

Program and Abstracts from the Fifth Annual Canadian Neuroscience Meeting

May 29 – June 1 2011

Quebec City Convention Center

Quebec Canada

Copyright 2011 Canadian Association for Neuroscience – Association Canadienne des
Neurosciences

Scientific Program

Special Guest Speakers:

Bert Sakmann (Max Plank Florida Institute)

William T. Newsome (Stanford University)

Fred H. Gage (Salk Institute for Biological Studies)

Program :

Sunday, May 29th 2011 :

09:00 – 17:00 Neurophotonics Satellite Symposium

18:00 – 20:00 Opening Reception

- Opening Comments by Yves De Koninck (President)
- Presentation of the CAN Young Investigator Award by Brian MacVicar (Past-President)
- Opening Address by Anthony Phillips, Director of the CIHR Institute of Neuroscience
Mental Health and Addiction

Monday, May 30th, 2011

07:30-08:30

Breakfast Meeting - Sponsored by NeuroDevNet

08:30 – 10:30

Neural stem cells in the developing, adult, and aging CNS: a lifetime of challenges

Chair: Karl Fernandes, U. Montréal

- Dare to be different: asymmetric cell divisions in the developing mouse retina (Michel Cayouette, IRCM, Montréal)

- Neural stem cells: from genes to cognition (Freda Miller, U. Toronto)

The fountain of youth: neural stem cells in the aging brain (Karl Fernandes, U. Montréal)

11:00 - 12:00

Fred H. Gage, Salk Institute: Neural Plasticity and Neuronal Diversity

12:00 - 13:30

Lunch and Exhibitor Events

13:30 - 15:30 Parallel Symposia - sponsored by Hotchkiss Brain Institute

I) Molecular and Cellular Mechanisms of Synapse Formation and Plasticity (Chair: Shernaz Bamji, UBC)

II) New insights on Pathogenesis of Amyotrophic Lateral Sclerosis (Chair: Jean-Pierre Julien, U. Laval)

III) Cortical Plasticity (Chair: Cam Teskey, U. Calgary)

IV) Non Invasive Brain Stimulation in Neurosciences and Mental Health (Chair: Cyril Schneider, U. Laval)

15:30 – 17:30 Posters

17:30 – 19:00 Presidential Forum

The Challenge of Studying the Neural Bases of Higher Brain Functions

(Moderators: Martin Paré, Queen's; Judy Illes, UBC)

(Participants: Bill Newsome, Stanford; Sharon Juliano, USUHS)

20:00 – 21:00 Public Lecture:

Le cerveau: exploration de la dernière frontière

(Rémi Quirion, McGill University)

Tuesday, May 31st, 2011

08:30 – 10:30

Systems/Cognitive Neuroscience - Chair: Doug Munoz

- Predictive Coding and Speech Intelligibility (Ingrid Johnsrude, Queen's)
- Determinants of Brain Plasticity Associated with Motor Sequence Learning (Julien Doyon, U. Montréal)
- Using Eye Movements to Probe Brain Function and Dysfunction (Doug Munoz, Queen's)

11:00 - 12:00

William T. Newsome, Stanford:

Linking Action to Reward: A Dynamic Systems Approach to Prefrontal Cortex Activity

12:00 - 13:30

Lunch and Exhibitor Events

CAN Annual General Meeting

13:30 - 15:30 Parallel Symposia

- I) Transcriptional Mechanisms of Neuronal Differentiation (Chair: Artur Kania, IRCM, Montréal)
- II) New Avenues for a Rational Psychopharmacology (Chair: Martin Beaulieu, U. Laval)
- III) The Dynamic Memory Trace (Chair: Paul Frankland, U. Toronto)
- IV) Brain Activities During Sleep and Waking States (Chair: Igor Timofeev, U. Laval)

15:30 – 17:30 Posters

17:30 – 18:30 Brainstar Awardee Presentations

18:30 - 19:30 Presidential Lecture: Brian MacVicar, UBC

Astrocyte-Neuron interactions to maintain brain function and cerebral blood flow

Wednesday, June 1st, 2011

07:30 - 08:30 Breakfast discussion:

Bridging animal and human studies for the understanding of psychiatric diseases

Sponsored by Université Laval and the IUSMQ (Institut Universitaire de santé mentale de Québec)

08:30 – 10:30

Microcircuits Chair: Len Maler, U. Ottawa

- Neural circuits for Optimization of Sensory Processing (Len Maler, U. Ottawa)
- Ontogeny of Neural Circuits for Motor Behaviour (Louis St Amant, U. Montréal)
- Understanding How GABAergic Interneurons Shape Theta and Gamma Oscillations in Hippocampus (Sylvain Williams, McGill)

11:00 - 12:00

Bert Sakmann, Max Planck Florida Institute:

Digital Neuroanatomy – 3D Reconstruction of Anatomically Realistic Neuronal Networks

12:00 - 13:30 Lunch and Exhibitor Events

13:30 - 15:30 Posters

15:30 - 17:30 Parallel Symposia

- I) Bidirectional Regulation of Mitochondrial Function and Neuronal Excitability (Chair: Roger Thompson, U. Calgary)
- II) Parkinson's Disease: Symptomatic and Neuroprotective Treatments (Chair: Thérèse Di Paolo, U. Laval)
- III) Roles of Synaptic Plasticity in Memory, Fear and Chronic Pain (Chair: Min Zhuo, U. Toronto)
- IV) Synaptic Integration and Plasticity in Developing and Mature Neural Circuitry (Chair: Melanie Woodin, U. Toronto)

Abstracts:

Poster Category A : Development

Title: Concurrent regulation of tau phosphorylation and splicing during development

Authors: Alexis Bretteville¹, Marie-Amélie Papon², François Marcouiller³, Joanie Baillargeon⁴, Emmanuel Planel⁵

Affiliation: 1-5 Centre de recherche du CHUQ - Université Laval

Abstract: Tau is a microtubule-associated protein extensively studied since intracellular aggregates of hyperphosphorylated tau are present in a group of neurodegenerative diseases called tauopathies, which include Alzheimer's disease. The microtubule-binding domain of tau mediates its most characterized biological function: to stabilize microtubules and promote their polymerization. In the human brain, tau is encoded by a single gene, generating 6 isoforms by splicing of exons 2, 3 and 10. Exons 9 to 12 encode the microtubule binding repeats, and alternate splicing of exon 10 generates tau with either three or four binding repeats. Thus, the carboxy-terminal region is characterized by the presence of 3 or 4 repeats that mediate the microtubule binding properties of tau. Understanding the regulation of tau phosphorylation and exon 10 splicing is important since imbalance in the ratio 3R to 4R has been shown to lead to certain forms of tauopathies, and because hyperphosphorylation can induce aggregation in vitro, and is thought to induce aggregates formation in the brain. In this study we used both primary cultures and developing mice to identify the molecular regulators of tau splicing and tau phosphorylation. We show that during embryonic development, tau is highly phosphorylated and its microtubule binding domain contains only 3 repeats. In post-embryonic development, phosphorylated tau and 3 repeats tau levels decrease, while 4 repeats tau gradually reaches adult levels. These changes correlate with differential regulation of several kinases and phosphatases, suggesting that they could influence tau phosphorylation and splicing, both during development and disease state.

Funding: CIHR, FRSQ, NSERC

Title: Expression of Conserved Transcription Factors (FoxP2, Otx1 and FoxO3) strongly suggest a Deep Homology between Neurons in Dorsocentral Telencephalon in Fish and Neocortical Neurons of Deep Layers in Mammals.

Authors: Erik Harvey-Girard¹, Ana Catarina Giassi²,

Affiliation: 1,2 U. Ottawa

Abstract: Objective: Pallial regions of teleost fish are associated with learning about social signal discrimination, spatial learning and emotional conditioning. We recently showed that neurons in the dorsal central part of the apteronotid pallium (DC) have similar features to neocortical neurons in deep layers (V/VI). We emit the hypothesis that apteronotid DC neurons show a deep homology with neurons in mammalian neocortical deep layers (V/VI). Materials and Methods We have cloned the homologs of FoxP2, Otx1 and FoxO3 in the weakly electric fish *Apteronotus leptorhynchus*. We also used in situ hybridization to examine the distribution of the mRNA of these genes in the apteronotid telencephalon. Results: There were, in the case of all three genes, good similarities between the apteronotid and human amino acid sequences: FoxP2 - 78%, Otx1 - 54%, FoxO3 - 71%. The functional domains of these genes was conserved to a far greater extent suggesting that the cellular functions of these genes is highly conserved. By in situ, we found that AptFoxP2 and AptOtx1 transcripts were expressed predominantly in DC; the dorsolateral pallium (DL) contained only weakly labeled neurons. In contrast, we found that most neurons in DL strongly expressed AptFoxO3 mRNA, while there was only weak expression in a small number of cells within DC. These expression patterns of FoxP2, Otx1 and FoxO3 are highly conserved between teleosts and mammals. Conclusion These results strongly suggest a deep homology between teleost DC neurons and neurons in neocortical layers V and VI which both express FoxP2 and Otx1."

Funding: CIHR

Title: Proteolytic processing of semaphorin 5B produces a diffusible repulsive guidance molecule

Authors: Kristen Browne¹, Wenyan Wang², QianQian Liu³, Robyn Lett⁴, Timothy O'Connor⁵

Affiliation: 1-5 University of British Columbia

Abstract: The Semaphorin family has well established roles as both secreted and transmembrane neural guidance cues. Here we describe the first evidence that the transmembrane Semaphorin 5B is proteolytically processed into a diffusible, repulsive guidance cue. Evidence is presented for processing into at least two fragments, the first as a result of pro-protein convertase activity, and the second from metalloprotease activity. The latter fragment repels sensory neurons in collagen co-culture and growth cone collapse assays. This data shows that transmembrane Semaphorin-

mediated axon guidance is subject to proteolytic regulation and suggests that these multi-domain proteins could be modulated to serve multiple functions.

Funding: CIHR, NSERC

Title: Age-dependent remodelling of inhibitory synapses onto hippocampal CA1 oriens-lacunosum moleculare interneurons

Authors: Charleen Salesse¹, Christopher Lacharité Mueller², Simon Chamberland³, Lisa Topolnik⁴

Affiliation: 1-4 Université Laval

Abstract: "Stratum oriens-lacunosum moleculare interneurons (O-LM INs) represent the major element of the hippocampal feedback inhibitory circuit, which provides inhibition to the distal dendritic sites of CA1 pyramidal neurons. Although the intrinsic conductance profile and the properties of glutamatergic transmission to O-LM INs have become a subject of intense investigation, far less is known about the properties of the inhibitory synapses formed onto these cells. Here, we used whole-cell patch-clamp recordings in acute mouse hippocampal slices to study the properties and plasticity of GABAergic inhibitory synapses onto O-LM INs. Surprisingly, we found that the kinetics of inhibitory postsynaptic currents (IPSCs) were slower in mature synapses (P26-40) due to the synaptic incorporation of the $\alpha 5$ subunit of the GABA_A receptor ($\alpha 5$ -GABAARs). Moreover, this age-dependent synaptic expression of $\alpha 5$ -GABAARs was directly associated with the emergence of long-term potentiation at IN inhibitory synapses. Finally, the slower time course of IPSCs observed in O-LM INs of mature animals had a profound effect on IN excitability by significantly delaying its spike firing. Our data suggest that GABAergic synapses onto O-LM INs undergo significant modifications during postnatal maturation. The developmental switch in IPSC properties and plasticity is controlled by the synaptic incorporation of the $\alpha 5$ -GABAAR subunit and may represent a potential mechanism for the age-dependent modifications in the inhibitory control of the hippocampal feedback inhibitory circuit."

Funding: This work was supported by the CIHR, the NSERC Discovery Grant, and the Savoy Foundation.

Title: Ca²⁺ transients in spinal progenitors during neuronal differentiation in vivo

Authors: Edna Brustein¹, Julien Ghislain², Pierre Drapeau³

Affiliation: 1-3 University of Montreal

Abstract: "Glycine and GABA are depolarizing during early development but the purpose is unclear. Altering depolarizing glycine signaling from the beginning of development in zebrafish embryos by over-expressing the potassium-chloride co-transporter type-2 (KCC2) to reverse the chloride gradient or by knocking down the embryonic glycine receptor caused a specific loss of spinal interneurons and an abnormal maintenance of progenitors. To determine whether progenitor differentiation was dependent on glycine signaling, spinal cells were labeled with AM-ester Ca²⁺ dyes (Oregon-green or Rhod-2) and spontaneous or evoked Ca²⁺ transients were recorded in-vivo in 19-22 hours old embryos in the presence of tricaine to block sodium currents and behaviorally related activity. In wild-type embryos 32%±12 of the labeled spinal cells (n=91) showed spontaneous Ca²⁺ transients. This number was reduced to 6%±4 (n=79) by bath application of 1-40 µM strychnine, but was not changed upon glycine application (33%±11, n=73, 0.5-2mM). Similar spontaneous Ca²⁺ transients were observed in spinal cells of the Neurogenin-1 (NGN) transgenic line (40%±14, n=79), in which only differentiated neurons express GFP from the NGN promoter and the others are progenitors. Ionophoresing glycine onto that progenitor population elicited Ca²⁺ transients (n=6). Spontaneous Ca²⁺ transients were also observed in spinal cells that expressed GFP from the GFAP promoter, a specific marker for radial glial progenitors (n=3/10). This demonstrates that spinal progenitors of zebrafish embryos express spontaneous, sodium resistant, Ca²⁺ transients mediated by glycine and block of which suppressed interneuron differentiation. Our results suggest a role for depolarizing chloride and spontaneous Ca²⁺ elevations during neural progenitor differentiation."

Funding: supported by the Canadian Institutes of Health Research and by the Fonds de recherche en santé du Québec

Title: Glycine promotes neural progenitor differentiation by calcium-dependent suppression of Notch

Authors: Sébastien Côté¹, Edna Brustein², Meijiang Liao³, Julien Ghislain⁴, Pierre Drapeau⁵

Affiliation: 1-5 Université de Montréal

Abstract: "Glycine and gamma-amino butyric acid (GABA) are paradoxically depolarizing during early development and the reason is unclear. Here we tested the effect of manipulating glycine signaling in zebrafish embryos by overexpressing the potassium-chloride co-transporter type 2 (KCC2) to reverse the chloride gradient or by blocking glycine receptors with strychnine or by the

selective knockdown of the embryonic glycine receptor (GlyR). In all three cases we observed a major reduction of all subtypes of spinal interneurons but not of motor or sensory neurons and a stalled progenitor population. In contrast, similar manipulations in the bandoneon mutant lacking synaptic but not extra-synaptic glycinergic signaling were without effect, indicating a paracrine action of glycine. We next showed that progenitor cells have spontaneous, glycine-mediated calcium transients. Raising embryos with the calcium channel blocker nifedipine also resulted in reduced interneuron differentiation and stalling of progenitors. Upon blocking calcium transients or glycinergic signaling we observed an increase in Notch 3 expression; blocking Notch signaling in general or suppressing Notch 3 expression partially rescued the phenotype. We conclude that paracrine glycinergic depolarization of neural progenitors evokes spontaneous calcium transients that enhance interneuron neurogenesis at least in part by selectively suppressing Notch 3 signaling. "

Funding: IRSC/CIHR, GRSNC

Title: Extracellular matrix glycoprotein Tenascin-R affects adult but not developmental neurogenesis in the olfactory bulb

Authors: Linda David¹

Affiliation: 1- Centre de Recherche Université Laval Robert-Giffard

Abstract: "In the adult rodent brain the subventricular zone (SVZ) of the lateral ventricle harbors stem cells of inhibitory interneurons generated for the olfactory bulb. About 30,000 neuronal precursors leave the SVZ every day bound to the olfactory bulb (OB), in a process known as adult neurogenesis. Intensive investigation has revealed myriad molecular cues involved in this form of neurogenesis, however molecules signaling exclusively for adult neurogenesis are yet to be described. Tenascin R (TNR) an extracellular matrix molecule known to take part in neurogenesis is expressed in an age dependent manner in the OB. Lack of TNR results in massive reduction in newly generated cells integrating in to the bulb. To understand the functional implications of adult neurogenesis, we used TNR KO and WT mice as a model system. Electrophysiological recordings were made from mitral cells (MC) the principal output neurons, targets of the newly generated interneurons in acute OB slices of WT and TNR KO mice A) Spontaneous inhibitory postsynaptic currents (sIPSC) and miniature currents (mIPSC) recordings revealed that adult TNR KOs have reduced frequency of mIPSCs. B) Evoked Dendrodendritic inhibition from MCs and C) local field

potential from the MC layer reiterates to us that MCs of adult TNR KOs receive reduced inhibition which subsequently results in reduced synchrony of bulbar output. Further; behavioral studies allowed us to conclude that the short term memory of adult KOs is significantly impaired. These data suggest that TNR specifically affects adult, but not early developmental neurogenesis in the OB. "

Funding: Centre de Recherche Universite Laval Robert-Giffard

Title: The integration of adult-born cells to the olfactory bulb is regulated by the principal cells activity.

Authors: Vincent Breton-Provencher¹, Daniel Côté², Armen Saghatelian³

Affiliation: 1-3 Centre de Recherche Universite Laval Robert-Giffard

Abstract: The interneurons of the olfactory bulb (OB) are continuously renewed during the whole lifespan of mammals. Until now, the understanding of the mechanisms controlling the integration and maturation of these adult-born interneurons into the pre-existing neuronal circuitry has remained unclear. By using time-lapse two-photon microscopy we analysed the spine formation and retraction of adult-born granule cells (GC) at different maturational stages. Our results demonstrate that adult-born cells display a high level of formation/retraction of filopodia-like structures and this dynamic reduces as the cell matures in the OB. Surprisingly, we also observed similar filopodia dynamic occurring directly on the spines and this dynamic is maintained even at later maturational stages of newborn GCs. The OB principal cells play a role in the orchestration of adult-born cells integration. Indeed, the electrical stimulation of principal cell activity increases the filopodia dynamic directly on dendrites of adult-born interneurons at an early maturational stage. The same stimulation of principal cells activity leads to the stabilization of filopodia appearing and retracting on spines of adult-born cells at a later maturational stage. We are currently investigating the possible role for glutamate in this control of integration via the principal cells activity. Our data reveals that integration of adult-born cells into the pre-existing neuronal network in the adult OB is accompanied by the constant morphological re-organization and is controlled by the activity of principal cells.

