (p=0.005) for spatial accuracy - the repeated sequence was learned under both conditions. Results indicate that acute moderate- and high-intensity exercise may have different effects on continuous motor skill learning.

2-F-156 Single-trial Decoding of Visual Attention from Local Field Potentials in the Primate Lateral Prefrontal Cortex

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Studies of attention have shown that the amplitude of local field potential (LFP) oscillations in several brain areas is modulated by visual attention, with

modulations in the gamma frequency band being the most commonly reported. However, it remains unclear whether LFP oscillations contain sufficient information to encode the allocation of attention within ecologically valid timeframes. Here we show that the allocation of attention can be decoded on a single-trial basis from gamma band LFP (60-256 Hz) recorded in prefrontal area 8A of macaque monkeys, but not from lower frequency bands (<60 Hz). Importantly, the information contained in the gamma band is fully redundant with the information contained in the spikes fired by neurons in the vicinity of the recording electrodes. Spikes and gamma LFP are highly similar both at a single-channel and at an ensemble level, allowing cross-signal decoding. Finally, the decoding of attention from gamma frequency bands is very stable across time, twice as much as the spiking signal. Our findings demonstrate that gamma-band LFP recorded in prefrontal area 8A are a potential source of information that can be used by cognitive brain-machine interfaces to signal the allocation of attention on a single-trial basis.

2-F-157 Targeted pharmacogenetic interrogation of a fear memory network

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The coordinated activation of a distributed network of brain regions is necessary to recall a consolidated memory trace. In recent work we used a global mapping approach to identify networks of brain regions co-activated during remote memory recall in mice (Wheeler et al [2013] PLoS Comp Biol). Expression analysis of the activity-regulated gene, c-fos, across 84 brain regions allowed us to identify regions that were co-active following memory recall, and presumably form a network that is engaged by long-term memory recall. Graph theoretical analysis indicated that the remote memory network included several highly-connected hub-like regions that may play privileged roles in memory expression. To address the possibility that these hub regions can play disproportionately important roles in memory consolidation we virally expressed the inhibitory designer receptor exclusively activated by designer drugs (DREADD) HM4Di in different brain regions. When bound to clozapine-N-oxide (CNO), this Gi-coupled DREADD induces membrane hyperpolarization and inhibition of spiking activity. Following contextual fear conditioning, CNO or vehicle was administered via drinking water for 10 days and then fear memory was tested. We independently inactivated 21/84 nodes during memory consolidation and we found that inactivation of hub but not non-hub nodes impaired memory consolidation and that "hubness" can predict consolidation deficits. These data support the idea that highly-connected hub regions play a disproportionately important role in the consolidation of contextual fear memories.

2-F-158 Long-term memory formation is required for training-associated changes in brain structure volume

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Learning and memory formation require alternations to the brain. Initially, learning involves changes in synaptic strength; long-term memory (LTM) formation, however, requires new protein synthesis, enabling structural changes. Recent imaging studies in humans and rodents have provided intriguing evidence that this type of plasticity may be able to be imaged with magnetic resonance imaging (MRI). For example, training mice on a maze induces volume changes in specific brain areas which can be detected with MRI. However, the biological basis of these volume changes, and whether they are driven by the cellular brain plasticity needed for LTM, is unknown. We used mice with a disruption in a key LTM signaling pathway to study whether training-associated volume changes depend on LTM. The transcription factor CREB is critical for LTM. We trained CREB mutant mice (CREB -/-, CREB +/-, & CREB +/+) on either the spatial or non-spatial water maze, and used MRI to detect volumetric brain changes associated with training. All mice learned the task, but CREB -/- mice had significant deficits in spatial memory. Consistent with prior studies, hippocampal volume was greater in spatially trained wildtype mice vs. controls. There was no effect of training on hippocampal volume in the CREB -/- mice, suggesting that the volume changes following spatial learning depend on LTM and CREB-dependent signaling. Intriguingly, spatially trained

CREB +/- mice had normal LTM, but no difference in hippocampal volume. This implies both LTM and CREB dosage underlie the volume changes seen with training.