Funding: Centre de Recherche Universite Laval Robert-Giffard

Title: Netrin-1 expression in mouse embryonic neural stem cells modulates their proliferation.

Authors: Pavel Gris¹, Joseph Kam², Reesha Raja³, Joanna Warziszinska⁴, Jennifer Sarah Goldman⁵, Timothy Edward Kennedy⁶, Jean-Francois Cloutier⁷

Affiliation: 1- 7 McGill University

Abstract: Netrin-1 is a secreted protein implicated in the regulation of multiple processes during development of the nervous system including axonal guidance and cell migration. Our detailed studies of the pattern of netrin-1 expression has revealed that it is expressed at high levels in neurogenic niches of the mouse embryo including the subventricular zone and the lining of brain ventricles suggesting it may play a role regulating neural stem cell function. Interestingly, the lateral brain ventricles are enlarged in netrin-1 mutant mice suggesting cell proliferation may be affected in the nervous system of these mice. We have used a neurosphere culture assay to examine the role of netrin-1 in regulating proliferation of neural stem cells. Our studies show that neural stem cells isolated from netrin-1 mutant mice form fewer and smaller neurospheres when cultured in vitro as compared to cells from wild-type embryos. The reduced proliferation observed is in part rescued by addition of recombinant netrin-1 protein to the cultures. Furthermore, addition of a netrin-1 neutralizing antibody to neural stem cell cultures from wild-type embryos also leads to the production of reduced numbers of neurospheres. Neurospheres produced from netrin-1 mutant embryos show reduced incorporation of BrDU and reduced ERK phosphorylation suggesting that netrin-1 affects proliferation of neural stem cells. In contrast, the levels of cleaved caspase-3 are unchanged in netrin-1 mutant neurospheres suggesting that netrin-1 is not required for survival of neural stem cells. Overall our results demonstrate that netrin-1 is expressed by neural stem cells and modulates their proliferation.

Funding: Center for Excellence in Commercialization and Research

Title: Immunohistochemical evidence of adult born neurons originating from oligodendrocyte progenitor cells throughout the adult rodent brain.

Authors: Jenna Boulanger¹, Carmen Dominguez², Claude Messier³

Affiliation: 1-3 University of Ottawa

Abstract: "In the context of adult neurogenesis, the hippocampus and the olfactory bulb are most commonly studied. Adult-born neurons within these two regions arise from neural precursors originating in the subgranular zone of dentate gyrus and the subventricular zone respectively.

However, there is increasing evidence suggesting that newly generated neurons may be found outside of these two areas. Additionally, recent research suggests that adult-born neurons can be generated from oligodendrocyte progenitor cells (OPC). The current study expands on previous research by demonstrating that adult-born neurons, as labeled by doublecortin (DCX), can be found in other areas of the adult rodent brain, including in the cerebellum, the caudate nucleus, the nucleus putamen, the piriform cortex, and the inferior and superior colliculi. Furthermore, a large proportion of these DCX-expressing cells also express the OPC markers Olig1 and PDGFR- α . This supports previous work that has shown that neurons can arise from OPCs. This study also demonstrates that a subset of DCX-expressing cells also express Pax2, a marker of GABAergic neurons. A subset of this population also expresses markers for OPCs. This suggests that some of the DCX-expressing cells originating from OPCs may mature into GABAergic neurons. "

Funding: NSERC Graduate Scholarship to J.B.

Title: Development of migration promoting vasculature scaffold in the postnatal brain

Authors: Lusine Bozoyan¹, Jivan Khlghatyan²

Affiliation: 1-2 Centre de Recherche Universite Laval Robert-Giffard

Abstract: New neurons that are continuously generated in the adult subventricular zone travel in the rostral migratory stream (RMS) to reach their final destination in the olfactory bulb. Recently, we have demonstrated that blood vessels (BVs) topographically outline the RMS and serve as a physical scaffold and provide essential molecular cues required for neuroblasts migration during adulthood. The molecular and cellular mechanisms leading to the formation of migration-promoting vasculature scaffold in the RMS remain unclear. We now reveal that astrocytes orchestrate the formation and structural re-organization of vasculature scaffold in the RMS. Our data demonstrate that during the early developmental periods, RMS contains only few BVs oriented randomly with regard to the migrating neuroblasts. The first parallel BVs appear at the outer border of RMS where most of, vascular endothelial growth factor, VEGF-expressing astrocytes are located. The gain-of-function and loss-of-function experiments with exogenously applied VEGF and siRNAs directed against this trophic factor, respectively, revealed the crucial role of astrocyte-derived VEGF in the formation and growth of new blood vessels. Finally, we demonstrate that migration of neuronal precursors in the developing RMS substantially differs from neuronal displacement in the adult migratory stream. While the speed of migration does not

differ, due to not yet fully developed vasculature scaffold in the migratory stream, neuroblasts have shorter migratory phases in the developing RMS. Altogether our results demonstrate that astrocytes secrete VEGF and thus orchestrate the formation and growth of parallel BVs, crucial migration promoting scaffold in the adult migratory stream.

Funding: CRCN (Centre de Recherche sur le Cerveau, le Comportement et la Neuropsychiatrie)

Title: The Role of Early Neuronal Activity in Motor Circuit Formation

Authors: Chris Law¹, Michel Paquet², Artur Kania³

Affiliation: 1-3 Institut de Recherches Cliniques de Montréal

Abstract: The formation of neural circuits is often thought of as being genetically hardwired, with the specification of cell types and the guidance of their axons being solely determined by expression of transcription factors and guidance molecules. Recent evidence, however, has demonstrated that a more complex system involving electrical activity also influences these early stages of neural circuit formation. Neurons of the developing nervous system are electrically active from a very early stage, and interference with this electrical activity perturbs the formation of neuronal circuits by altering transcriptional identity and guidance receptor expression. We are examining the role of electrical activity in motor circuit development and the identity of the molecular mechanisms underlying it by studying the developing spinal motor neurons preceding the formation of neuromuscular synapses. We developed an experimental paradigm that allowed us to record intracellular calcium concentration changes in large populations of motor neurons throughout the development of the spinal motor circuit. Simultaneous recording of voltage and calcium concentration changes provide evidence that imaged calcium transients accurately represent electrical activity. Using antibody staining, we were able to assign motor neuron subtype identities to the neurons whose calcium transients were imaged, thus relating their molecular identity to their activity patterns. Currently, we are examining how changing electrical activity patterns through overexpression of specific ion channels, affects cell identity and axon guidance.

Funding: Canadian Institute of Health Research

Title: Ephrin-mediated cis-attenuation of Eph receptor signalling is essential for spinal motor axon guidance

Authors: Tzu-Jen Kao¹, Artur Kania²

Affiliation: 1-2 Institut de recherches cliniques de Montréal

Abstract: Objective: Axon guidance receptors guide neuronal growth cones by binding to axon guidance ligands in the developing nervous system. Interestingly, some ligands are co-expressed “in cis” with their receptors raising the possibility that such ligands might modulate the function of axon guidance receptors. Spinal motor axons use Eph receptors to chart their trajectories in response to ephrins expressed in the limb. Additionally, spinal motor neurons also express ephrins. Our recent in vivo studies show that changes in motor neuron ephrin expression result in trajectory selection defects. To test more directly the possibility that ephrins in motor neurons affect the function of co-expressed Eph receptors, we analyzed the sensitivity of motor axon growth cones to ephrins in vitro and assayed directly whether ephrins expressed by motor axons can attenuate Eph signalling “in cis”. Methods and results: The challenge of cultured motor neurons explants or dissociated neurons with ephrin stripes, in the context of ephrin gain and loss of function, results in changes of growth cone sensitivity to these stripes, arguing for ephrin attenuation of Eph function in motor neurons “in cis”. Furthermore, we demonstrate that the degree of cis-attenuation is modulated by the extent of localization of ephrins to membrane patches containing Eph receptors. Conclusion: Our observations argue that cis-attenuation of Eph signalling by co-expressed ephrins contributes to the fidelity of motor axon trajectory selection. More generally, co-expression of axon guidance ligands and their receptors might be a mechanism for expanding the diversity of axon guidance signalling responses.

Funding: CIHR and the EJLB foundation to A. Kania (MOP-77556 and IG-74068).

Title: Neogenin regulates the differentiation of olfactory sensory neurons

Authors: Joseph W.K. Kam¹, David Mendes da Silva², Jean-Francois Cloutier³

Affiliation: 1-3 Montreal Neurological Institute

Abstract: The birth and differentiation of sensory neurons is critical for our ability to detect and decode information from the environment. In the olfactory epithelium, new olfactory sensory neurons (OSNs) are generated throughout the life of the organism to compensate for cell death caused by toxic chemicals in the air. The olfactory epithelium therefore represents a good model system to study mechanisms that regulate neurogenesis. The transmembrane receptor Neogenin has been implicated in the regulation of a wide variety of processes during development of the

nervous system including axonal guidance, cell migration, and cell differentiation. Furthermore, multiple ligands have been identified for this receptor including Netrin, RGMs, and BMPs. We have examined the role of Neogenin during development of the olfactory system. We find that Neogenin is expressed in progenitor cells and subsets of mature OSNs in the olfactory epithelium but is not expressed in immature OSNs. Interestingly, RGM-b expression in the olfactory epithelium appears complementary to Neogenin expression with high levels in immature OSNs suggesting that RGMb-Neogenin signaling may regulate OSN differentiation. Mice carrying a hypomorphic allele of Neogenin have reduced numbers of mature OSNs and increased numbers of proliferating progenitor cells, suggesting that Neogenin expression is essential for differentiation of progenitor cells into immature OSNs. While Neogenin is required for the differentiation of OSNs, it is dispensable for the guidance of OSN axons to specific glomeruli in the olfactory bulb. Our findings therefore define a new role for Neogenin in olfactory neurogenesis.

Funding: CIHR Operating Grant

Title: Semaphorin 5B is critical to axonal guidance in the chick spinal cord

Authors: Qian Q. Liu¹, Wenyan Wang², Arthor Legg³, Timothy O'Connor⁴

Affiliation: 1- 4 University of British Columbia

Abstract: Semaphorins, characterized by a conserved sema domain at the N-terminus, are a large family of molecules that guide growing axons to their targets during nervous system development. Thus far, little is known about the function of the transmembrane class 5 semaphorins which are distinguished from other classes by the presence of seven type-1 and type-1-like thrombospondin repeats C-terminal to the sema domain. Here we present the expression and function of one class 5 semaphorin, Sema5B, in the developing chick spinal cord. The dynamic expression of Sema5B in the developing spinal cord is intriguing as it appears to correlate with sensory axon targeting in the grey matter. Our research addresses the hypothesis that Sema5B regulates the timing and extent of penetration of sensory axons into the dorsal horn of the developing spinal cord. We show that sensory axons of the dorsal root ganglia avoid Sema5B-expressing HEK293 cells in vitro, therefore confirming that Sema5B can function as a guidance cue for these neurons. We also test the function of Sema5B in vivo by electroporating vectors that express short hairpin RNAs (RNAi) targeted against Sema5B into one side of the developing spinal cord grey matter.

Immunohistochemical examination of proprioceptive sensory axons show aberrant projections

into the side of the spinal cord where Sema5B expression was reduced. Thus, Sema5B is a guidance cue that is dynamically expressed throughout the embryonic spinal cord and it plays a critical role in the correct circuit formation of sensory axons during development.

Funding: This work was funded by a CIHR (MOP-13246) operating grant to TOC.

Title: CAR, a cell adhesion molecule in the developing brain, undergoes ectodomain shedding and regulated intramembrane proteolysis

Authors: Nadia Hour¹, Kuo-Cheng Huang², Luyu Zheng³, Josephine Nalbantoglu⁴

Affiliation: 1- 4 McGill University/Montreal Neurological Institute

Abstract: The Coxsackie and Adenovirus Receptor (CAR) is a cell adhesion molecule originally discovered to be a receptor for certain viruses, including ones used in gene therapy. CAR is highly expressed in the developing brain, peaking at birth and disappearing by adulthood. Results from our laboratory and others indicate that the extracellular domain of CAR enhances outgrowth of developing neurons. An increasing number of cell surface proteins have been discovered to undergo ectodomain shedding and regulated intramembrane proteolysis (RIP), often with consequences on cell signaling. We found that CAR is shed from a variety of human and murine cell types, including developing neurons, and that the metalloprotease ADAM10 is a candidate sheddase. In vitro assays and mass spectrometry revealed a putative cleavage site in CAR's ectodomain, which was confirmed by generating point mutations and expressing the mutant in cells. Similar to what has been reported for L1, metalloprotease activity was found to be required for the enhanced outgrowth of developing cerebellar neurites on CAR ectodomain substrate. Using pharmacological inhibitors and MEF knockout cells, we also found that CAR undergoes RIP by presenilin/gamma-secretase. As RIP of Notch, APP and other cell surface proteins produces cytosolic fragments that enter the nucleus and affect gene expression, we are currently investigating whether the cytoplasmic domain of CAR translocates to the nucleus following proteolysis, and if yes, whether this translocation can affect transcription. These studies will increase our understanding of how CAR is regulated and of non-canonical signaling pathways it may be involved in.

Funding: CIHR grant to J.N. CIHR Neuroinflammation Strategic Training Program and McGill Principal studentships to N.H.

Title: Glial cells decode synaptic strength of competing nerve terminals at developing mammals neuromuscular junctions

Authors: Houssam Darabid¹, Richard Robitaille²

Affiliation: 1-2 Département de Physiologie and GRSNC group FRSQ, Université de Montreal

Abstract: The maturation of synapses relies on the specific elimination of redundant connections during early developmental stages. At the neuromuscular junction (NMJ), this refinement is the result of an activity-dependant synaptic competition of two nerve terminals for the same postsynaptic site. At this developmental stage, perisynaptic Schwann cells (PSCs), glial cells at the NMJ, are apposed to competing nerve terminals. Although the role of glial cells in synaptic transmission is now recognized at mature synapses, their role during activity-dependant competition remains ill defined. We performed intracellular recordings from dually innervated NMJs simultaneously with PSCs Ca²⁺ imaging on P7 mice Soleus muscle. First, we observed that a single PSC detects synaptic activity of each competing terminals. Importantly, the size of PSCs Ca²⁺ responses is tightly related to the synaptic strength of each input. Using PSC receptors desensitisation, we found that PSCs detect transmitter release from each individual input via differentiated sets of receptors. In addition, activation of PSC is mediated by P2 receptors since bath application of a P2 receptor antagonist (RB2, 20 uM) prevented all glial Ca²⁺-responses. Finally, PSC ability to differentiate each inputs activity does not solely depend on the level of transmitter release since PSC Ca²⁺ responses were unaltered when neurotransmitter release of the weak inputs was increased using TEA (0.5 mM). In conclusion, these data indicate that PSCs decode synaptic competition at dually innervated NMJs. This suggests that a single PSC may differentially modulate each competing input and influence the dynamic process of synaptic competition and elimination.

Funding: CIHR, FRSQ, CFI, NSERC

Title: The involvement of neogenin in spinal motor neuron development

Authors: Louis-Philippe Croteau¹

Affiliation: 1- Institut de recherches cliniques de Montréal (IRCM)

Abstract: "The means by which the CNS generates the astonishing diversity among neuronal types remains obscure. In the developing spinal cord, morphogen gradients specify generic motor neuron precursors but less is known about the mechanisms responsible for further motor neuron

subtype diversification. neogenin, a member of an evolutionary conserved subfamily of immunoglobulin receptors, and its ligands from the netrin and RGM families, are widely expressed in the CNS including developing spinal motor neurons and their precursors. We are investigating the role of neogenin in chick spinal motor neuron diversification by studying the effects of electroporation of siRNAs directed against neogenin, in the developing spinal cord. While the loss of neogenin function does not affect the total number of limb innervating motor neurons, their subtype identity is altered. This phenotype is consistent with the observed differential expression of neogenin ligands in motor neuron subtypes implying that neogenin signaling promotes the specification of one motor neuron subtype at the expense of another. To test this idea, we are continuing the analysis of the effects of gain and loss of function of neogenin, its ligands and putative downstream effectors in the chick spinal cord and will begin a detailed characterization of a mouse Neo1 knockout. Interestingly, neogenin, its ligand RGMa, as well as a putative downstream effector, LMO4, have been implicated in vertebrate neural tube closure defects. Therefore, our studies, in addition to addressing the question of neuronal diversity generation, might shed some light on the molecular defects underlying a common human malformation. "

Funding: CIHR

Title: E2f3 Isoforms Balance Precursor vs Neurogenic States and Bind a Network of Neurogenic Gene Promoters

Authors: Lisa Julian¹, Catherine Pakenham², Renaud Vandenbosch³, Kelly McClellan⁴, Yubing Liu⁵, David Park⁶, Gustavo Leone⁷, Alexandre Blais⁸, Ruth Slack⁹

Affiliation: 1-3,6,9 Department of Cellular and Molecular Medicine, University of Ottawa 4- University of Ottawa 5,8- Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology 7- Human Cancer Genetics Program, Department of Molecular Virology, Immunology and Medical Genetics

Abstract: "Mutations within the pRb/E2F pathway are common events in tumourigenesis; however, we have described expanded roles for these proteins in neural development. We have recently shown that the E2F3 transcription factor is an important regulator of multiple aspects of neural precursor cell function. We now show that distinct E2f3 isoforms perform reciprocal functions in neural precursor populations, with E2f3a regulating self-renewal and E2f3b modulating progenitor cell proliferation, leading to opposing effects on neuronal output. These

intriguing results, highlighted by the observation that E2f3 activities in neural precursor cells stretch beyond basic cell cycle regulation, prompted us to ask which genes are regulated by E2f3 in these cells. We employed ChIP-on-chip technology to determine the target genes of E2f3 isoforms in neural precursors, and found that E2f3 binds over 3000 genomic promoter sites. We find that E2f3 isoforms bind a largely overlapping set of genes involved in classical and novel E2f directed activities, most markedly neurogenesis and CNS development, including multiple genes known to regulate neural precursor cell identity, self-renewal, proliferation, differentiation, death, neuronal maturation, and growth factor responsiveness. Furthermore, we show that E2f3, as well as the pRb family member p107, bind the promoter region of the neural precursor maintenance gene Sox2, and we show that knocking down Sox2 levels in E2f3a mutant cells restores normal self-renewal function. We propose that E2f3 isoforms regulate the balance between neural precursor identity and differentiation by transcriptionally regulating Sox2 itself, and other potential interacting pathways such as Notch and Shh."