2-F-173 Effects of pre and post-training administration of glucose and fructose: the importance of non-specific interpretations for memory consolidation

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Drug addiction is well-known, but addiction to other substances like sugar, is less clear. Therefore, to test the sugar addiction hypothesis a framework was applied that hypothesized that the addictive potential of a substance is determined in part by its ability to promote memory consolidation (MC). But, results with MC for sugars are mixed; thus experiments tested the difference in ability to promote MC for glucose (G) and fructose (F). Exp 1 administered G and F (2, 4, 6g/kg) 30 mins prior to self-administration (SA) as a manipulation check. Injections dose dependently reduced intake, as expected, but also reduced locomotion. Exp 2 showed that intragastric infusion of the same doses in the same task had minimal effects on locomotion, which suggested these infusions reduced intake due to a non-specific effect. Exp 3, administered 2g/kg of G and F by intra-gastric infusion 5, 30 and 90 mins prior to SA and found locomotion was significantly reduced at the 5 min time point and was significantly larger for F, while insulin was identical. This confirmed a non-specific effect, not satiety. Experiment 4, which tested 100mg/kg and 2g/kg of G/F administered following a win-shift memory task, found the 2g/kg dose of F significantly increased time to complete the task and reduced pellets eaten, suggesting the previous effects were due to malaise. While 100mg/kg G enhanced MC, this same dose of F did not. This together suggests that high doses may mask effects of MC due to malaise, but low dose G can enhance MC more than F, and thus G may be potentially more addictive than F.

G - Novel Methods and Technology Development

2-G-160 Development of an intravital multi-plane multiphoton microscopy platform for functional cellular imaging in living mice

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Pain sensation is propagated from the periphery to the central nervous system by dorsal root ganglion (DRG) neurons. These neurons detect different stimuli and convey information to the dorsal horn where they form synapses. The project focuses on the development of an intravital multi-plane multiphoton microscopy platform for functional cellular imaging in living mice, to study calcium dynamics of DRG neurons stimulated peripherally. We image DRG neurons labelled with the Ca2+ indicator GCaMP6s directly through a laminectomy and stimulate these with feedback-controlled thermal and mechanical stimulators applied to the paw. Subsequent Ca2+ responses are identified using a home-made software to find responsive neurons in real-time. The platform we developed allows online adjustments of the digital zoom level, image size, spatial sampling, acquisition speed and to trigger or be triggered by external devices; all essential capabilities for live animals physiological experiments. The sensory-evoked activity from DRG neurons stimulated peripherally is recorded at video-rate, and multi-plane capability is ensured by mounting the objective on a piezoelectric actuator allowing nearly whole-DRG functional microscopy. Because animals' movement can seriously degrade acquisitions, we register the resulting movies offline using graphic card acceleration. Also, we developed an automated pipeline for data processing in addition to a database in the context of Big Data. The system we built is therefore tailored to fit the specific needs of in vivo whole-organ functional microscopy.

2-G-161 Self-directed, high-throughput, and automated mouse motor-learning home cage assays

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Automated assessment of rodent behaviour is desired as it reduces experimenter bias, facilitates

high throughput, avoids disrupting circadian rhythms and eliminates stress in animals introduced by experimenter handling. We developed an automated mouse lever pulling task that mice can perform 24/7 in the home cage to assess forelimb function. The apparatus consists of a small chamber attached to the outside of the home cage, which contains a recessed lever that the mice can access with their forelimb. The lever is attached to the axle of a rotary encoder allowing us to measure lever position during each pulling event. A sensor on the roof of the training compartment detects a unique RFID tag that is implanted subcutaneously in each mouse at the nape of the neck. Upon successful pulling of the lever a solenoid dispenses a ~10uL water drop. In the initial phase of training mice receive a water reward for just entering the training compartment, while subsequent phases require the mice to hold the lever in a rewarded position for incrementally longer durations and a smaller range of movement. Individualized performance is logged and task parameters are controlled by custom software written in Python running on a Raspberry Pi. Preliminary data show that mice perform over 500 trials once they have acquired the task. The number of successful trials increased during the first week of training, and on average it takes ~7 days of unsupervised training for mice to acquire a 1 second hold duration. Future experiments will introduce lever perturbations to assess voluntary motor control.

2-G-162 Assessing connectivity in real neuronal networks from cellular activity

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Getting the ability to assess connectivity maps of neuronal networks is a crucial step toward understanding the rules that govern information encoding, circuit formation, and learning. Current light microcopy technologies allow to record calcium activity of large neuronal networks, but the evaluation of the connections, from activity data only, remains a challenging problem. Yet, statistical methods for the inference of connectivity already exist, such as cross-correlations, Granger Causality, and Transfer Entropy (TE). In order to compare these methods based on their accuracy and numerical cost, we generated activity data from computer simulations of populations of 10 to 500 neurons and tested the inferred connections against those used for the simulations. We observed that repeated stimulations of selected neurons drastically increase the accuracy, especially with TE. We also applied the inference methods to in vitro data. We infected rat hippocampal cultures with gCamP6, a fluorescent marker for intracellular calcium. Then, we electrically and selectively stimulated one neuron in the culture while measuring the calcium response in the adjacent neurons, where we were able to detect calcium transients. Together these results indicate that a new strategy of connectivity inference, which combines TE and neuronal stimulation, could be both theoretically acceptable and experimentally achievable. Further development of this strategy will allow to test the formation/removal of connections under different paradigms of development, learning, and pathological conditions.

2-G-163 MiniPromoters Driving PAX6-like Retinal Expression Designed from Bioinformatically Predicted Regulatory Regions

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Purpose: Although gene therapies may benefit from tissue- and cell-type specific promoters, capturing the expression of a human gene in a small MiniPromoter (MiniP) is challenging. Paired box six (PAX6) causes the vision-loss disorder aniridia when mutated, and has a complex retinal expression pattern driven by discrete regulatory regions (RRs). Here we describe the bioinformatically driven design of MiniPs from the PAX6 gene. Methods: PAX6 RRs were predicted bioinformatically. MiniPs were cloned into AAV genomes containing emGFP and WPRE, packaged into AAV2(Y272F, Y444F, Y500F, Y730F, T491V), and injected intravitreally into postnatal day 14 mice. Eyes were collected 30 days after injection and emGFP expression was evaluated by confocal microscopy. MiniPs driving PAX6-like expression were examined for unique transcription factor binding sites (TFBSs) using oPOSSUM3. Results: At the PAX6 locus, 31 RRs were bioinformatically predicted and 9 were used to

construct 7 MiniPs. Two of the MiniPs did not drive interesting expression in the retina and 5 drove expression in the 2 retinal layers that express PAX6 (ganglion cell and inner nuclear). Unique to these 5 MiniPs were 21 TFBSs. One MiniP (Ple255) expressed in all three cell types that express PAX6 (ganglion, amacrine, and horizontal). Conclusions: MiniPs that recapitulate the tissue-specific endogenous expression of a gene can be designed using bioinformatic approaches. Such MiniPs may be of general use in the study of the retina, and Ple255 in particular could be an important element in a PAX6 gene therapy for aniridia.