Funding: This work is funded by a CIHR operating grant to RSS and a CIHR CGS award to LMJ.

Title: Pharmacological and deprivation-induced reinstatement of juvenile-like long-term potentiation in the primary auditory cortex of adult rats

Authors: Laura Rosen¹, Jennifer Hogsden², Hans Dringenberg³

Affiliation: 1-3 Queen's University

Abstract: "Sensory cortices show a decline in synaptic plasticity (e.g., long-term potentiation, LTP) during postnatal maturation. We demonstrate a partial reversal of this decline in rat primary auditory cortex (A1) by pharmacological manipulations or modifications of the acoustic environment. In adult, anesthetized rats, field postsynaptic potentials (fPSPs) in A1 elicited by medial geniculate nucleus (MGN) stimulation consisted of two sequential peaks. Simultaneous application in A1 of a γ -Aminobutyric acid (GABA)(A) receptor agonist (muscimol) and GABA(B) receptor antagonist (SCH 50911), thought to result in a preferential inhibition of intracortical activity while preserving thalamocortical inputs, suggested that these two fPSP components largely reflect thalamocortical and intracortical synapses, respectively. Rats (postnatal day [PD]60-70) showed moderate LTP of fPSPs following theta-burst stimulation (TBS) of the MGN. Interestingly, repeated episodes (PD10-20 & 50-60) of patterned sound deprivation by continuous white noise exposure resulted in substantial LTP, an effect not seen with single exposure (PD10-

20 or 50-60), or two episodes during adulthood (PD50-60 & 100-110). Thus, early sensory deprivation acts as a "prime", allowing subsequent deprivation to reinstate juvenile-like levels of LTP. Older (>PD200) rats that no longer exhibit LTP in A1 showed LTP of the first fPSP peak when TBS occurred during cortical zinc application. We conclude that the age-related decline of plasticity in A1 can be partially reversed by pharmacological techniques or manipulations of the acoustic environment during specific periods of postnatal life. "

Funding: This work was supported by a NSERC Discovery Grant to H.C.D. and a NSERC Postgraduate Scholarship to J.L.H.

Title: Cloning of a novel, non-chordate retinoic acid receptor and its role in development and growth cone guidance

Authors: Christopher J Carter¹, Christopher D Rand², Robert L Carlone³, Gaynor E Spencer⁴

Affiliation: 1- 4 Brock University

Abstract: Retinoic acid (RA) is an important molecule during neural development and regeneration of the nervous system in vertebrates and invertebrate chordates. In non-chordate species, there are limited reports providing evidence for a similar role of RA, possibly due to the reported absence of any retinoic acid receptors (RARs). We now report here, the cloning of the first full-length, non-chordate RAR (termed LymRAR) from the adult CNS of the protostome *L. stagnalis*. The LymRAR protein shares high amino acid similarity with other known vertebrate RARs and we also determined that this protein is expressed in the early stages of *Lymnaea* embryogenesis. Treatment of early embryos with various RAR antagonists resulted in shell malformations, eye defects and even halted development. Immunostaining of cultured neurons revealed RAR signals in the cytoplasm of the soma, but also in regenerating neurites and growth cones. We have previously shown that all-trans RA (atRA) can induce neurite outgrowth and growth cone turning of cultured *Lymnaea* neurons. We report here that in the presence of a RAR pan-antagonist, LE540, there is a significant reduction in atRA-induced growth cone turning (n=10). However, the RAR pan-agonist, TTNPB, was unable to mimic the effects of atRA in the growth cone response (n=15). Taken together, these data clearly show that RARs must have originated as early as bilaterians and strongly suggest an important role for LymRAR in molluscan development and growth cone guidance.

Funding: "NSERC Discovery Grant to G.E. Spencer; NSERC CGS-D to C.J. Carter."

Title: Fragile X Mental Retardation Protein Regulates Synaptic Targeting in *Drosophila*

Authors: Vedrana Cvetkovska¹, Alexa Hibbert², Brian Chen³

Affiliation: 1-3 Centre for Research in Neuroscience, McGill University

Abstract: Fragile X syndrome is the most common heritable cause of mental retardation and is the result of silencing of the Fragile X Mental Retardation gene *Fmr1*. *Fmr1* protein product, FMRP, binds RNA and suppresses its translation, and loss of FMRP results in increased protein synthesis. Our study investigates how loss of FMRP impairs synaptic targeting and identifies *Dscam* as an FMRP mRNA target whose overexpression may underlie the targeting errors. We use the stereotyped axonal branching pattern of identifiable *Drosophila* mechanosensory neurons to examine how loss of FMRP alters sensory circuit connectivity. We imaged the axonal arbors of single mechanosensory neurons in *Drosophila Fmr1* (*dFmr1*) homozygous loss of function mutants and observed specific, identifiable axonal branch targeting errors. We also found by co-immunoprecipitation that *Dscam* mRNA is selectively bound to FMRP, and flies with an extra copy of the *Dscam* gene have mechanosensory axonal arbors that phenocopy the targeting errors observed in *dFmr1* nulls. We assessed mechanosensory circuit function by eliciting a cleaning reflex in mosaic animals expressing dsRNA against *dFmr1* in mechanosensory neurons. We found that specific branches of the mechanosensory arbor are required for proper circuit function whereas others are not. Our results demonstrate that *dFmr1* is required for precise synaptic targeting and function of a hard-wired neural circuit and have identified the cell-surface molecule *Dscam* as a mediator of these effects. These results will provide important insight into how dysregulation of *Dscam* expression in Fragile X syndrome impairs neural wiring.

Funding:

Title: The Role of MicroRNA miR-133a During Caudal Spinal Cord Regeneration in *Notophthalmus viridescens*

Authors: Amanda Lepp¹, Robert Carlone²

Affiliation: 1-2 Brock University

Abstract: "Adult urodele amphibians possess the unique ability to regenerate a number of lost structures such as limbs and spinal cord following tail amputation. Caudal tail regeneration begins with the formation of a blastema, and outgrowth occurs as ependymal cells lining the central canal of the spinal cord proliferate and extend as an ependymal tube. Regeneration requires rapid global

changes in gene expression, and one current focus involves identifying molecules and signalling pathways that contribute to the formation of the tail blastema, as well as outgrowth and patterning of the regenerating spinal cord. MicroRNAs (miRNAs) are attractive candidates as regulators of regeneration in the urodele; they are small non-coding RNAs that regulate gene expression at the translational level. Recent efforts in our lab have focused on identifying miRNAs in the newt, *Notophthalmus viridescens*, which exhibit differential expression in response to injury that may contribute to caudal spinal cord regeneration after tail amputation. We have demonstrated that miR-133a is significantly downregulated during the first 14 days following tail amputation. In the intact tail spinal cord, miR-133a expression appears to be localised to the ependymal cells surrounding the central canal, and expression decreases in these cells following amputation. In the zebra fish, *Danio rerio*, miR-133a has been shown to act as a “regenerative break”, where expression is very high in the uninjured fin and then significantly decreases following fin amputation. Our data provides evidence to miR-133a acting as a “regenerative break” for spinal cord repair, and further analysis of its function is underway."

Funding: NSERC Discovery Grant

Title: Mismatched structural and functional remodeling at the developing calyx of Held-MNTB synapse following unilateral removal of sound-evoked activity

Authors: Giovanbattista Grande¹, Yi-Mei Yang², Lu-Yang Wang³

Affiliation: 1-3 Hospital for Sick Children Toronto

Abstract: Synapse development of auditory circuits is driven genetically to form initial connections but subsequently refined by spontaneous and sound-evoked activity from the cochlea. After hearing onset, ~P12 in mice, synapses between principal cells of the medial nucleus of the trapezoid body (MNTB) and the calyx of Held, a large glutamatergic nerve terminal originating from the contralateral cochlear nucleus, undergo parallel morphological and physiological transformations achieving high-fidelity transmission required for sound localization. Because these transformations occur after P12, it is generally assumed that such parallel remodeling is tightly coupled and driven by sound-evoked activity. To investigate this, we examined morphological and physiological properties of calyces and principal MNTB neurons in brainstem slices taken from P16-19 mice that underwent unilateral surgery to remove middle ear ossicles at P5. Surprisingly, we found that both ipsilateral and contralateral calyx of Held-MNTB synapses are

underdeveloped in their physiological parameters versus age-matched normal hearing animals, including smaller EPSCs (5.2 vs 5.7 vs 9.1 nA) slower decay times (1.4 vs 1.5 vs 1.2 ms), exacerbated short-term depression and prominent NMDAR-EPSCs typical of immature synapses. Morphologically, only calyces located in the MNTB contralateral to the surgical ear appeared affected, but show significant overdevelopment based on larger surface areas and volumes and more structural complexity than expected versus normal hearing animals. This striking mismatch in symmetric physiological underdevelopment and asymmetric morphological overdevelopment after unilateral ablation suggests that structural and functional remodeling at this synapse may be driven by independent mechanisms after the onset of sound-evoked activity at both ears.

Funding: CIHR

Title: Regulation of cadherin trafficking and intercellular adhesion by the GTPases Rab35 and Arf6: implication in glioma

Authors: Sarah Konefal¹, Patrick Allaire², Mohamad Seyed Sadr³, Emad Seyed Sadr⁴, Rolando Del Maestro⁵, Brigitte Ritter⁶, Peter McPherson⁷

Affiliation: 1-7 McGill University

Abstract: Arf6 is an intensely studied GTPase that promotes decreased intercellular adhesion and increased cellular migration by disrupting endosomal recycling of cadherins and enhancing recycling of integrins. Consistent with these activities, Arf6 expression is upregulated in invasive glioma. How decreased cadherin recycling is coordinated with increased integrin recycling is not clear. We recently demonstrated that Rab35 is required for endosomal recycling of MHCII (Allaire et al., Mol. Cell, 2010), a trafficking event that involves inactivation of Arf6 on endosomes. We now demonstrate that Rab35 localizes to an Arf6 compartment and that Rab35-GTP binds an Arf6 GAP (GTPase activating protein). Furthermore, Arf6-GTP binds and recruits a Rab35 GAP, suggesting that Rab35 and Arf6 provide reciprocal regulation on an adhesion molecule recycling compartment. Consistent with this hypothesis, Rab35 knock down leads to decreased surface levels and intracellular accumulation of cadherins and interferes with intercellular adhesion. Moreover, Rab35 loss of function leads to enhanced cellular migration, accompanied by increased lamellapodia formation, membrane ruffling and Erk activation. These properties are consistent with sustained Arf6 activation and the epithelial to mesenchymal transition seen in cancer. Using quantitative PCR we found that Arf6 expression was increased and Rab35 expression was

decreased as a function of tumor grade in surgically retracted human glioma. Our data indicate that Rab35 and Arf6 function in a mutually antagonistic manner to regulate cell adhesion and migration with important implications for cancer.

Funding: Canadian Institute of Health Research (CIHR)

Title: Early hypoxic rearing promotes neural stem cell properties in cortical astroglial cells of juvenile mice

Authors: Natalina Salmaso¹, Baoyuan Bi², Mila Komitova³, Maria Simonini⁴, John Silbereis⁵, Elise Cheng⁶, Janice Kim⁷, Suzannah Luft⁸, Tomas Horvath⁹, Laura Ment¹⁰, Michael Schwartz¹¹, Flora Vaccarino¹²

Affiliation: 1- 12 Yale University

Abstract: Glial fibrillary acidic protein (GFAP)+ astroglial cells give rise to new neurons during development. In the intact juvenile and adult brain, however, this process is thought to be limited to the neurogenic niches. Although astroglial cells derived from adult brains having undergone stab injury are able to generate neurons in vitro, whether GFAP+ astroglial cells are able to generate neurons in the cortical parenchyma following hypoxic injury in vivo is not known. Here, we use a genetic fate mapping technique in mice to examine the progeny of GFAP+ cells following exposure to postnatal hypoxia: a model for the brain injury observed in premature children. Following hypoxia, immature cortical GFAP+ astroglia underwent a shift towards neuronal fate and generated cortical excitatory neurons that appeared morphologically mature and synaptically integrated into the circuitry. Fate mapped GFAP+ cells derived ex vivo from hypoxic, but not normoxic mice were able to form pluripotent, long-term self-renewing neurospheres. Similarly, exposure to low oxygen conditions in vitro induced stem cell-like potential in immature cortical GFAP+ cells. Our data support the conclusion that hypoxia promotes long-term pluripotency in GFAP+ cells in the cortical parenchyma. Such plasticity possibly explains the cognitive recovery observed over time in a proportion of preterm children

Funding: This was funded by NIH (P01-NS062686) to FV, an FRSQ fellowship to NS, a Swedish Brain Foundation fellowship to MK

Title: 14-3-3 proteins regulate axonal growth cone cytoskeletal dynamics through the control of myosin II

Authors: Tadayuki Shimada¹, Christopher Kent², Dominique Guillet³, Paul Wiseman⁴, Alyson Fournier⁵

Affiliation: 1,2,5 Montreal Neurological Institute 3,4- Physics Department McGill University

Abstract: The growth cone is a critical structure regulating the speed and direction of neuronal outgrowth during development. How the growth cone spatially and temporally regulates signals from guidance cues to alter the underlying cytoskeletal dynamics is not fully known. We have previously identified several isoforms of the 14-3-3 family of adaptor proteins as major constituents of the growth cone. 14-3-3 proteins bind and regulate the activity of multiple proteins through interactions with phospho-serine and phospho-threonine containing motifs. Functional analysis of 14-3-3 proteins through the use of a peptide inhibitor and live-imaging studies revealed an important role for 14-3-3 proteins in regulating the actin and microtubule cytoskeleton. We observe that inhibition of 14-3-3 leads to a decrease in the rate of retrograde flow of F-actin in the peripheral domain, increases in filopodial length, and increases in the frequency of growing microtubules entering into the peripheral domain. These 14-3-3 loss of function phenotypes phenocopy the effects of myosin II inhibition. Furthermore, consistent with a role in myosin regulation, 14-3-3 inhibition alters myosin II distribution in the growth cone, and the phosphorylation status of the regulatory myosin light chain. Together our data suggests 14-3-3 proteins play a critical role in regulating cytoskeletal dynamics in growth cones by controlling myosin II activity.

Funding:

Title: Expression profile of three Neurofilament subunits (Light, Medium and Heavy) in the developing rodent somatosensory cortex

Authors: Alex Crocker-Buque¹, Peter Kind², Kevin Duffy³

Affiliation: 1-2 The University of Edinburgh 3- Dalhousie University

Abstract: "Molecular/biochemical markers within primary sensory cortices often reveal distinct patterns that represent aggregations of neurons with similar structure and/or function. For example, in the primate visual cortex cytochrome oxidase (CO) 'blobs' identify clusters of neurons with high metabolic activity that share thalamocortical axon (TCA) input and color processing. In contrast, neurofilament, a cytoskeletal protein involved in the maintenance of neuron shape, is predominant in neurons located outside of blobs. The rodent somatosensory cortex (SI) is also

characterized by highly metabolically active (CO positive) “patches” that receive TCA input, and regions lacking TCA input called septa. We examined whether mouse SI exhibits a non-uniform pattern of neurofilament labeling around regions with high metabolism and strong thalamic input. Using immunohistological labeling of three neurofilament subunits (light: NF-L; medium: NF-M; and heavy: NF-H) we demonstrate that in adult mice all three neurofilament subunits are preferentially located to septal regions, analogous to the pattern found in primate visual system. However, our investigation into the development of neurofilament subunits revealed divergent patterns. NF-M and NF-H initially identify a pattern consistent with TCA patches that subsequently transitions to the adult septal pattern during the second postnatal week. In contrast, NF-L identifies septa from its first appearance in layer IV at P5. Our findings suggest that molecules that underlie the organization of primary sensory regions are conserved between species. They also reveal a rather dynamic developmental expression profile for neurofilament subunits suggesting a distinct role for this cytoskeletal element during the acquisition of mature neuronal phenotype. "

Funding:

Title: Netrin-1 and DCC Promote Synapse Formation and Organize Synaptic Specializations

Authors: Jennifer Goldman¹, Mohammed Ashour², Pavel Gris³, Nicolas Tritsch⁴, Jean-Francois Bouchard⁵, Timothy Kennedy⁶

Affiliation: 1-6 McGill University

Abstract: Netrin-1 and DCC are expressed in the cortex during synapse formation and are enriched at cortical synapses in vivo. We provide evidence that netrin-1 signalling through DCC regulates cortical axon and dendrite branch complexity and the density of synapses between cortical neurons. Stimulating neurons with netrin-1 increases filopodia and branch density on developing axons and dendrites and triggers a DCC dependent increase in the density of glutamatergic synapses. In addition to enhancing the probability of initial contact between neurons, netrin-1 and DCC locally reorganize actin at sites of contact through rapamycin-sensitive protein synthesis and src family kinase signalling. Netrin-1 induced reorganization of actin is required for adhesive effects of netrin-1/DCC signalling on cortical neurons and for local enrichment of both pre- and post-synaptic protein. Our findings indicate that netrin-1 and DCC regulate cortical synapses by promoting arborisation and inducing local effects on synaptic

architecture.

Funding: CIHR.

Title: Vision Restored and Brain Abnormalities Ameliorated by Single-Copy Knock-in of Human NR2E1 in Null Mice

Authors: Jean-François Schmouth¹, Kathleen G. Banks², Anthony Mathelier³, Cheryl Y. Gregory-Evans⁴, Mauro Castellarin⁵, Robert Holt⁶, Kevin Gregory-Evans⁷, Wyeth W. Wasserman⁸, Elizabeth M. Simpson⁹

Affiliation: 1-3,8,9 Centre for Molecular Medicine and Therapeutics at the Child and Family Research Institute, University of British Columbia 4,7 Department of Ophthalmology and Visual Science, University of British Columbia 5-6 Canada's Michael Smith Genome Sciences Centre, British Columbia Cancer Agency

Abstract: NR2E1 (TLX) encodes for an orphan nuclear receptor involved in stem cell regulation of the developing forebrain and retina. Nr2e1-null mice suffer from brain and retinal disorders that can be ameliorated using multiple copies of the human NR2E1 gene. In the current study, we characterized the gene expression of a novel single-copy human bacterial artificial chromosome (BAC) NR2E1-lacZ reporter mouse strain, in the developing and adult mouse. Another mouse strain, carrying the reporter-less version of this NR2E1 BAC, was bred onto the mouse Nr2e1-null background and used to assess the complementation capacity of the human gene. Unexpectedly, cortex and olfactory bulb hypoplasia, hallmarks of the Nr2e1-null phenotype, were not fully corrected in these animals. Brain histological analyses showed reduced adult neurogenesis in the subventricular zone (SVZ) of the lateral ventricles and dentate gyrus (DG) of hippocampus. These results correlate with an absence of expression of the human NR2E1-lacZ reporter gene in the dorsal pallium in embryos and proliferative cells in the adult brain. Surprisingly, retinal analyses demonstrated histological and functional correction of the null phenotype in animals harbouring the functional BAC. These results correlate with appropriate expression of the NR2E1-lacZ reporter gene in developing and adult retina. Thus, the human genomic BAC used in this study contains all the elements allowing appropriate expression of the NR2E1 gene in the mouse retina while apparently missing regulatory regions important for proper spatio-temporal brain expression. Comparative genomic analyses suggest the discovery of candidate brain-specific stem-cell regulatory elements important for proper expression of NR2E1.

Funding: This work was funded by the U.S.A. National Institute for Mental Health (NIMH) and by genome Canada (Pleiades Promoter Project).

Title: E2F3 REGULATES NEURAL STEM CELL POPULATIONS BY MODULATING POLYCOMB GROUP PROTEINS

Authors: Catherine Pakenham¹, Lisa Julian², David Park³, Gustavo Leone⁴

Affiliation: 1-3 University of Ottawa 4- Ohio State University

Abstract: The E2f family of transcription factors are well known essential regulators of cell cycle progression. Recently, we have found that E2f3 performs unique, cell cycle independent functions, including the regulation of neural precursor cell populations. Neurosphere assays revealed that in the absence of E2f3, there is an initial increase in the number of neural precursors and that these cells have a greater self-renewal capacity compared to their wild-type counterparts. We determined by ChIP that the promoter regions of p16INK4a, a marker of cellular senescence and gene target of the polycomb group (PcG) proteins, was also a target of E2f3 and its expression was deregulated in the absence of E2f3. The PcG proteins regulate the epigenetic repression of numerous genes involved in differentiation and maintenance of stemness. Further ChIP results suggested that the PcG protein Mel-18, which has been implicated in stem cell regulation, is eluted from the p16INK4a locus in the absence of E2f3, despite consistent expression of Mel-18. E2f3 ChIP experiments also demonstrated that E2f3 binds at the promoter region of several PcG proteins, including Ezh2, the enzymatic component of polycomb repressive complex 2 (PRC2) responsible for trimethylation of histone 3 at lysine 27 (H3K27me3). Moreover, in the absence of E2f3, expression of Ezh2 and abundance of the repressive H3K27me3 mark are similarly deregulated. Taken together, these results suggest that E2f3 controls neural precursor populations by regulating the expression of p16INK4a, possibly in collaboration with PcG protein complexes, or as a result of downstream effects on PcG protein expression.

Funding: CIHR, OGSST, OGS

Title: Blockade of chemical neurotransmission reveals emergence of intermediate spontaneous behavior prior to swimming in zebrafish embryos

Authors: Joel Ryan¹, Louis Saint-Amant²

Affiliation: 1-2 Université de Montréal

Abstract: Spontaneous embryonic activity is a common trait among vertebrates. While it has been shown that spontaneous activity contributes to motor circuit formation, how the spinal cord circuitry matures from this to a coordinated motor output is unknown. In the zebrafish, activity begins around 17 hours, with slow alternating contractions of the tail, mediated by an electrically coupled motor network in the spinal cord. At 21 hours, embryos become responsive to touch, a behaviour which requires glutamatergic neurotransmission. Finally, after 28 hours, the embryo is capable of swimming, which requires glutamatergic neurotransmission, as well as input from the hindbrain. Given the increasing dependence of motor activity on chemical synapses, we investigated the role of glycinergic and glutamatergic neurotransmission in spontaneous activity prior to the onset of swimming, by analysing high-speed video recordings of behaviour at that time of development. As a result, we uncovered the emergence of an intermediate hindbrain dependent spontaneous motor behaviour occurring between the early spontaneous coils and swimming. Studying the circuitry underlying this intermediate behaviour will increase our understanding of the integration between the circuits of the hindbrain and spinal cord during maturation of the motor network.

Funding: Groupe de recherche sur le système nerveux central (GRSNC)

Title: A genetic approach to evaluate the role of DSCAM as a Netrin-1 receptor in vertebrates.

Authors: Elena Palmesino¹, Patrick Haddick², Marc Tessier-Lavigne³, Artur Kania⁴

Affiliation: 1,4 IRCM 2- Genentech, Inc. 3- Genentech, Inc. and Rockefeller University

Abstract: Down Syndrome Cell Adhesion Molecule (DSCAM) has mainly been characterized for its function as an adhesion molecule in axon growth and in self-recognition between dendrites of the same neuron. Recently, it has been shown that DSCAM can bind to Netrin-1 and that downregulation of DSCAM expression by siRNAs in chick and rat spinal cords lead to impaired growth and turning response of commissural axons to Netrin-1. In order to investigate the effect of complete genetic ablation of DSCAM to Netrin-1 induced axon guidance, we analysed commissural neurons in DSCAM mutant mice. DSCAM is expressed by commissural neurons at the time at which they extend their axon. Commissural neurons of DSCAM mutant mice are able to reach and cross the floor plate and show normal expression levels of the Netrin receptors DCC and Neogenin. Dorsal spinal cord explants of DSCAM mutant embryos, containing commissural neurons, show normal outgrowth in response to Netrin. We conclude that DSCAM is not required

for Netrin-induced axon outgrowth in vitro or for overall guidance to the floor plate in vivo in mice. Studies of mutant tissue are in progress to evaluate whether there are any defects in turning (as opposed to outgrowth) responses to Netrin in vitro, and whether there are any subtle guidance defects in vivo.

Funding:

Title: Activity Induced Plasticity in AMPAR composition at the Developing Calyx of Held Synapse

Authors: Lee Stephen Lesperance¹, Lu-Yang Wang²

Affiliation: 1-2 The Hospital for Sick Children and The University of Toronto

Abstract: The development of high fidelity synaptic transmission at the calyx of Held synapse requires the postsynaptic switch of slow-gating GluA1 dominant to fast-gating GluA4 dominant AMPARs, but the signal initiating this switch remains unknown. The onset of sound evoked neural responses (P11-12) coincides with the beginning of the AMPAR gating switch, raising the possibility that activity dependant mechanisms drive the shift in AMPAR composition. To test this, pre-hearing synapses (P7-10) were stimulated with patterned trains mimicking acoustically evoked activity. When postsynaptic neurons were held in the cell-attached mode coupled with an extended expression phase (>30 min) before membrane rupture to establish whole-cell recording mode, we found that tetanus burst stimulation significantly accelerates the fast decay time constant (τ -f) of whole-cell evoked EPSCs (eEPSCs). Furthermore, distribution histograms of miniature EPSC (mEPSC) τ -f values for control and tetanized synapses show two populations of mEPSCs with τ -f being 0.4 and 0.7 ms, respectively, and that the relative weight of the τ -f=0.4 ms population increased dramatically in tetanized synapses. Such changes in eEPSCs and mEPSCs are blocked by NMDAR or mGluR antagonism as well as inhibitors of Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) which are implicated in post-translational AMPAR regulation. Finally, these kinetic changes are absent in GluA4 knockout synapses, suggesting GluA4 is an indispensable substrate underlying this gating switch in postsynaptic neurons. These results demonstrate that activity works through NMDARs/ mGluRs and downstream signalling mechanisms to facilitate high fidelity synaptic transmission at the calyx of Held synapse in vitro.

Funding: provided by CIHR

Title: The guanine nucleotide exchange factor beta-Pix is required for netrin-1 mediated chemoattraction

Authors: Karen Lai Wing Sun¹, Sonia Rodrigues², Nicolas Stifani³, Stefano Stifani⁴, Timothy Kennedy⁵

Affiliation: 1-5 Montreal Neurological Institute

Abstract: Netrin-1 is a secreted chemoattractant that guides the axons of spinal commissural neurons to the ventral midline in the developing spinal cord. Although many molecules downstream of the netrin-1 receptor Deleted in colorectal cancer (DCC) have been identified, the precise signalling events that promote the cytoskeletal rearrangements that underlie chemoattraction are not fully understood. We have previously demonstrated that netrin-1 recruits several signalling molecules, including the Rho GTPases Cdc42 and Rac1, and their downstream effector, the serine-threonine kinase Pak1, to the intracellular domain of DCC in commissural neurons (Shekarabi et al., 2005). The formation of this signalling complex is required for DCC-mediated remodelling of the actin cytoskeleton. We then investigated the mechanisms that regulate Cdc42 and Rac1 activation downstream of DCC. Our current findings demonstrate that the Cdc42 and Rac1 specific guanine nucleotide exchange factor (GEF), beta-Pix, and its adaptor protein Git2, associate with DCC upon netrin-1 stimulation in embryonic rat spinal commissural neurons. We report that the dimerization of beta-Pix and its association with its downstream effector Pak1 are required for filopodia formation and growth cone expansion induced by netrin-1. Additionally, we provide evidence implicating beta-Pix function in commissural axon extension to the floor plate of embryonic spinal cord. These results suggest that beta-Pix plays a key role in mediating netrin-1 dependent chemoattraction of commissural neurons.

Funding: This work is supported by the Canadian Institutes of Health Research (CIHR).

Title: Characterization of the Signaling Adapter Protein Fibroblast Growth Factor Receptor Substrate 3 and its Role in Cortical Neurogenesis

Authors: Sarah Gamble¹, James MacDonald², Todd Hryciw³, Robert Grant⁴, Susan Meakin⁵

Affiliation: 1-5 The University of Western Ontario/Robarts Research Institute

Abstract: The neuronal cytoskeleton is responsible for governing dynamics such as axonal growth and cortical neurogenesis. In particular, microtubule associated proteins (MAPs), and molecular motors, are critical in many neuronal processes such as neurotransmitter and organelle transport,

as well as cortical neurogenesis. Cortical neurogenesis is initiated in mouse at E10 within the subventricular and ventricular zones, which use microtubule tracts for proper migration. The fibroblast growth factors (FGFs) act as powerful morphogens in regulating cortical development. Fibroblast growth factor receptor substrate 3 (FRS3) has been shown to interact with activated FGF receptors to mediate downstream signaling cascades. In addition to FRS3's role in signaling, preliminary evidence suggests that FRS3 is a novel microtubule binding protein, and interacts with MAPs and molecular motors (kinesin, dynein). Furthermore, FRS3's expression coincides with migrating cortical neurons in E12 cortical slices via immunohistochemistry. It is hypothesized that FRS3 regulates the migration and differentiation of neurons as well as post-mitotic neuron function by regulating microtubule stability and intracellular transport of cargo and organelles. Here, we describe FRS3 to be a novel Tau binding protein. In addition, FRS3 has been found in purified post synaptic density preparations, suggesting that FRS3 is actively transported. Lastly, FRS3 siRNA studies in E12 and E18 cortical and hippocampal cultures have suggested that FRS3 may play a role in neurite outgrowth and/or microtubule stability. Future experiments using FRS3 floxed mice will assess FRS3's role in cortical neurogenesis and migration in vivo, thereby gaining insight into FRS3's endogenous role in mouse brain development.

Funding: Krembil Foundation Canadian Institute of Health Research (CIHR)

Title: Developmental distribution of NMDA receptor subunit mRNA in auditory brainstem of rat

Authors: Enakshi Singh¹, Jane Foster², Deda Gillespie³

Affiliation: 1-3 McMaster University

Abstract: To compute interaural sound level differences, principal neurons of the lateral superior olive (LSO) integrate converging excitatory inputs from the cochlear nucleus with inhibitory inputs from the medial nucleus of the trapezoid body (MNTB). This computation requires precise tonotopic organization, and major functional tonotopic refinement is accomplished during the first postnatal week. During this period of refinement, synapses in the nascent inhibitory MNTB-LSO pathway release glutamate, which may mediate developmental plasticity through NMDA receptors (NMDARs). The GluN2A and GluN2B NMDAR subunits confer widely different properties on NMDARs, substantially affecting plasticity. We assessed postnatal developmental expression of these NMDAR subunits in the LSO and MNTB using quantitative in situ hybridization in tissue from 10 litters, ages postnatal day 1 to 36 (P1-36). In the LSO, GluN1 expression was stable P1-15,

and declined by ~50% between P15 and P36 (adult). GluN2A expression increased 100% P1-P15 whereas GluN2B expression decreased 70% P1-36, with GluN2A/2B ratio increasing 3-fold between P8 and P15. In the MNTB, GluN1 expression increased 50% from P1 to a peak at P15 and subsequently declined to P1 levels at P36. GluN2A was expressed earlier and at higher levels in the MNTB than in LSO, and the rise in GluN2A/2B ratio occurred earlier in MNTB, between P1 and P8. These data corroborate electrophysiological results showing high levels of functional GluN2B in the first postnatal week and point to a GluN2B >> GluN2A subunit switch at P8, similar to the subunit switch seen in other areas of the nervous system.

Funding: Supported by the CIHR and an Ontario Early Researcher Award.

Title: Calcium-dependent calmodulin kinase modulates AMPA currents in developing zebrafish Mauthner neurons.

Authors: Declan Ali¹, Birbickram Roy²

Affiliation: 1-2 University of Alberta

Abstract: "Glutamate AMPA receptors (AMPA receptors) are major excitatory receptors in the vertebrate CNS. Phosphorylation of AMPARs has been shown to be an important mechanism for controlling the function of these receptors in synaptic transmission and synaptic plasticity. We have previously shown that fast excitatory synaptic transmission in zebrafish Mauthner neurons is mediated predominantly by AMPARs. We are investigating the mechanisms that underlie the maturation of glutamatergic synapses and wanted to determine if CaMKII, which is expressed in developing zebrafish, might play a role in the normal development of AMPA receptor-containing synapses. Western blot analysis indicates that zebrafish embryos express CaMKII (~50 kDa in size). We used whole cell patch clamp electrophysiology to record AMPA miniature excitatory postsynaptic currents (AMPA-mEPSCs) from Mauthner cells (M-cells) of developing zebrafish. mEPSCs were recorded in the presence of elevated K⁺ (10 mM K⁺), which was used to enhance synaptic activity. Bath application of elevated K⁺ increased the amplitude and frequency of AMPA mEPSCs. Pipette application of the CaMK inhibitors, KN-62 (10 μM) and AIP (5 μM), blocked the effects of high K⁺ on mEPSC amplitude, but not frequency. We are currently performing single cell quantitative RT-PCR on Mauthner cells to determine the relative expression of various isoforms of CaMKII. Our results suggest that CaMKII may be involved in the synaptic activity-induced

enhancement of AMPA mEPSC amplitude in zebrafish."

Funding: This research was funded by grants from NSERC and CFI to DWA.

Title: Interactions between the developing cortical and peripheral sensorimotor system examined with VSD imaging in vivo

Authors: David McVea¹, Majid Mohajerani², Timothy Murphy³

Affiliation: 1-3 University of British Columbia

Abstract: Activity in the developing mammalian brain contributes to cortical maturation. Here, we focus on the role of this activity in the sensorimotor cortex by using voltage-sensitive dye (VSD) imaging to study activity in the developing rodent cortex in vivo. Studying postnatal day 4-6 rats, we exposed the cortex via a large (50 mm²) craniotomy. We collected spontaneous changes in VSD fluorescence which reflect underlying membrane potential changes. To determine how peripheral limb twitches affect activity across the cortex, we also collected video signals of peripheral movements. Initially we examined the dominant pattern of activity in the developing cortex, the spindle burst. Consisting of a fast (~20 Hz) oscillation atop a slower (~3 Hz) burst, this pattern follows afferent input caused by peripheral twitches. We compared activity following a spontaneous twitch with activity evoked by sensory stimulation, as similarities between these two signals would support the hypothesis that spontaneous twitching provides a reliable template of ascending sensory pathways during development. We showed that the spatial distribution of cortical activity following a spontaneous twitch closely resembled that which follows external sensory stimulation. Dynamic properties, however, were different, with activity following spontaneous twitches traveling more across the cortex. We next examined slower components of cortical activity, focusing on very slow (0.01-0.1 Hz) bands. We found that slow activity in the sensorimotor cortex preceded peripheral limb twitches. We propose that the coexistence of this slow activity with the subsequent fast, sensory-driven bursts may serve to calibrate and connect sensory and motor systems during development.

Funding: "Funded by CIHR operating grant to Timothy Murphy; CIHR Vanier scholarship to David McVea; CIRH, MSFHR fellowship to Majid Mohajerani."