2-G-164 Localized light-induced stimulation of hippocampal neurons with cell surface?bound gold nanoparticles

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Light-induced stimulation of whole neurons or neuronal networks with optogenetic tools is a well-established and powerful approach to study circuit function. On the other hand, local membrane stimulation at the nm to µm scale, which would be useful to study the cellular rules governing neuronal and dendritic functions, remains a challenge. To address this problem we used off-resonance plasmonic excitation of 100 nm gold nanoparticles (NPs) bound to cultured rat hippocampal neurons. While non-specific binding of NPs onto the neurons was possible through passive sedimentation, we designed a specific binding approach via surface functionalization of the NPs with antibodies targeting membrane receptors. To induce plasmonic excitation of the NPs, we applied femtosecond laser pulses at 800 nm in regions of interest on somato-dendritic domains with one or more NPs. We monitored the neuronal response with confocal imaging of Ca2+ fluctuations using GCaMP6 and with patch clamping. We were able to induce localized or widespread Ca2+ elevation inside the neurons, depending on i) laser intensity, ii) area of illumination, or iii) number of functionalized NPs on targeted cells. We also observed light-induced whole-cell electrical response. Furthermore, we show variable levels of subcellular translocation of the GFP-tagged Ca2+ signal decoder CaMKII. Our results suggest that localized, controlled light-based stimulation of a single functionalized NP on neurons can be used to characterize the impact of local or widespread Ca2+ transients on downstream signaling.

2-G-165 A Cortical Thickness Gowth Model for Neurodegenerative Analysis

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Widespread decline in cortical thickness across the cerebral cortex have been observed during normal aging and in subjects with cognitive impairment. We propose a vertex-wise cortical thickness growth model to analyze the dynamic of cortical thickness across the cerebral cortex. The growth model is formulated as a series of differential equations using the subject's age and local geometrical structure of cortical surface, based on cortical thicknesses extracted from three longitudinal MRI scans (about 6 months inter-scan interval). This study focused on 20 mild cognitive impairment (MCI) (age 72.7±2.3 years) and 20 matched cognitive normal (CN) subjects (age 70.2±2.8 years) from ADNI cohort. Whist a mixed of increasing and declining cortical thickness change rates were detected across the entire cortex in both MCI and CN subjects, MCI subjects exhibited greater cortical thickness absolute change rates (0.11±0.02mm vs 0.07±0.02mmm per year) over CN subjects. In addition, MCI subjects showed higher absolute change rate on temporal lobe (0.13±0.02mm vs 0.09±0.02mm per year in CN) and cingulate cortex (0.11±0.02mm vs 0.08±0.02mm per year in CN). This sequence of changes corresponded to in vivo observation of cortical atrophy during degenerative process. With a natural exponentially growth trajectory, our proposed growth model enables estimation of cortical thickness change rate within a continuous time frame. It allows vertex-wise and region-wise analysis of cortical thickness development for individual and population study.

2-G-166 NeuroFluor CDr3: A Novel Tool for the Detection of Live CNS and Human Pluripotent Stem Cell-Derived Neural Stem and Progenitor Cells

Carmen Mak[1], Vivian Lee[1], Sam Lloyd-Burton[1], Allen Eaves[2], Terry Thomas[1], Sharon Louis[1]

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Conventional methods for identifying neural cell types are heavily dependent upon antibodies and mRNA probes. These approaches are labour-intensive, often require optimization of individual reagents, and frequently require fixation and permeabilization steps that limit further use of the cells. We have developed NeuroFluor CDr3, a fluorescent membrane-permeant probe that selectively labels live neural progenitor cells (NPCs). NeuroFluor CDr3 is based on the boron-dipyrromethane (BODIPY) derivative compound CDr3, which specifically binds to the NPC marker fatty acid binding protein 7 (FABP7, also known as brain lipid binding protein, BLBP; Yun et al. 2012; Leong et al. 2013). Here, we demonstrate that NeuroFluor CDr3 labels NPCs from the rodent central nervous system (CNS) and further demonstrate its application in the detection of human pluripotent stem cell (hPSC)-derived NPCs. NeuroFluor CDr3 labeled 99% of cells in NPC cultures (97% Nestin%2B) generated from the adult mouse subventricular zone or the E18 rat cortex, as assessed by flow cytometry. It also labeled Nestin%2B hPSC-derived NPCs within neural rosette structures, but not surrounding non-NPC cells, and also labeled single NPCs in subsequent passages. Together, our findings indicate that NeuroFluor CDr3 is a simple and effective detection tool for identifying rodent CNS- and hPSC-derived NPCs in live cultures. NeuroFluor CDr3 is compatible with flow cytometry and can be used to enrich NPCs directly from live cultures and tissue samples.