Title: Wengen, the sole TNF Receptor family member in Drosophila, regulates cell autonomous and non-cell autonomous axonal targeting.

Authors: Wenjing Ruan¹, Julie Desberats², Edward Fon³, Philip Barker⁴

Affiliation: 1-4 McGill University

Abstract: Photoreceptor neurons (R cells) in the Drosophila retina define a map of visual space by connecting to targets in distinct layers of the optic lobe, with R1-R6 cells connecting to the lamina and R7 and R8 cells connecting to distinct layers in the medulla. Here, we show that Wengen (Wgn), the sole Drosophila member of the tumor necrosis factor (TNF) receptor (TNFR) superfamily, is required for two distinct aspects of axonal targeting. wgn loss of function disrupts R8 axon targeting due to loss of a cell autonomous wgn function that requires a physical interaction with the cytosolic ERM family member Moesin. wgn mutants also show defects in R2-5 targeting but interestingly, these are non-cell autonomous effects secondary to R8 disruption. These studies reveal that the fly TNFR plays a key role in photoreceptor target field innervation through cell autonomous and non-cell autonomous mechanisms.

Funding: This work was supported by CIHR grants (to EF and PB) and by a Parkinson Society Canada Fellowship (to WR).

Title: Membrane type matrix metalloproteinase-3 regulates neuronal responsiveness to myelin through Nogo Receptor 1 cleavage

Authors: Gino B. Ferraro¹, Charlotte J. Morrison², Christopher M. Overall³, Stephen M. Strittmatter⁴, Alyson E. Fournier⁵

Affiliation: 1,5 McGill University 2,3 University of British Columbia 4- Yale University School of Medicine

Abstract: "Nogo-66 receptor 1 (NgR1) is a glycosylphosphatidylinositol-anchored receptor for myelin-associated inhibitors (MAIs) that restricts plasticity and axonal regrowth in the CNS. NgR1 is cleaved from the cell surface of SH-SY5Y neuroblastoma cells in a metalloproteinase-dependent manner; however, the mechanism and physiological consequence of NgR1 shedding has not been explored. We now demonstrate that NgR1 is shed from multiple populations of primary neurons. Through a loss of function approach we find that membrane type matrix metalloproteinase-3 (MT-MMP3) regulates NgR1 shedding in primary cortical neurons. . Recombinant MT-MMPs 1, 2, 3 and 5 promote NgR1 shedding from the surface of primary neurons and this treatment renders neurons resistant to MAIs. Introduction of a cleavage resistant form of NgR1 reconstitutes the neuronal response to these inhibitors demonstrating that specific metalloproteinases attenuate

neuronal responses to myelin in an NgR1-dependent fashion. We are now exploring the impact of CNS injury on NgR1 shedding focusing on immune cell activation. Co-culturing human peripheral blood monocytes with cortical neurons promotes shedding of NgR1 and N-cadherin into the media. Our data suggests that MMP activity on infiltrating immune cells can modulate neuronal activity by cleaving cell surface receptors including NgR1. "

Funding: This study was supported by a grant to AEF from CIHR. Studentship to GF by the FRSQ.

Title: Subchronic peripheral neuregulin-1 administration increases adult hippocampal neurogenesis and induces antidepressant effects

Authors: Ian Mahar¹, Stephanie Tan², Maria Antonietta Davoli³, Sergio Dominguez-Lopez⁴, Gustavo Turecki⁵, Naguib Mechawar⁶

Affiliation: 1-3,5,6 Douglas Mental Health University Institute, McGill University 4- McGill University

Abstract: "Objectives: Neuregulin-1 (NRG1) is a growth factor involved in neuronal migration and differentiation in the brain. Abnormal NRG1 signaling has been implicated in psychiatric conditions. Having reported that short-term peripheral NRG1 administration increases cell proliferation in the ventral dentate gyrus (DG) of the adult hippocampus, we investigated whether this translates into an increased number of adult-born mature neurons. Our hypothesis was that DG neurogenesis would be increased in the ventral hippocampus, accompanied by antidepressant behavior. Methods: Adult mice were implanted s.c. with osmotic mini-pumps chronically delivering either saline (controls; n=7) or NRG1 (n=7; 10 µg/d) for 3d. BrdU (50 mg/kg, i.p.) was injected twice daily. Behavioral tests (locomotor and forced swim task (FST)) were conducted 28d after administration. Additional mice (n=2) were injected with BrdU 2h, 24h, 7d or 28d prior to sacrifice. Perfused animals were processed for BrdU, NeuN, ErbB3, or ErbB4 immunohistochemistry. Results: New DG cell numbers increased 28d after NRG1 administration (31%; p=0.042). As with cell proliferation, only caudal DG cells increased significantly (50%, p=0.013; rostral: p=0.20). Neuronal cell-fate was unaffected (ps>=0.9). NRG1 animals showed decreased immobility (p=0.0062) and increased swimming (p=0.0062) in the FST, without locomotor differences (duration: p=0.32; distance: p=0.26). BrdU+ DG cells were found to be ErbB3+ but not ErbB4+ at all time points. Conclusion: Peripheral subchronic NRG1 administration increases DG cell proliferation in a subregion involved in affective regulation. ErbB3 may mediate

this effect. Increased hippocampal neurogenesis and antidepressant effects occurred four weeks post-treatment. Endogenous peripheral NRG1 may regulate hippocampal plasticity and mood."

Funding: NSERC Discovery and RTI grants (NM), FRSQ (IM, NM) and CONACYT (SDL).

Title: The Role of Rb In the Regulation of Dentate Gyrus Neurogenesis

Authors: Alysen Clark¹, Renaud Vandebosch², David Park³, Ruth Slack⁴

Affiliation: 1-4 The University of Ottawa

Abstract: The retinoblastoma protein (pRb) is a key cell cycle regulator that controls the G1/S transition. Telencephalic-specific deletion of pRb results in ectopic neuroblast proliferation and abnormal migration of GABAergic neurons into the cortex. Here, we have investigated the role of pRb in hippocampal neurogenesis during late embryonic development using a Foxg1-Cre to specifically delete pRb in the telencephalon. We show that, in these pRb deficient mice, the development of the hippocampus is disrupted. For instance, at E18.5, despite the identity of hippocampal neurons seems to be conserved, the hippocampus exhibits hyperplasia and ectopic proliferation throughout the Ammon's Horn (CA) and the dentate gyrus (DG). Interestingly, that phenotype in the DG does not come from an increase of proliferation of Sox2+ precursors, but rather from a dramatic increase in the number of dividing cells expressing the bHLH transcription factor NeuroD1 in addition to those expressing the granule cell marker, Prox1. Together, these data suggest that, during hippocampal development, pRb intrinsically represses proliferation of neuronally committed cells and induces their exit from the cell cycle. We are currently investigating whether pRb plays a similar role during adult hippocampal neurogenesis.

Funding: Supported by CIHR to RSS, OGS to AMC and the University of Ottawa Cellular and Molecular Medicine Department.

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

Title: Memantine Modulation of Excitation-Inhibition Balance in Rat Hippocampus

Authors: Marzia Martina¹, Tanya Comas²

Affiliation: 1-2 National Research Council of Canada

Abstract: The hippocampus is a complex network that consists of tightly regulated interaction between excitation and inhibition. In Alzheimer's disease (AD) and other neurodegenerative disorders in which cognitive functions such as learning and memory are severely impaired, the inhibition-excitation balance in the hippocampal neuronal circuitry is impaired. Increasing evidence also suggests that, due to the role of N-methyl-D-aspartate receptor (NMDAR) in learning and memory, dysfunctions in the NMDAR may play a pivotal role in the pathogenesis of numerous neurodegenerative disorders. The uncompetitive NMDAR antagonist, memantine, clinically used for the treatment of AD may alter the inhibition-excitation balance in the hippocampus. However, the mechanism by which memantine exerts its action is not clear. To better understand the effect of memantine on hippocampal neuronal circuitry, we studied the pharmacology of memantine on the NMDAR currents of pyramidal cells (PCs) and interneurons (Ints) in the CA1 region of the hippocampus. Using whole-cell patch-clamp on acute rat hippocampal slices, we found that memantine antagonism of NMDAR currents was more effective on PCs than Ints. In particular, using specific NMDAR subunit antagonists, we found that the effect of memantine depends on the different types of NMDAR subunits expressed in PCs and Ints. Our results could help identify new targets for pharmacological exploration and could contribute to the development of refined and better-suited therapeutic strategies for the treatment of neurodegenerative disorders.

Funding:

Title: A Sodium Leak Current Regulates Pacemaker Activity of Adult Central Pattern Generator Neurons in *Lymnaea stagnalis*.

Authors: Zhong-Ping Feng¹

Affiliation: 1- University of Toronto

Abstract: The resting membrane potential of the pacemaker neurons is one of the essential mechanisms underlying rhythm generation. In this study, we described the biophysical properties

of an uncharacterized channel (U-type channel) and investigated the role of the channel in the rhythmic activity of a respiratory pacemaker neuron and the respiratory behaviour in adult freshwater snail *Lymnaea stagnalis*. Our results show that the channel conducts an inward leak current carried by Na⁺ (I_{Leak-Na}). The I_{Leak-Na} contributed to the resting membrane potential and was required for maintaining rhythmic action potential bursting activity of the identified pacemaker RPeD1 neurons. Partial knockdown of the U-type channel suppressed the aerial respiratory behaviour of the adult snail *in vivo*. These findings identified the Na⁺ leak conductance via the U-type channel, likely a NALCN-like channel, as one of the fundamental mechanisms regulating rhythm activity of pacemaker neurons and respiratory behaviour in adult animals.

Funding: The work was supported by an operating grant to ZPF from National Sciences and Engineering Research Council of Canada.

Title: "Ethanol potentiation of glycine receptors is blocked by peptide that interferes with Gβγ "

Authors: Leonardo Guzman¹, Loreto San Martin², Gustavo Moraga-Cid³, Gonzalo Yevenes⁴, Jorge Fuentealba⁵, Fabian Cerda⁶, Luis Aguayo⁷, Veronica Jimenez⁸, Jose Martinez⁹

Affiliation: 1-9 Universidad de Concepcion

Abstract: "Glycine Receptor (GlyR) is the most important inhibitory ion channels in the spinal cord and brain stem, regulating processes of pain transmission, motor coordination and respiratory rhythms. Ethanol is the major recreational drug used in the history of mankind and an important pharmacological modulator of this and other ion channels. In control condition using the patch clamp technique with transfected HEK cells the potentiation of GlyR by ethanol reach up to a 51%. It has been demonstrated that this potentiation can be inhibited by the use of Gβγ scavengers. In this work we have design peptides using the sequence of the amino terminal region of the Cytoplasmic Domain (CD) of GlyR in order to interfere with the ethanol potentiation. A peptide of 17aa (RQH) inhibited both the ethanol potentiation and the Gβγ binding *in vitro*. Smaller peptides of 10aa (RQH-C10) and 7aa (RQH-C7) caused the same inhibition of the ethanol effect decreasing to a 10%. However a peptide from a region nearby (RQH-N) did not caused any interference, either with ethanol potentiation or Gβγ binding. Throught *in silico* analysis we determined the regions and the amino acids of Gβγ involved in the interaction with these peptides. in this way we have described the specific region in GlyR and GGβγ that participate in this functional interaction.

These results are the primary step in future research with therapeutical relevance."

Funding: FONDECYT Grant 11080145, NIH Grant ROIAA 15150-01

Title: Salt-loading reverses chloride gradient via downregulation of KCC2 in rat supraoptic neurons

Authors: Katrina Choe¹, Charles Bourque²

Affiliation: 1-2 McGill University

Abstract: "The K-Cl cotransporter 2 (KCC2) maintains the reversal potential (E_{rev}) of GABA_A receptors below action potential threshold so that synaptic activation of these receptors promotes inhibition. Chronic systemic hyperosmolality induces numerous changes in the supraoptic nucleus (SON) to facilitate excitation, vasopressin secretion and osmoregulation. We tested the hypothesis that 7-day salt-loading (SL) might attenuate GABA-A mediated inhibition of SON neurons using intracellular recordings from rat hypothalamic explants in the presence of the AP5A receptor antagonist CNQX (20μM). In control explants, electrical stimulation of the diagonal band of Broca (DBB) evoked bicuculline-sensitive postsynaptic potentials (PSPs) with an E_{rev} of -59.4±1.6mV and peri-stimulus histogram (PSH) analysis showed that these resulted in a transient inhibition of action potential firing (-100.0±0%; n=6; P<0.05). In contrast, DBB stimulation in explants from SL rats evoked PSPs reversing at -31.7±5.2mV (n=13; P<0.001 vs control) and PSH analysis revealed that these functionally increased the probability of firing (+1043.4±567.9%; n=6). The E_{rev} of spontaneous PSPs were also more depolarized in SL rats (-36.4±1.9mV; n=10) than controls (-57.2±1.5mV; n=14; P<0.001). Moreover, application of the GABA-A receptor antagonist bicuculline (10μM) increased the basal firing rate of SON neurons in control explants (dHz=0.62±0.18Hz; n=9) but inhibited those in SL preparations (dHz=-1.02±0.69Hz; n=14). Immunohistochemistry and Western blotting analyses showed that KCC2 protein levels are significantly reduced in the SON of SL rats compared to controls. These results suggest that chronic SL can abolish synaptic inhibition via downregulation of KCC2 in the SON. "

Funding: CIHR

Title: A Sodium Leak Current Regulates Pacemaker Activity of Adult Central Pattern Generator Neurons in *Lymnaea stagnalis*.

Authors: Tom Lu¹, Zhong-Ping Feng²

Affiliation: 1-2 University of Toronto

Abstract: The resting membrane potential of the pacemaker neurons is one of the essential mechanisms underlying rhythm generation. In this study, we described the biophysical properties of an uncharacterized channel (U-type channel) and investigated the role of the channel in the rhythmic activity of a respiratory pacemaker neuron and the respiratory behaviour in adult freshwater snail *Lymnaea stagnalis*. Our results show that the channel conducts an inward leak current carried by Na⁺ (I_{Leak-Na}). The I_{Leak-Na} contributed to the resting membrane potential and was required for maintaining rhythmic action potential bursting activity of the identified pacemaker RPeD1 neurons. Partial knockdown of the U-type channel suppressed the aerial respiratory behaviour of the adult snail in vivo. These findings identified the Na⁺ leak conductance via the U-type channel, likely a NALCN-like channel, as one of the fundamental mechanisms regulating rhythm activity of pacemaker neurons and respiratory behaviour in adult animals.

Funding: The work was supported by an operating grant to ZPF from National Sciences and Engineering Research Council of Canada (NSERC-249962-09).

Title: Distinct sodium channel isoforms contribute to enhanced excitability within lamina I/II spinal cord neurons

Authors: Michael Hildebrand¹, Janette Mezeyova², Paula Smith³, Elizabeth Tringham⁴, Michael Salter⁵, Terry Snutch⁶

Affiliation: 1, 5 Hospital for Sick Children 2-4 Zalicus Pharmaceuticals 6- Michael Smith Laboratories

Abstract: Voltage-gated sodium channels and superficial dorsal horn (lamina I/II) neurons play key roles in acute and chronic pain processing. In fact, increased sodium channel expression and a resultant hyperexcitability within lamina I/II neurons has been directly linked to increased pain responses during neuropathic pain states. Despite this, investigations into the roles of sodium channel function in nociceptive signalling have almost exclusively been limited to recordings from recombinant channels or peripheral nociceptors. We utilize recordings from lamina I/II neurons pulled from the surface of spinal cord slices to systematically characterize the functional properties of sodium channels within these neurons. Sodium channel currents within lamina I/II neurons have relatively hyperpolarized voltage-dependent properties and fast kinetics of both inactivation and recovery from inactivation, enabling small changes in neuronal membrane

potentials to have large effects on intrinsic excitability. By combining the biophysical and pharmacological sodium channel properties with quantitative real-time PCR results, we demonstrate that functional sodium channel currents within lamina I/II neurons are predominantly composed of both NaV1.2 and NaV1.3 isoforms. Overall, lamina I/II neurons express a unique combination of functional sodium channels that are highly divergent from the sodium channels found within peripheral nociceptors, and these spinal cord sodium channels may form a potential complementary or distinct target for future pain therapeutics.

Funding: "NSERC Industrial Research and Development Fellowship; CIHR Postdoctoral Fellowship "

Title: Synaptic DCC: Novel roles in long-term potentiation and memory formation

Authors: Katherine Horn¹, Stephen Glasgow², Sarah-Jane Bull³, Tamarah Luk⁴, Jacklyn Girgis⁵, Marie-Eve Tremblay⁶, Danielle Mceachern ⁷, Jean-Francois Bouchard⁸, Michael Haber⁹, Paul Krimpenfort¹⁰, Keith Murai¹¹, Edith Hamel¹², Anton Berns¹³, Guy Doucet¹⁴, C. Andrew Chapman¹⁵, Timothy Kennedy¹⁶,

Affiliation: 1,3,4,5,7,12,16- McGill University, Montreal Neurological Institute 2,15 Concordia University 6, 8, 14 Universite de Montreal 9,11- McGill University 10,13 - Netherlands Cancer Institute

Abstract: The role of the canonical axon guidance cue netrin-1 and its receptor DCC in adult neurons is not understood. Here we demonstrate that DCC is enriched in the dendritic spines of forebrain neurons and ask whether DCC plays a role in activity-dependent changes in synaptic efficacy. Using a cre-lox genetic approach, we show deficient LTP induction, intact LTD, and impaired spatial and recognition memory as a result of conditionally deleting DCC only in adult forebrain neurons. DCC deletion also reduced dendritic spine length, and increased levels of NMDAR subunit NR2B, consistent with regression of synapses to a relatively immature state. Src activation regulates NMDAR function, and is necessary and sufficient to induce LTP. Netrin-1 binding DCC activates Src in neurons. DCC depletion results in severely reduced levels of Src activation. We conclude that DCC activation of Src in neurons is required for NMDA receptor-dependent LTP and for learning and memory.