2-G-167 Effective Gene Silencing in Brain and Spinal Cord In Vivo Models Mediated by Lipid Nanoparticle Technology

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Lipid nanoparticles (LNP) are the leading systems for in vivo delivery of short interfering RNA (siRNA) for therapeutic applications. The lack of an appropriate protocol to administer LNP into the brain and spinal cord has limited the investigation of their potency to silence neuronal genes in vivo. Here, we attempt to bridge this gap in neuroscience research by describing the use of siRNA-LNP to efficiently knockdown gene expression in brain and, for the first time, in spinal cord in vivo models. The effectiveness of the siRNA-LNP system was assessed in the brain by direct injection into the cortex. LNPs were seen to be present in cortical neurons, where delivery of the siRNA against PTEN exerted the desired changes in gene expression. Subsequently, LNPs were injected at the site of cervical spinal cord injury and a decrease in expression of the target PTEN was observed in the vicinity of the injury/injection site 10 days later. Moreover, the presence of LNPs in the neurons of the red nucleus located in the brainstem, suggests that the lipid nanoparticles are taken up by axons and retrogradely transported. With no previous reports on this phenomenon, this study reflects the potential of siRNA-LNPs in advancing our understanding of the central nervous system. Furthermore, the ability of this relatively novel siRNA-delivering LNP technology to successfully affect gene silencing in brain and spinal cord in vivo, presents a promising prospect for the development of new gene therapies to treat neurological disorders.

2-G-168 Neuromodulatory opto-fMRI

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Visualising the functional connectivity of specific neural circuits in real-time would represent a new opportunity in neuroscience and medicine to probe how predefined circuits are altered as a function of life experience and pharmacological treatment. Here we present the development of a whole-brain readout of functional serotonergic connectivity in the mouse originating from the dorsal raphe. The method is achieved by merging serotonergic neuron-restricted deep-brain optogenetics and small animal fMRI. We demonstrate the feasibility and specificity of the system, provide a mechanistic explanation for the observed fMRI readout at the level of brain oscillations and neuronal spiking, and find specific changes in serotonergic functional connectivity as a consequence of behavioural experience.

2-G-169 A deterministic, rapid-access microscope and monitoring system for high-throughput data acquisition of neuron activity in the awake brain

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In this work, we describe a novel two-photon, laser-scanning, rapid-access microscope, combined with calcium imaging, to investigate how sensory activity is temporally and spatially coordinated within the full, dendritic arbor of single neurons located in the optic tectum of the awake brain of a Xenopus Laevis. Random-access microscopy, is a burgeoning technology, and cutting edge instrumentation is used for high-throughput data acquisition making it possible to capture activity throughout the arbor providing real-time feedback indicating computational states of the neuron. This versatile technology features a highly coordinated combination of optics, sensors and mechanical actuators for data-acquisition at ultra-fast rates. Our system achieves high rates of data acquisition by combining a new custom, deterministic, software system with a hardware platform designed using high-speed, piezo-actuator Z-stage and acoustic-optic deflectors to scan preselected and scheduled points on the dendritic arbor. Using this system, we monitored the activity and the morphology in tips and branches of individual neurons using randomized stimulation while constrained within a stimulation chamber.

2-G-170 Plasma Soluble Prion Protein as a Potential Biomarker for the Traumatic Brain Injury

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Traumatic brain injury (TBI) is a neurological disturbance caused by an external application of force to the head. Globally, TBI is a major burden to public health and the leading cause of mortality and neurological disabilities in individuals under the age of 45. A vast majority of TBI patients (~ 80%) suffer mild, non-penetrating closed head injuries (mTBI) and experience a short loss of consciousness (< 20 min). Often these patients do not seek medical attention or their diagnosis is challenging as medical imaging may not be able to detect pathology. Appropriate treatment in a timely manner is therefore not delivered. Significant changes in the concentration of certain proteins in biological fluids following TBI can be used as biomarkers for diagnosis and prognosis as well as guide therapy. In our study we hypothesized that an external force to the head could dislodge almost entirely extarcellularly oriented cellular prion protein (PrPC)to the extracellular compartment which eventually enters into circulation. Using a modified ELISA kit, we discovered that blast-TBI is associated with an elevation of soluble cellular prion protein (PrPC) levels in the plasma of rats exposed (heads only) to various blast force magnitudes. We have further supported our hypothesis from a pilot study on the University of Saskatchewan Huskies Athletic Teams. We detected higher levels of PrPC in post-concussion plasma samples compared with the plasma collected from healthy, non-concussive students. We therefore conclude that the plasma soluble PrPC may serve as a potential TBI biomarker.

2-G-171 Genome-wide association for sensory neuron function in C. elegans using an automated behavioural tracking system.

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Nervous and muscular systems allow animals to sense, and appropriately respond to, the environment. Understanding the genetic basis of these processes requires an integrative approach, probing the molecular mechanisms of distinct components acting in complex neuronal circuits. Typical circuits include sensory neurons bearing cilia (sensory organelles), interneurons, motor neurons, and synapses that collectively transmit signals to muscle cells which impart movement and thus behaviour. The systematic study of such behavioural circuits on a large scale, termed 'behavioural phenomics', represents a sought-after area of research, but which due to technical limitations in most animals, remains virtually unexploited. We are working to establish a nematode, Caenorhabditis elegans, as a platform for behavioural phenomics. This is only now possible with the very recent development of a deep-sequenced C. elegans multi-mutation library and newly developed automated behavioural tracking system. For each deep-sequenced multi-mutant strain, sensory-related phenotypes (> 20 morphological/behavioural features) are combined to create a phenotypic 'fingerprint'. Next, we test for genome wide-associations (GWAS) between behavioural and

genetic variants to link genes to behaviour. We anticipate that these innovative behavioural phenomic studies will successfully uncover many components and functional mechanisms of sensory neuron function. Importantly, our findings are of distinct biomedical relevance, pertinent to a wide array of clinical ailments such as ciliopathies and sensory neuropathies.

2-G-172 Mesoscale transcranial cortical imaging with fast kinetic genetic-encoded glutamate sensor - iGluSnFr

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Wide field mesoscopic cortical imaging with genetic encoded sensors enables decoding regional activity and connectivity in anesthetized and behaving mice (Vanni and Murphy, 2014). Fast and sensitive sensors are required to improve linkages to behavior. We report that mesoscopic imaging of virally transduced or transgenic expression of glutamate-sensing fluorescent reporter (iGluSnFr) can robustly report sensory stimulation-evoked and spontaneous cortical activity through intact transparent skull (Vanni and Murphy, 2014; Silasi et al., 2013) in anesthetized or head-fixed awake mice (Guo et al., 2014). Compared to EMX1:creRosa26-GGCaMP3 transgenic mice, EMX1:tTA-CamkII: iGluSnFr (Ai85, Allen Institute for Brain Science) transgenic and AAV1-synapsin-iGluSnFr virally transduced mice showed faster kinetics for reporting regional cortical activity, being comparable to the speed of RH1692 voltage sensitive dye (VSD), but with larger signals. The seed pixel correlation maps of iGluSnFr spontaneous activity indicated functional circuits in cortex that were generally consistent with the maps generated from EMX-GCaMP3 mice and VSD spontaneous activity. In the current study, we present a mesoscopic imaging strategy using a fast genetic-encoded sensor that is reporting regional signals of extracellular glutamate concentration in the cortex. It may help the study of cortical circuits following behavior training or brain injury.