Funding: - KEH: CGS Doctoral Award (CIHR), SDG: NSERC, CAC: NSERC and FRSQ, MET: FRSQ, TEK: FRSQ & Killam Foundation.

Title: Role of Sec8 in the Regulation of Dense-Core Vesicle Secretion in PC12 Cells

Authors: Fauzia Akbary¹, Lijun Li², Danny Bin³

Affiliation: 1-3 University of Toronto

Abstract: "The exocyst complex is composed of eight subunits, Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84, and shown to be critical for tethering secretory vesicles to the plasma membrane of yeast. Despite its conservation from yeast to mammals, its function in secretory pathways has not been characterized in mammalian cells. It was previously demonstrated that the interaction between the exocyst complex and RalA GTPase is the key to GTP-dependent exocytosis from Dense-Core Vesicles (DCV). However, its role in other regulated exocytosis, namely Ca²⁺-dependent, remains obscure. Furthermore, it is unknown whether this octameric complex exerts its functions as a single complex or as subcomplexes. To elucidate its role in GTP- and Ca²⁺-dependent exocytosis, we generated Sec8 knockdown (KD) PC12 cells. Like its Sec8 counterpart, expressions of Sec5, Sec6, and Sec3 were also markedly reduced, unlike that of Exo70 and Exo84 whose expressions were unaltered. Furthermore, Ionomycin and HighK⁺-induced Ca²⁺-dependent, and nonhydrolyzable GTP-stimulated secretion from DCV was impaired in intact and permeabilized Sec8 KD cells, respectively. Electron microscopy analysis revealed that the ratio of docked to undocked DCV were significantly reduced in these cells. Our results suggest that exocyst complex is essential for the proper function of both GTP- and Ca²⁺-dependent exocytosis probably through regulation at the tethering stage. Lastly, we speculate that the exocyst could be exerting its function possibly as subcomplexes; one constituting Sec8, Sec5, Sec6, and the other Exo70 and Exo84. "

Funding: NSERC

Title: Synaptic effects of a cholesterol chelator depend on temperature-acclimatization

Authors: Kiel G Ormerod¹, Tatiana Rogasevskaia², Jens Coorssen³, Joffre Mercier⁴

Affiliation: 1,4 Brock University 2- University of Calgary 3- University of Western Sydney

Abstract: Treatment with the 'cholesterol chelator' methyl- β -cyclodextrin (M β CD) alters synaptic function in many systems. At crayfish neuromuscular junctions (nmj), exposure to M β CD is reported to reduce excitatory junctional potentials (EJPs) by impairing impulse propagation to synaptic terminals, even though it enhances transmitter release from directly stimulated synaptic terminals. We report that effects of M β CD on synaptic properties in crayfish nmj depend on

thermal acclimatization. Treatment with M β CD impaired impulse propagation and decreased EJP amplitude in preparations from crayfish acclimatized to 14°C but not from those acclimatized to 21°C. The reduction in EJP amplitude in the cold-acclimatized group was associated with a reduction in quantal content. M β CD had no effect on input resistance in muscle fibers but decreased sensitivity to the neurotransmitter L-glutamate. This effect occurred in both warm- and cold-acclimatized crayfish, but the effect was less pronounced and reversible in the warm-acclimatized group. Treatment with M β CD reduced cholesterol in cold- and warm-acclimatized groups by an equivalent amount, and this effect was reversible by cholesterol loading only in warm-acclimatized crayfish. Thus, the effects of M β CD on glutamate-sensitivity correlated with its ability to remove cholesterol. Effects on impulse propagation and resulting EJP amplitude, however, did not correlate with cholesterol reduction. These results suggest that M β CD can affect both presynaptic and postsynaptic properties, and that the latter are related to cholesterol removal but the former are not, or are complicated by the presence of M β CD during recording.

Funding: Supported by NSERC

Title: A Consensus Phosphoinositide-Binding Motif in the P2X Receptor Family

Authors: Louis-Philippe Bernier¹, Dominique Blais², Eric Boue-Grabot³, Philippe Seguela⁴

Affiliation: 1,2,4 McGill University 3- Universite Bordeaux Segalen

Abstract: Phosphoinositides (PIPn) have been shown to be direct modulators of many types of ion channels, including the ATP-gated P2X receptors. Most P2X receptor channels are positively regulated by PIPn, but little is known concerning the molecular mechanisms of this interaction. While the involvement of specific residues on P2X has been shown in some cases, no consensus binding site has been identified for PIPn-channel interactions. Here, we demonstrate that PIPn mediate regulation of P2X receptors by binding to a dual cluster of positively-charged amino acids located on their cytosolic C-terminus. For all known P2X subtypes, the number of basic residues in these clusters determines the binding affinity of the protein to the lipid. Through mutational studies, we show that neutralizing the charge of either of the basic clusters affects P2X function in a predictable manner. In the case of P2X₄ homomers, a prototypical subtype that is regulated by PIPn through direct binding, we show that a loss-of-binding mutation leads to changes in its current phenotype similar to what is seen following pharmacological PIPn depletion. On the other

hand, P2X5 does not contain the consensus motif, P2X5 homomers do not bind to PIPn and their function is not PIPn-dependent. We show that the addition of basic residues at specific positions in the C-terminal domain dramatically affects P2X5 current amplitude and renders it sensitive to intracellular PIPn levels. Taken together, our data demonstrate that a conserved pattern of basic residues in the cytosolic C-terminal domain is responsible for P2X receptors' sensitivity to phospholipids.

Funding: This work is supported by operating grants from CIHR (PS) and CNRS (EBG). LPB holds a CIHR Canada Graduate Scholarship.

Title: Tranexamic acid inhibits glycine receptors: Insights into seizures caused by tranexamic acid

Authors: Irene Lecker¹, Dianshi Wang², David C. Mazer³, Beverley Orser⁴

Affiliation: 1-2 University of Toronto 3- St. Michael's Hospital 4- Sunnybrook Health Sciences Centre

Abstract: "Tranexamic acid (TXA), an antifibrinolytic drug used to reduce blood loss during surgery, is associated with an increased risk of post-operative seizures. The mechanisms underlying these seizures are unknown. TXA is a structural analogue of the major inhibitory neurotransmitter glycine (Gly) whose receptors (GlyRs) are expressed throughout the central nervous system. GlyRs mediate both synaptic and tonic inhibition and their pharmacological blockade is associated with seizures. Here, we tested the hypothesis that TXA inhibits GlyR function and examined whether TXA-mediated inhibition can be reversed. Whole-cell voltage clamp techniques were used to record Gly-evoked currents from cultured cortical and spinal cord neurons. Results: TXA rapidly and reversibly inhibited GlyRs in cortical and spinal cord neurons. Gly currents, evoked by half-maximal concentrations of Gly (EC50 cortex: 97.9 ± 43.5 μ M ; EC50 spinal cord: 30.9 ± 14.6 μ M), were blocked by TXA (IC50 cortex: 1.1 ± 0.04 mM ; IC50 spinal cord: 1.4 ± 0.1 mM). In addition, TXA acted as a competitive antagonist (EC50 Gly control: 93.9 ± 6.8 μ M ; EC50 Gly + TXA: 207.1 ± 38.9 μ M) and TXA-mediated inhibition of GlyR was voltage- and use-independent. Tonic Gly current was particularly sensitive to TXA inhibition (IC50 tonic: 0.09 ± 0.03 mM) and co-application of isoflurane, but not propofol or midazolam, fully reversed the inhibition. Our results provide the first evidence that TXA, at clinically relevant concentrations, inhibits GlyRs. Additionally, the reversal of TXA-mediated inhibition by isoflurane offers a plausible prevention and/or reversal strategy for TXA-induced seizures in patients."

Funding: Canadian Institutes of Health Research (MOP-38028, MOP-79428), Canadian Anesthesiologists' Society and CRC.

Title: Anoxic regulation of Ca²⁺-activated K⁺ currents in turtle cortex

Authors: Corinne Rodgers-Garlick¹, Leslie Buck²

Affiliation: 1-2 University of Toronto

Abstract: "In response to low ambient oxygen levels facultative anaerobes, such as the western painted turtle (*Chrysemys picta bellii*), undergo a large depression in metabolic rate thereby reducing ATP demand. This involves ion channel arrest (CA) via decreased AMPAR and NMDAR currents in turtle cortical neurons; however, an anoxia-induced decrease in activity of other ion channels has not been demonstrated in turtle cortical neurons. In a search for other channels we discovered a Ca²⁺-activated K⁺ channel (KCa) that was responsive to changes in oxygen tension. Cortical brain sheets were bathed in either normoxic or anoxic aCSF, and K⁺ and Ca²⁺ concentrations were manipulated in both bath and pipette solutions. Single-channel recordings of KCa activity in pyramidal neurons were performed in cell-attached and inside-out excised patch configurations. We showed that the channels are selective for K⁺, have a conductance of 214 pS, and are controlled by [Ca²⁺] on the intracellular side of the cell membrane. We also showed a reversible 4-fold reduction in Popen of these channels following 30 min anoxia. We speculate that arrest of KCa channels during anoxia prevents a rise in extracellular [K⁺] ([K⁺]_o), thereby promoting maintenance of transmembrane ion gradients and reducing susceptibility to anoxic depolarization (AD)."

Funding: This work was supported by an NSERC Discovery Accelerator Supplement to L.T. Buck.

Title: CaM-Ca²⁺ Regulation of TRPM2 currents

Authors: Brian Lockhart¹, Oies Hussein², John MacDonald³, Michael Jackson⁴

Affiliation: 1-4 University of Western Ontario

Abstract: TRPM2 (transient receptor melastatin-like type 2), a non-selective cation channel with substantial permeability for Ca²⁺, has been implicated in the death of neurons following oxidative stress. Gated through binding of ADP-ribose to a site residing within the intracellular C-term, channel opening is also reliant on Ca²⁺-calmodulin binding to the N-term TRPM2. Ca²⁺ has been shown to promote channel inactivation however the mechanisms involved are not understood. We

therefore examined the possibility that inactivation may similarly involve Ca^{2+} -calmodulin. Using whole-cell voltage-clamp, currents were recorded from T-Rex-293 cells stably expressing TRPM2 under a tetracycline inducible promoter. The dependence of TRPM2 currents on intracellular Ca^{2+} and CaM were determined by either adding the Ca^{2+} chelator BAPTA or CaM to the patch-pipette. The effects of varying voltage as well as the actions of altering extracellular concentrations of Ca^{2+} were also tested. CaM binding to TRPM2 was investigated using CaM-Sepharose beads together with TRPM2 truncation mutants stably expressed in cell lines. We found that TRPM2 currents were activated in part by elevations in intracellular Ca^{2+} concentrations, and that buffering intracellular Ca^{2+} abolished the current. Currents underwent a time-dependent inactivation process that required intracellular CaM. Furthermore, inactivation was dependent on extracellular Ca^{2+} concentrations, and decreased proportionally as membrane potential was set closer to the reversal potential for Ca^{2+} . CaM bound to both the N- and C-terminals of TRPM2 suggesting multiple possible binding sites for regulation the channel. This information may help to identify potential novel therapeutic treatments that target TRPM2.

Funding: CIHR

Title: "Modulation of Cav3.2 activity through a physical and functional interaction with nNOS; a potential inhibitory feedback circuit for neuronal excitability."

Authors: Kirk Mulatz¹, Anamika Singh², William K. Milsom³, Terrance P. Snutch⁴

Affiliation: 1-4 University of British Columbia

Abstract: Modulation of neuronal activity is an essential component in providing adaptive responses to external stimuli. Redox regulation of the Cav3.2 T-type voltage-gated calcium channel has been shown to affect the overall excitability and firing patterns of distinct types of central and peripheral neurons. A potential source of oxidizing molecules within neurons is neuronal nitric oxide synthase (nNOS), a calcium dependent enzyme which produces nitric oxide (NO). The carboxyl terminus of the Cav3.2 channel possesses a motif which is compatible with the PDZ3 domain of nNOS and we hypothesized that the physical and functional coupling between Cav3.2 and nNOS may contribute to the T-type channel-dependent modulation of neuronal activity. Transiently transfected HEK cells were utilized to demonstrate that nNOS co-immunoprecipitates with the wild type Cav3.2 channel but not when the carboxyl PDZ3 motif is mutated. We further demonstrated that the PDZ3 mediated interaction is required for the activation of nNOS by

Cav3.2-mediated calcium influx by measuring NO production with an NO-sensitive fluorometric compound incorporated into a chemical depolarization assay. Additionally, using whole cell patch clamp to study Cav3.2 T-type channel functional activity in cells co-transfected with nNOS we found that Cav3.2 channels are inhibited by NO although independently of soluble guanylyl cyclase. Currently, we are investigating the role of the Cav3.2/nNOS complex in vivo by examining the effect of disrupting the PDZ3 interaction on the hypoxic and hyperoxic ventilatory response.

Funding: Michael Smith Foundation for Health Research/BC Epilepsy Society, Canadian Institutes of Health Research

Title: Fragile X Related Protein 1 Controls Spine Size by Regulating Local Protein Synthesis in Dendrites and at Spines

Authors: Denise Cook¹, Maria del Rayo Sanchez-Carbente², Claude Lachance³, Danuta Radzioch⁴, Edouard Khandjian⁵, Luc DesGroseillers⁶, Keith Murai⁷

Affiliation: 1,3,4,7 McGill University 2,6 Université de Montréal 5- Université Laval

Abstract: Local protein synthesis at synapses is important for forming and storing memories in neuronal networks. New proteins are required for long-lasting changes in synapse strength and size in response to high levels of synaptic activity. To ensure that proteins are made at the appropriate time and location to support these synaptic changes, messenger RNA translation is tightly controlled by dendritic RNA-binding proteins. Fragile X Related Protein 1 (FXR1P) is an RNA-binding protein with high homology to Fragile X Mental Retardation Protein (FMRP) that is known to repress and activate protein synthesis in non-neuronal cells. However, unlike FMRP, very little is known about the role of FXR1P in the central nervous system. To determine whether FXR1P controls spine size by regulating local protein synthesis in neurons, we studied the expression and targeting of FXR1P in neurons as well as the effect of loss-of-function and gain-of-function of FXR1P on dendritic spine size. We find that FXR1P co-localizes with ribosomes and messenger RNAs on dendrites and at a subset of dendritic spines in hippocampal pyramidal neurons. We further demonstrate that loss of FXR1P reduces spine density and spine size on CA1 apical dendrites, whereas over-expression of FXR1P results in targeting of FXR1P to large spines. These results suggest that FXR1P may regulate the translation of messenger RNAs important for activity-dependent changes to spine size.

Funding: CIHR Frederick Banting and Charles Best Canada Graduate Scholarship FRSQ Doctoral Research Award

Title: Mechanism of chemical transmission between a glial cell and its associated neuron in the chick dorsal root ganglion

Authors: Gabriela Rozanski¹, Elise Stanley²

Affiliation: 1-2 Toronto Western Research Institute

Abstract: Glial cells are closely associated with chemical synapses throughout the nervous system. Although their structurally supportive roles in processes such as axon myelination and synaptogenesis are relatively well characterized, less is known about the involvement of glial cells in neuronal function. In this study we explore the mechanism of chemical transmission between a glial cell and its associated neuron in the chick dorsal root ganglion (DRG). DRG neurons were gently dissociated to retain connections with their ensheathing satellite glial cells (SGCs). The patch clamp technique was used to record ion currents from cells and pharmacological agents were applied to dissect the signaling pathways. We found that BzATP, a P2X7 receptor agonist, puffed onto a SGC can generate a dose duration-dependent increase in inward current in the ensheathed neuron. The purinergic antagonist suramin inhibited the drug's effect. BzATP puffed directly onto a fully isolated neuron had little or no direct effect on DRG neuron current fluctuations, suggesting that the neuronal response can be attributed to SGC secretion. This was verified by the finding that transmission was inhibited in the intact structure in the presence of nicotinic antagonists d-tubocurarine and pancuronium bromide to block neuronal acetylcholine (ACh) receptors. Our results suggest that activation of purinergic receptors on SGCs triggers the secretion of ACh which activates the associated DRG neuron.

Funding: CIHR

Title: "Modulation of Cav3.2 activity through a physical and functional interaction with nNOS; a potential inhibitory feedback circuit for neuronal excitability."

Authors: Kirk J Mulatz¹, Anamika Singh², William K. Milsom³, Terrance P. Snutch⁴

Affiliation: 1-4 University of British Columbia

Abstract: Modulation of neuronal activity is an essential component in providing adaptive responses to external stimuli. Redox regulation of the Cav3.2 T-type voltage-gated calcium channel

has been shown to affect the overall excitability and firing patterns of distinct types of central and peripheral neurons. A potential source of oxidizing molecules within neurons is neuronal nitric oxide synthase (nNOS), a calcium dependent enzyme which produces nitric oxide (NO). The carboxyl terminus of the Cav3.2 channel possesses a motif which is compatible with the PDZ3 domain of nNOS and we hypothesized that the physical and functional coupling between Cav3.2 and nNOS may contribute to the T-type channel-dependent modulation of neuronal activity. Transiently transfected HEK cells were utilized to demonstrate that nNOS co-immunoprecipitates with the wild type Cav3.2 channel but not when the carboxyl PDZ3 motif is mutated. We further demonstrated that the PDZ3 mediated interaction is required for the activation of nNOS by Cav3.2-mediated calcium influx by measuring NO production with an NO-sensitive fluorometric compound incorporated into a chemical depolarization assay. Additionally, using whole cell patch clamp to study Cav3.2 T-type channel functional activity in cells co-transfected with nNOS we found that Cav3.2 channels are inhibited by NO although independently of soluble guanylyl cyclase. Currently, we are investigating the role of the Cav3.2/nNOS complex in vivo by examining the effect of disrupting the PDZ3 interaction on the hypoxic and hyperoxic ventilatory response.