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2-IBRO-175Nociceptive hypersensitivity induced by Herpes Simplex-1 is a consequence of leukocyte migration and production of inflammatory mediators at infected dorsal root ganglia

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Herpes Zoster (HZ) is a disease caused by reactivation of latent herpesvirus Varicella Zoster in the sensory ganglion, characterized by dermal rash and pain. A murine model of HSV-1 infection on the hind paw skin has been used to study HZ, since mice develop HZ-like skin lesions and pain-related responses. The aim of this study was to evaluate cells and inflammatory mediators present in DRGs and its relationship with hiperalgesia during HSV-1 infection. Mice developed hyperalgesia from 3 to 14 dpi only in the ipsilateral paws. A higher viral load was detected in DRGs L4, L5 and L6 at 7 dpi. The intrathecal treatment of infected mice with the steroidal anti-inflammatory drug dexamethasone (5 ug) or fucoidin resulted in a reduction of hyperalgesia, consistent with the inhibition of leukocyte migration to infected DRGs. The leukocyte infiltrate was composed by neutrophils and macrophages. The mRNA expression of COX-2 and TNF-a was found to be up regulated at 7 dpi. Moreover, the pharmacological blockage of COX-2 and TNF-a resulted in the reduction of hyperalgesia. Corroborating these data, the genetic deficiency of TNF-a receptor type I was associated with the absence of hyperalgesia in infected mice. Our results show the presence of an inflammatory infiltrate in DRGs of infected mice, and the early expression of inflammatory mediators that contribute for the induction of herpetic hyperalgesia. Financial support: FAPESP (2010/12309-8).

2-IBRO-176 Effects of Cold Exposure on Behavioral and Electrophysiological Parameters.Related with Hippocampal Function in Rats

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This study examined spatial memory as measured by Morris Water Maze(MWM) performance and hippocampal long-term potentiation(LTP) in the dentate gyrus after exposure to cold in a repeated stress condition for 2 h/day for 5 days. Three to four month old rats were randomly divided into four groups to form a control group and a cold stress group for each sex. The groups of cold stressed animals were placed in a cold room (ambient temperature of (4oC) for 2 hours day. Adrenal glands and body weight (g) were recorded in control and stressed rats during the cold exposure Spatial learning (acquisition phase) and memory (probe trial) were tested in the Morris water maze immediately after daily exposure. Latency to locate the hidden platform, distance moved, mean distance to platform, swim speed and time spent in the platform quadrant were compared between genders and treatments. Field potential recordings were made, under urethane anesthesia, from the DG granule-cell layer, with stimulation of the medial perforant pathway 2 hours after the probe trial. The cold-exposed female rats needed less time to find the hidden platform on day 1, day 2 and on day 4, while cold-exposed male rats showed a decreased escape latency on day 1 only. Cold-exposed male rats spent less time in the target quadrant than the control male rats. 2h cold exposure decreased population spike potentiation during both induction and maintenance intervals in male rats. Meanwhile cold exposure did not affect the body weight but it impacts the adrenal gland relative weight.