Funding: Michael Smith Foundation for Health Research/BC Epilepsy Society, Canadian Institutes of Health Research

Title: N-type Ca²⁺ channels carry the largest current: implications for nanodomains and transmitter release

Authors: Fiona K Wong¹, Alexander M Weber², Adele R Tufford³, Lyanne C Schlichter⁴, Victor Matveev⁵, Elise F Stanley⁶

Affiliation: 1-4,6 Toronto Western Research Institute 5- New Jersey Institute of Technology

Abstract: A number of studies support the conclusion that single Ca_v channel Ca²⁺ nanodomains gate molecular signaling pathways. Thus, at presynaptic terminals single Cav2.2 channels trigger fusion of synaptic vesicles (SVs) by saturating a nearby calcium sensor. It is generally accepted that Cav1, Cav2, and Cav3 families (L, N and T, respectively) exhibit a decreasing order of single channel conductance. Since nanodomain dimensions are proportional to single channel current amplitude (*i*), high-conductance L type channels would be expected to be favoured over the intermediate conductance N-type. Since the L>N>T hierarchy was determined with high Ba²⁺+EXT, we tested the idea that this sequence may differ at physiological Ca²⁺+EXT. We recorded *i* values

for all three CaV families across a broad range of Ca²⁺EXT, spanning the physiological range. We focused on i(-65mV) to avoid non-linear current-to-voltage relationship complications and for direct relevance to the gating of synaptic transmission. A Cav2.2>Cav1>Cav3.2 hierarchy was determined for i(-65mV) at 1-2 mM Ca²⁺EXT. Mathematical modeling predicts that the Cav2.2 Ca²⁺ nanodomain is ~25% more extensive than that generated by CaV1. We also calculated single channel 'SV fusion' domains, defined as the radii where the channel would saturate >50% of 5-binding site calcium sensors. With a sensor binding affinity of 10uM a single Cav2.2 can activate a calcium-fusion sensor located on the proximal face of the synaptic vesicle. These findings may explain why CaV2 family channels are preferred for transmitter release site gating.

Funding:

Title: Insulin induces endocannabinoid-mediated long-term depression of ventral tegmental area dopaminergic neurons.

Authors: Gwenael Labouebe¹, Stephanie Borgland²

Affiliation: 1-2 University of British Columbia

Abstract: Excitatory synaptic transmission of dopamine (DA) neurons of the ventral tegmental area (VTA) mediates reward seeking behaviours. Plasticity of this system may override the homeostatic systems controlling food intake. Little is known about how insulin modulates the activity of VTA DA neurons. Insulin receptors are expressed on VTA neurons and direct action of insulin in this area can reduce palatable food eating. However, functional evidence for direct insulin receptor signaling on DA neurons has not been reported. Using electrophysiological recordings in mouse midbrain slices, we demonstrated that insulin induced a long-term depression (LTD) of AMPA receptor-mediated synaptic transmission of VTA DAergic neurons. Insulin-induced LTD was initiated by activation of the insulin receptor and required mTOR signaling. Activation of this pathway led to an endocannabinoid-mediated inhibition of glutamate release as a CB1 receptor antagonist, AM251, inhibited the insulin-mediated suppression of mEPSCs frequency and blocked LTD. A dysfunction of insulin-induced LTD may contribute to the pathogenesis of obesity. Thus, we found that in the hyperinsulinemic BTBR mice genetically prone to obesity, insulin-mediated suppression of synaptic transmission was significantly compromised. To determine if insulin in the VTA modifies ingestive behavior, insulin was microinjected in VTA before fasted chow feeding or sated sweetened-high fat (hedonic) feeding. Intra-VTA insulin

significantly reduced hedonic feeding, but did not affect regular chow intake. Taken together, insulin suppresses synaptic transmission onto VTA DAergic neurons via endocannabinoid-mediated LTD. These results add new insight to how insulin may control normal and pathogenic feeding by suppressing synaptic transmission of mesolimbic DA neurons.

Funding: Canadian Institutes of Health Research (CIHR) Swiss National Science Foundation (SNSF)

Title: "Modulation of Cav3.2 activity through a physical and functional interaction with nNOS; a potential inhibitory feedback circuit for neuronal excitability."

Authors: Kirk J Mulatz¹, Anamika Singh², William K. Milsom³, Terrance P. Snutch⁴

Affiliation: 1-4 University of British Columbia

Abstract: Modulation of neuronal activity is an essential component in providing adaptive responses to external stimuli. Redox regulation of the Cav3.2 T-type voltage-gated calcium channel has been shown to affect the overall excitability and firing patterns of distinct types of central and peripheral neurons. A potential source of oxidizing molecules within neurons is neuronal nitric oxide synthase (nNOS), a calcium dependent enzyme which produces nitric oxide (NO). The carboxyl terminus of the Cav3.2 channel possesses a motif which is compatible with the PDZ3 domain of nNOS and we hypothesized that the physical and functional coupling between Cav3.2 and nNOS may contribute to the T-type channel-dependent modulation of neuronal activity. Transiently transfected HEK cells were utilized to demonstrate that nNOS co-immunoprecipitates with the wild type Cav3.2 channel but not when the carboxyl PDZ3 motif is mutated. We further demonstrated that the PDZ3 mediated interaction is required for the activation of nNOS by Cav3.2-mediated calcium influx by measuring NO production with an NO-sensitive fluorometric compound incorporated into a chemical depolarization assay. Additionally, using whole cell patch clamp to study Cav3.2 T-type channel functional activity in cells co-transfected with nNOS we found that Cav3.2 channels are inhibited by NO although independently of soluble guanylyl cyclase. Currently, we are investigating the role of the Cav3.2/nNOS complex in vivo by examining the effect of disrupting the PDZ3 interaction on the hypoxic and hyperoxic ventilatory response.

Funding: Michael Smith Foundation for Health Research/BC Epilepsy Society, Canadian Institutes of Health Research

Title: Investigating the roles of N-cadherin and nectin in synapse formation using in vivo approaches

Authors: Wiam Belkaid¹, Omar De Faria Junior², Ajit Dhaunchak³, David Colman⁴

Affiliation: 1-4 Montreal Neurological Institute

Abstract: "The cell adhesion molecules family includes a plethora of molecules each of them playing essential roles at different junctions in the central nervous system. Formation of synapses, generally occurring between an axon and a dendrite from two nerve cells, requires specific alignment and attachment of the pre- and post-synaptic sides. Several molecules have been implicated in this process; two that we are interested in are the neuronal-cadherin (N-cadherin) and Nectins. N-cadherin, a Ca²⁺-dependent adhesion molecule, is critical for neuronal migration and axonal pathfinding during development within the mouse central nervous system (CNS). In the absence of N-cadherin, the brain is malformed and mice die prematurely. Nectins belong to a class of Ca²⁺-independent cell-adhesion molecules and these molecules have been shown to play an important role in recruiting N-cadherin at puncta adherens junctions. Abnormal dendritic spine morphology and male-specific infertility are the main phenotypes observed in both nectin 1- and nectin 3-null mice. In order to better understand the function of these molecules in synaptogenesis and memory formation we generated two transgenic mice with altered levels of N-cadherin and nectins in the hippocampus. With these mice, the roles of N-cadherin and nectins in synapse formation are being investigated. Here we present the characterization of these two mice and a biochemical analysis of synaptic molecules expression for the N-cadherin and the nectin overexpressing mice. "

Funding:

Title: Beta-adrenergic Receptors Engage Transcription and Epigenetic mechanisms to Enhance LTP

Authors: Sabyasachi Maity¹, Steven A. Connor², Jonathan Wong³, Kian Parseyan⁴, Peter V. Nguyen⁵

Affiliation: 1-5 University of Alberta

Abstract: Norepinephrine (NE) stimulates beta-adrenergic receptors (BARs) in the hippocampus, a brain structure crucial for episodic and spatial memory formation. Activation of hippocampal BARs facilitates long-term potentiation (LTP), an activity-dependent increase in synaptic strength

believed to underlie declarative learning and memory consolidation. Previous research has shown that BAR-mediated LTP requires synthesis of new proteins to maintain enhanced synaptic responses. It is unclear whether BAR-LTP requires the synthesis of new mRNA or engages epigenetic mechanisms to enhance synaptic strength. These epigenetic processes involve post translational modifications of chromatin which can alter patterns of gene expression. Thus, we assessed the hypothesis that transcription and epigenetic mechanisms are required for the persistence of BAR-dependent LTP. Extracellular field EPSPs were recorded to measure synaptic strength at CA1-Schaeffer collaterals in mouse hippocampal slices. To determine if transcription is required for BAR-LTP, we inhibited transcription with either actinomycin-D or DRB. Blocking transcription significantly reduced the duration of BAR-LTP. Recently, epigenetic mechanisms have been implicated in synaptic plasticity, learning, and memory (Levenson et al., 2004). To determine if epigenetic mechanisms regulate BAR-LTP, we used a DNA methylation inhibitor, 5'-Aza-2'-deoxycytidine (5-AZA), which blocks methylation of cytosine in DNA. Our data suggest that BAR-LTP persistence requires DNA methylation, as maintenance of LTP was decreased in the presence of 5'-AZA. Our results suggest that transcription and epigenetic mechanisms are engaged by BAR stimulation that boosts the duration of LTP. We speculate that epigenetic recruitment could help boost the formation of resilient memories of key events during an organism's life span.

Funding: CIHR, NSERC, AHFMR

Title: Impact of overexpressed vesicular acetylcholine transporter (VACHT) on other components of the cholinergic system

Authors: Paul Nagy¹, Isabelle Aubert²

Affiliation: 1-2 Sunnybrook Research Institute 2- Sunnybrook Research Institute

Abstract: Septohippocampal cholinergic neurons produce the neurotransmitter acetylcholine and are essential to critical brain functions, including memory and learning. Acetylcholine is synthesized by the enzyme choline acetyltransferase (ChAT) and is localized to synaptic vesicles by the vesicular acetylcholine transporter (VACHT). Appropriate ChAT and VACHT expression are required for adequate function of cholinergic neurons, however the detailed mechanisms that regulate cholinergic tone are not fully understood. Here we used transgenic mice modified to express the enhanced green fluorescence protein under control of the ChAT promoter. We show that this transgenic modification leads to a triplicate in the VACHT gene copy number compared to

non-transgenic controls. This modification led to significant increases in VACHT mRNA expression in both embryonic (29%) and adult (117%) septal brain tissue compared to controls. In embryonic tissue, increased VACHT expression results in a significant reduction in ChAT and high affinity choline transporter (CHT1) mRNA expression. In contrast, we found that adult septal cholinergic gene expression was not significantly different compared to controls. Consistent with these findings, ChAT protein and enzymatic activity were not significantly different at the adult stage. These data suggest that in response to VACHT overexpression, ChAT and CHT1 can be modified at the transcriptional level at embryonic stages, perhaps as a mechanism to maintain normal cholinergic homeostasis. These particular regulatory events were not observed in the adult septum. Taken together, this study contributes to our understanding of cholinergic regulation during development, specifically in response to increased VACHT expression.

Funding: CIHR, NSERC

Title: TRPV1 and TRPV4 channels contribute toward steady state levels of excitability in the mouse supraoptic nucleus at physiological temperatures in situ

Authors: Jessica Sudbury¹

Affiliation: 1- McGill University

Abstract: "Neuronal TRPV channels are thought to function as thermosensors; however few studies have addressed the effects of natively expressed TRPV channels on neuronal excitability at physiological temperatures in situ. Here, we compared the excitability of supraoptic nucleus (SON) neurons maintained at a fixed physiological temperature (37°C) in acute hypothalamic slices prepared from wild-type (WT), *trpv1* (TRPV1^{-/-}), *trpv4* (TRPV4^{-/-}) and *trpv1* and 4 (TRPV1,4^{-/-}) gene knockout mice. During whole-cell patch clamp recordings, SON neurons were stimulated with 2-second current pulses. Membrane excitability parameters were compared across genotypes: resting membrane potential, membrane input resistance, firing frequency, and rheobase current. Steady state voltage-current analysis indicated that TRPV1,4^{-/-} neurons are significantly hyperpolarized compared to WT neurons, and TRPV1^{-/-} and TRPV4^{-/-} neurons are intermediate to these groups (at I= 0 pA in mV: WT, -43.5 ± 1.6 n= 22; TRPV1,4^{-/-}, -68.4 ± 2.2 n= 6; TRPV1^{-/-}, -56.8 ± 1.7 n= 15; TRPV4^{-/-}, -52.8 ± 4.1 n= 9; one-way ANOVA p< 0.001). Knockout neurons exhibited significantly higher input resistance than WT neurons. Further, compared to WT neurons, knockout neurons showed rightward-shifted spike frequency-current (F-I) relations,

and significantly lower firing frequencies (at $I=0$ pA in Hz: WT, 15.9 ± 3.4 n= 21; TRPV1,4-/-, 1.0 ± 0.3 n= 6, TRPV1-/-, 1.3 ± 0.7 n= 15; TRPV4-/-, 3.7 ± 1.4 , n= 9; one-way ANOVA $p < 0.05$). Finally, rheobase current was significantly lower in WT compared to knockout neurons. In general, we conclude that TRPV1 and TRPV4 channels in part determine SON neuronal excitability at physiological temperatures in situ. "

Funding: CIHR Canada Graduate Scholarship FRSQ Doctoral Award

Title: A Modeling Study of AMPA and NMDA Mediated Responses in Thalamocortical Neurons

Authors: Francis Lajeunesse¹, Helmut Kröger², Igor Timofeev³

Affiliation: 1-3 Laval University

Abstract: Thalamocortical (TC) neurons of the ventroposterolateral (VPL) nucleus of the thalamus receive excitatory inputs from lemniscal fibers at thick proximal dendrites, but excitatory synapses are also established at thin distal dendrites by corticothalamic (CT) fibers. Lemniscal synapses activate mainly AMPA receptors, while CT fibers activate both AMPA and NMDA receptors. Using a multicompartment model based on reconstructed TC cells from the VPL nucleus of the cat, we investigated the amplitude of synaptic responses to proximal and distal inputs for both AMPA and NMDA mediated currents. Computations were performed on the NEURON simulation environment. EPSPs induced at the site of stimulation were larger for thin dendrites than for thick ones. Due to the low-pass properties of passive dendrites, EPSPs were dramatically attenuated during their propagation toward the soma, particularly in thin dendrites. Therefore the amplitude of somatic responses for AMPA mediated currents was higher for synapses located on thick proximal dendrites. In contrast, due to longer time courses, EPSPs induced by NMDA mediated currents attenuated much less and were therefore more efficient in depolarizing the soma when synapses were located at thin distal dendrites. Despite highly variable EPSPs at the site of stimulation, the variability of somatic responses to both AMPA and NMDA mediated currents was small within each cell, but high among different neurons. The model predicts that propagating EPSPs induced at different branches have similar impacts on the somatic compartment.

Funding: NSERC, CIHR, NIH and FRSQ

Title: States of vigilance modulation of synaptic responses in the thalamocortical somatosensory system.

Authors: Sylvain Chauvette¹, Igor Timofeev²

Affiliation: 1-2 Laval University, Centre de Recherche Université Laval Robert-Giffard

Abstract: "Waking state is characterized by rich and diverse sensory experiences, while during slow-wave sleep (SWS) virtually no sensory information is perceived and during REM sleep sensory perception is illusory. Here we investigated how states of vigilance modulate the shape of cortical responses from both local field potential (LFP) and from intracellular recordings in somatosensory cortex of non-anaesthetized head-restrained cats. States of vigilance were controlled by simultaneous LFP, EOG and EMG recordings. Electrical stimuli were delivered to the medial lemniscus (1Hz) and the amplitude of stimulation was adjusted just above threshold intensity for evoked potential generation. LFP recordings showed the largest averaged cortical response amplitude during REM, a bit smaller during wake, and the smallest during SWS. Intracellular recordings also revealed the highest amplitude of excitatory responses during REM sleep and the lowest variability in latency; stimuli during WAKE showed a low variability in latency and a high amplitude, while SWS showed the highest variability in latency of responses and the smallest amplitude. Excitatory responses were often preceded by an IPSP and followed by a long period of quiescence. We conclude that during brain activated states (REM and WAKE) the latency of responses is quite fixed and because cortical cells are depolarised more simultaneously, LFP responses are ampler. The membrane potential of cortical neurons being similar in all states of vigilance, our results suggest that the variation in response latency observed during SWS depends on the membrane potential of thalamocortical neurons. "

Funding: Supported by CIHR, NIH, FRSQ, and NSERC

Title: Monoaminergic firing activity in the VTA and the DR across the light-dark cycle

Authors: Sergio Dominguz-Lopez¹, Rebecca Howell², Martha Graciela Lope-Canul³, Marco Leyton⁴, Gabriella Gobbi⁵

Affiliation: 1-5 Department of Psychiatry, McGill University

Abstract: "In this work we characterized the firing activity of dopaminergic (DA) and serotonergic (5-HT) neurons in the ventral tegmental area (VTA) and the dorsal raphe (DR) nuclei, respectively, across the light-dark cycle. Rats kept under a constant 12/12h light-dark cycle (lights on at 7h)

were used to perform in vivo single-unit extracellular recordings at 6 different time intervals (7-11h, 11-15h, 15-19h, 19-23h, 23-3h and 3-7h). In the VTA, DA firing rate oscillates between intervals but not significantly (7-11h: 3.5 ± 0.4 Hz; 11-15h: 2.7 ± 0.2 Hz; 15-19h: 2.8 ± 0.3 Hz; 19-23h: 3.6 ± 0.3 Hz; 23-3h: 2.9 ± 0.3 Hz; 3-7h: 2.8 ± 0.4 Hz; $F(5,203)=0.792$, $p=0.556$). At 15-19h and 19-23h, a significant decrease in the number of spontaneously active neurons was observed ($p < 0.05$ in both cases), compared with the peak observed at 23-3h (7-11h: 2.2 ± 0.3 neurons/track; 11-15h: 2.4 ± 0.4 neurons/track; 15-19h: 1.6 ± 0.1 neurons/track; 19-23h: 1.5 ± 0.2 neurons/track; 23-3h: 2.9 ± 0.2 neurons/track; 3-7h: 2.2 ± 0.3 neurons/track; $F(5,96)=3.065$, $p=0.013$). In the DR, the 5-HT neuronal activity decreases during the dark phase. In particular, 5-HT firing rate significantly decreases at 3-7h (7-11h: 0.9 ± 0.1 Hz; 11-15h: 0.85 ± 0.1 Hz; 15-19h: 0.98 ± 0.09 Hz; 19-23h: 0.75 ± 0.1 Hz; 23-3h: 0.65 ± 0.06 Hz; 3-7h: 0.53 ± 0.09 Hz; $F(5,253)=2.841$, $p=0.016$) and the number of spontaneously active neurons decreases at 19-23h and at 23-3h (7-11h: 3.0 ± 0.6 neurons/track; 11-15h: 3.7 ± 0.2 neurons/track; 15-19h: 3.8 ± 0.3 neurons/track; 19-23h: 2.6 ± 0.3 neurons/track; 23-3h: 2.4 ± 0.2 neurons/track; 3-7h: 2.6 ± 0.3 neurons/track; $F(5,79)=3.331$, $p=0.009$), compared with the peak detected at 15-19h in both parameters ($p < 0.05$, in all cases). These data suggest that DA and 5-HT neuronal populations have distinct diurnal rhythms of firing activity. "

Funding: SDL was supported by a scholarship from the Mexican National Council for Science and Technology (CONACYT, Reg. 193202/302017).