2-IBRO-177 The neuroprotective effects of recombinant erythropoietin isoform with low glicosilation is mediated by activation of BCL2 pathway

Carolina Castillo[1], Angela Hidalgo[1], Tiare Silva-Grecchi[1], Jorge Fuentealba[1], Jorge Toledo[1]

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Erythropoietin is a glycoproteic hormone that weighs 34 KDa. Its principal function is to regulate the erythropoiesis, however, since the discovery of its receptor (EpoR) in non erythroids tissues like the central nervous system (SNC), their function has been related with another pleiotropic effects. In the SNC has been observed a neuroprotective effect of this hormone that's related with the upregulation of antiapoptotic pathways that directly related with activation of its receptor. The objective of this proyect is evaluate the neuroprotective effect in vitro and their dependence of the activation of EpoR of a new isoform of Erythropoietin with low glicosilation obtained from adenoviral transduction of mammary gland of goats (EpoL). For this purpose we preincubated PC12 cells with EpoL for an hour and then we incubated this cells with β-amyloid peptide or with an uncoupling of respiratory chain of electrons in mitochondria (FCCP), like a model of oxidative stress. The cells preincubated with EpoL shown an increase of the cellular viability of 32% more than the control cells, and an increase of 26% more than cells incubated with an inhibitor of activation of EpoR. That difference was not shown in cells that incubated with a native isoform of Epo. Finally this results agree with the 50% of upregulation of BCL2 gene observed in the same treatments by qRTPCR experiments and with an increase of immunorreactivity of EpoR in immunoflurescence data. These results suggested that EpoL has a neuroprotective effect more potent than native Epo.

2-IBRO-178 Cell-type specific chloride dynamics in epilepsy.

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Epilepsy is the most common disorder of the nervous system in sub-Saharan Africa with devastating socioeconomic effects. The central feature of all epileptic activity is a failure of inhibitory signaling in the brain to contain the generation and spread of excessive neuronal excitation. Neuronal inhibition is primarily mediated by chloride permeable GABAA receptors making the neuronal transmembrane chloride gradient critical for setting the properties of inhibitory signaling. My research is investigating how dynamic changes in chloride concentration relate to the development of abnormal neural activity. Firstly, I am investigating the conditions under which transient changes in neuronal intracellular chloride concentration have pathological effects on the strength of synaptic inhibition within cortical circuits. Whilst astrocytes have long been implicated in the regulation of extracellular ionic environments in the brain, their role in maintaining/modulating chloride gradients is not well understood and forms a further aim of this research. Techniques, used for the first time in

Africa, including patch clamp and optogenetics have been set up. In promising preliminary data patch-clamp recordings have shown that during seizure-like events in hippocampal brain slices, the astrocytic membrane potential closely mirrors that of surrounding neurons. Further research utilizing transgenic mice is under way and will allow for the measurement of chloride regulation and changes to synaptic inhibition in both neurons and astrocytes, particularly in the context of epileptic seizures.

2-IBRO-179 Characterization of TDP43 misfolding in an experimental model of ALS/FTD

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Introduction: TDP43 is a nuclear protein that has important cellular functions related to the regulation of RNA metabolism. TDP43 has been described as the major component of the pathologic inclusions present in post-mortem tissues of most cases of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In this work, we characterized the pathogenesis of TDP43 in tissue extracts of a transgenic mouse model that overexpresses a mutant form of TDP43 (A315T) in the CNS. We also evaluated the expression of Unfolded Protein Response (UPR) markers in brain and spinal cord tissue. Methods: We obtained protein extracts and mRNA from cortex and spinal cord from transgenic animals (TDP43A315T) in a symptomatic stage and non-transgenic controls. Protein extracts were analyzed by western blot and filter trap. Finally, we did histological analysis in brain and spinal cord of TDP43A315T mice in symptomatic stage and non-transgenic littermates. Results: Biochemical analysis indicated the presence of disulfide-dependent TDP43 protein aggregates in cortex and spinal cord of TDP43A315T mice in symptomatic stage. We found altered levels of UPR markers in tissue extracts of TDP43A315T cortex compared with controls. Finally, by histological analysis we found the presence of ubiquitinated aggregates in cortex and spinal cord of symptomatic TDP43A315T mice. Discussion: The presence of TDP43 ubiquitinated aggregates could be generating alterations in ER proteostasis of neural cells, affecting the UPR machinery and the adaptive capacity against stress.

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