Title: Short-term plasticity during wake-like and sleep-like presynaptic pattern of stimulation

Authors: Josée Seigneur¹, Igor Timofeev²

Affiliation: 1-2 Centre de recherche Université Laval Robert-Giffard

Abstract: Recent studies demonstrated that slow-wave sleep is implicated in memory processing. Long-term and short-term synaptic plasticity occur at the same synapses. We tested the existence of a link between short-term plasticity occurring during slow-wave sleep and subsequent long-term plasticity. We hypothesized that slow-wave pattern during sleep could affect short-term plasticity of the cortical neurons and could promote long-term potentiation. Using field potential recordings from cats during natural sleep and waking states we extracted single unit firing and slow wave periods. Then, rat slices were used for whole-cell recordings in cortical neurons depolarized close to firing threshold. Stimuli were delivered every 5 sec to set the minimal intensity in control and in extension periods. Then, slices were stimulated for 10 min with the

same intensity using either wake-like or sleep-like stimuli combined with somatic hyperpolarization or not. Interspike interval (ISI) was used to analyze short-term plasticity. In 50% of cells, a sleep-like pattern induced either short-term facilitation for ISI less than 200ms or moderate short-term depression for ISI up to 2 sec. The wake-like pattern induced either short-term facilitation for ISI of 10-20ms only or short-term depression for ISI up to 3 sec. We did not see a link between short-term and long-term plasticity. We conclude that the mechanisms of short-term are distinct from those for long-term plasticity.

Funding: Supported by CHIR, NSERC, NIH and FRSQ

Title: Switch in GABA(A) receptor subunit composition in the rat spinal dorsal horn after peripheral nerve injury

Authors: Louis-Etienne Lorenzo¹, Antoine Godin², Dominic Boudreau³, Paul Wiseman⁴, Alfredo Ribeiro-da-Silva⁵, Yves De Koninck⁶

Affiliation: 1-3,6 Centre de Recherche Universite Laval Robert-Giffard^{4,5} McGill University

Abstract: "Altered inhibition at the spinal level appears to be a substrate of pain hypersensitivity characteristic of neuropathic pain, but the molecular underpinnings remains poorly understood. Here, we tested whether a change in composition of GABA(A) receptors (R) in the spinal dorsal horn occurs following peripheral nerve injury. We found that, in adult rats with chronic constriction injury of the sciatic nerve (PNI), there is a selective increase in GABA(A)R α 2-3 subunits (SU) immunostainings within gephyrin-IR clusters at inhibitory postsynaptic sites as the global immunoreactivity of the β 2/3 GABA(A)R did. These changes were not due to a change in size of the gephyrin clusters which remained very stable. In contrast, the number of gephyrin clusters decreased, as did the number of GABAergic boutons. The results indicate a decrease in number of inhibitory synapses in the superficial dorsal horn after nerve lesion counterbalanced by an increase in number of GABA(A)R at synapses, suggesting a homeostatic compensation. The increase in α 2-3 SU, but not of other α SUs indicates a switch in receptor composition. Consistent with this, we found a change in the kinetics of GABA(A)R mediated mIPSCs. We found that BDNF applied to spinal slices from control animals caused similar changes in GABA(A)R expression and composition as well as in the kinetics properties of the GABA(A) mIPSCs. The change in expression and composition of GABA(A)R may explain the efficacy of treating neuropathic pain with enhancing the affinity of selective GABA(A)R SU using, for example, subunit-selective

benzodiazepines."

Funding: CIHR

Title: Monoaminergic firing activity in the VTA and the DR across the light-dark cycle

Authors: Rebecca Howell¹, Sergio Dominguez-Lopez², Martha Graciela Lopez-Canul³, Marco Leyton⁴

Affiliation: 1-4 Department of Psychiatry, McGill University

Abstract: "In this work we characterized the firing activity of dopaminergic (DA) and serotonergic (5-HT) neurons in the ventral tegmental area (VTA) and the dorsal raphe (DR) nuclei, respectively, across the light-dark cycle. Rats kept under a constant 12/12h light-dark cycle (lights on at 7h) were used to perform in vivo single-unit extracellular recordings at 6 different time intervals (7-11h, 11-15h, 15-19h, 19-23h, 23-3h and 3-7h). In the VTA, DA firing rate oscillates between intervals but not significantly (7-11h: 3.5 ± 0.4 Hz; 11-15h: 2.7 ± 0.2 Hz; 15-19h: 2.8 ± 0.3 Hz; 19-23h: 3.6 ± 0.3 Hz; 23-3h: 2.9 ± 0.3 Hz; 3-7h: 2.8 ± 0.4 Hz; $F(5,203)=0.792$, $p=0.556$). At 15-19h and 19-23h, a significant decrease in the number of spontaneously active neurons was observed ($p < 0.05$ in both cases), compared with the peak observed at 23-3h (7-11h: 2.2 ± 0.3 neurons/track; 11-15h: 2.4 ± 0.4 neurons/track; 15-19h: 1.6 ± 0.1 neurons/track; 19-23h: 1.5 ± 0.2 neurons/track; 23-3h: 2.9 ± 0.2 neurons/track; 3-7h: 2.2 ± 0.3 neurons/track; $F(5,96)=3.065$, $p=0.013$). In the DR, the 5-HT neuronal activity decreases during the dark phase. In particular, 5-HT firing rate significantly decreases at 3-7h (7-11h: 0.9 ± 0.1 Hz; 11-15h: 0.85 ± 0.1 Hz; 15-19h: 0.98 ± 0.09 Hz; 19-23h: 0.75 ± 0.1 Hz; 23-3h: 0.65 ± 0.06 Hz; 3-7h: 0.53 ± 0.09 Hz; $F(5,253)=2.841$, $p=0.016$) and the number of spontaneously active neurons decreases at 19-23h and at 23-3h (7-11h: 3.0 ± 0.6 neurons/track; 11-15h: 3.7 ± 0.2 neurons/track; 15-19h: 3.8 ± 0.3 neurons/track; 19-23h: 2.6 ± 0.3 neurons/track; 23-3h: 2.4 ± 0.2 neurons/track; 3-7h: 2.6 ± 0.3 neurons/track; $F(5,79)=3.331$, $p=0.009$), compared with the peak detected at 15-19h in both parameters ($p < 0.05$, in all cases). These data suggest that DA and 5-HT neuronal populations have distinct diurnal rhythms of firing activity. "

Funding: SDL was supported by a scholarship from the Mexican National Council for Science and Technology (CONACYT, Reg. 193202/302017).

Title: The CUB domain protein Neto1 is an auxiliary protein of native synaptic kainate receptors

Authors: Man (Cristina) Tang¹, Kenneth Pelkey², David Ng³, Evgueni Ivakine⁴, Chris McBain⁵,

Michael Salter⁶, Roderick McInnes⁷

Affiliation: 1,3-7 Hospital for Sick Children 2- NICHD

Abstract: Neto1 and Neto2 are homologous single-pass transmembrane proteins encoding 2 extracellular CUB domains, an LDLa domain and a cytoplasmic domain that ends with a PDZ binding motif. Both Neto1 and Neto2 have overlapping regions of expression in the developing and mature brain (layer V cerebral cortical neurons, limbic system, basal ganglia, pons, and retina). In adult mice, both proteins are expressed in neurons, and are localized to the postsynaptic density (PSD). Loss of Neto1 and Neto2 in mice results in axon guidance defects at the embryonic stage and in numerous abnormal behavioural phenotypes in the adult suggesting important roles for these two proteins in neuronal development and function. Here, we show that Neto1 plays a critical role at the synapse by acting as an auxiliary subunit for endogenous kainate-type glutamate receptors (KARs). We find that Neto1 interacts with KARs in brain lysates and in postsynaptic density (PSD) fractions. In addition, loss of Neto1 leads to a ~50% reduction of kainate receptors in hippocampal PSDs, and to a selective deficit in KAR-mediated synaptic transmission with altered decay kinetics at mossy fiber-CA3 pyramidal cell synapses. By contrast, loss of the homologous protein Neto2, which also interacts with KARs, had no effect on the synaptic abundance of KARs or on KAR-mediated EPSCs. Neto1 has been previously shown to be a key component of the NMDA receptor protein complex. Together, our findings establish Neto1 as an important auxiliary protein required for the synaptic function of both NMDA and kainate receptors in the brain.

Funding: CIHR grant NICHD Intramural award

Title: Thalamic Burst Firing in a Rodent Model of Absence Epilepsy: Altered Calcium Channel and I_h Conductances

Authors: Stuart Cain¹, John Tyson², Terrance Snutch³

Affiliation: 1-3 University of British Columbia

Abstract: The Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model displays a well-characterized absence epilepsy phenotype. We previously described the first genetic mutation in the GAERS Cacna1h gene encoding the Cav3.2 T-type calcium channel and which correlates strongly with seizure expression. In Cav3.2 channels exogenously expressed in HEK cells the GAERS missense mutation induces a robust gain-of-function, although only in (+)25 exon-

containing Cav3.2 splice variant channels. We recently found that neurons from the reticular thalamic nucleus (nRT) in GAERS, but not control animals, exhibit significantly enhanced oscillatory burst-firing. While nRT neurons express both Cav3.2 and Cav3.3 T-type calcium channels, the enhanced burst-firing appears to occur as a result of modified Cav3.2 biophysical properties. The expression of the GAERS mutation-sensitive (+)25 exon-containing Cav3.2 splice variant was found to increase with development, peaking at 60 days when the animals begin to experience seizures. In a different region of the thalamocortical network, thalamic relay neurons send information between the cortex and periphery and also receive GABAergic inputs from the nRT. Distinct from nRT neurons, thalamocortical relay neurons predominantly express Cav3.1 T-type calcium channels. Using acute thalamic brain slices we find that GAERS ventral posterior thalamocortical neurons exhibit significantly attenuated burst-firing as a result of an enhanced I_h current. Furthermore, both T-type and high voltage-activated calcium current densities are enhanced. Together, these findings reveal a further complexity underlying the neurophysiology of absence epilepsy in the GAERS model.

Funding: Supported by the Canadian Institutes of Health Research

Title: Olfactory bulb glomerular NMDA receptors mediate odor preference learning in the neonate rat

Authors: Rebecca Lethbridge¹, Qinlong Hou², Carolyn Harley³, Qi Yuan⁴

Affiliation: 1-4 Memorial University of Newfoundland

Abstract: "Early odor preference learning is an associative learning model whereby neonatal rats learn to prefer a novel odor following a single pairing with olfactory bulb β -adrenoceptor activation. We propose a critical role of NMDA receptors (NMDARs) in mediating mitral cell LTP in the olfactory bulb and subsequently allowing a memory trace to form between olfactory nerve to mitral cell synapses. In vitro, pairing theta burst stimulation of the olfactory nerve with β -adrenoceptor activation through isoproterenol (10 μ M) significantly potentiated mitral cell evoked spike rate. Mitral cell spike potentiation was dependent on NMDAR activation, as it was blocked by D-APV, an NMDAR antagonist. To test if isoproterenol promotes NMDAR activation in mitral cells via suppression of the glomerular inhibitory network, muscimol, a GABAA receptor agonist, was locally applied to the glomerular layer. Muscimol application prevented mitral cell spike potentiation. Furthermore, local glomerular application of gabazine, a GABAA receptor antagonist,

was sufficient to produce mitral cell potentiation. When translated to the in vivo learning paradigm, bilateral glomerular-targeted infusion of D-APV or muscimol blocked isoproterenol infusion induced learning. Furthermore, infusion of gabazine, when paired with odor, was sufficient to produce learning. This was again dependent on NMDAR activation.

Immunohistochemical analysis revealed elevated phosphorylation of the NMDA GluN1 subunit 5 min post-training within the mid-lateral glomerular layer, where we targeted our drug infusion. Western blot analysis of synaptic NMDAR showed reduced expression of the GluN1 and the GluN2B subunits post-training. Together, these experiments demonstrate critical roles of the NMDAR in mediating early odor preference learning. "

Funding: CIHR & RDC to Dr Qi Yuan. NSERC CGS to Rebecca Lethbridge.

Title: Expression and Regulation of GDNF Receptor GFR-alpha1 in the Basolateral Amygdala of Depressed Suicides

Authors: Marissa E. Maheu¹, Juan Pablo Lopez², M.A. Davoli³, Gustavo Turecki⁴, Naguib Mechawar⁵

Affiliation: 1-5 Douglas Research Centre

Abstract: "Glial cell line-derived neurotrophic factor (GDNF) is a potent pro-survival factor for dopaminergic neurons, and several studies suggest that changes in its expression may be associated with depressive behaviour. However, despite evidence indicating decreased dopaminergic transmission in limbic brain regions of depressed suicides, little attention has been paid to central GDNF expression in mood disorders. In this study, we measured protein (Western blotting) and mRNA (real-time PCR; qPCR) levels of both GDNF and its principal receptor, GFR-alpha1, in the basolateral amygdala (BLA) of well-characterized depressed suicides (DS) and matched sudden-death controls (SDC). While GDNF protein and mRNA did not differ between groups, the DS group displayed a significant reduction in GFR-alpha1 protein compared to SDC (fold change: -1.24; p = 0.002). Unexpectedly, no difference in GFR-alpha1 gene expression was found between groups, suggesting post-transcriptional regulation. Using qPCR, we then tested the hypothesis that microRNAs (miRNAs) regulate GFR-alpha1 protein expression through translational repression. Two miRNAs predicted to target the 3' UTR of GFR-alpha1 were found to be significantly up-regulated in the DS group: hsa-miR-511 (fold change: 1.6; p = 0.049) and hsa-miR-340 (fold change: 1.8; p = 0.028). Additionally, there was a trend toward increased hsa-miR-511 expression being associated with decreased GFR-alpha1 protein levels (r = -0.46; p = 0.054).

Taken together, these results suggest that the decreased GFR-alpha1 expression observed in the BLA of DS individuals may be mediated by miRNA regulation of protein translation."

Funding: AFSP, FRSQ

Title: Correlated Visual Activity Stabilizes Axon Arbors in the Developing Retinotectal System

Authors: Jessie Poquérousse¹, Martin Munz², Edward S. Ruthazer³

Affiliation: 1-3 McGill University

Abstract: In the developing visual system, correlated activity across neighbouring retinal ganglion cells (RGCs) that receive information from overlapping receptive fields has been proposed to drive the stabilization of synaptic contacts and axonal arbors. To determine how correlated visual activity regulates the development of RGC axonal projections, we reared albino *Xenopus laevis* tadpoles under visual stimulation conditions designed to increase or decrease correlated firing of RGCs. In vivo multiphoton time-lapse images of developing axon terminals of RGCs, electroporated to express Green Fluorescent Protein, were collected for up to ten days. While the vast majority of RGCs project contralaterally in tadpoles, a very small fraction of axons ectopically innervate the ipsilateral optic tectum. These rare ipsilaterally projecting RGC axons terminate among axons originating from the contralateral eye that represent the opposite visual hemifield. Animals were either strobe-reared or exposed to a pattern of randomly moving dots to respectively enhance or decrease the degree of correlated activity across RGCs. Rearing with moving dots produced significantly larger, more complex and dynamic arbors in ipsilaterally projecting RGCs within 24 hr of altering visual experience. These experiments constitute a direct demonstration of the effects of patterned sensory input on the dynamics and morphology of developing axon arbors.

Funding: CIHR grants MOP-77567 and NHG-99088. MM holds a DAAD Jahresstipendium. ER is a tier 2 CRC and MNI Killam Fellow.

Title: TGF- β 1 derived from myeloid cells contributes to early pathogenesis after spinal cord injury.

Authors: Isabelle Pineau¹, Dave P Stirling², Steve Lacroix³

Affiliation: 1,3 CHUL Research Center and Department of Molecular Medicine 2- Department of Clinical Neurosciences, Hotchkiss Brain Institute

Abstract: The cytokine transforming growth factor (TGF)- β 1 plays a critical role in many of the pathophysiological responses that occur after neural injury. While some studies have suggested that TGF- β 1 may exert protective effects on neural cells, others have shown that neutralizing TGF- β 1 after spinal cord injury (SCI) reduces inhibition associated with the glial scar and facilitates axonal regeneration. However, because TGF- β 1 signaling can modulate the activity of multiple CNS and immune cell lineages, it is not clear from these studies whether interfering with the TGF- β 1 system will be beneficial or detrimental after SCI. By taking advantage of the Cre-loxP recombination system, we were able to bypass embryonic lethality and developmental defects normally seen in mice lacking TGF- β 1 or its type II receptor (TGF β RII), and created mice with a conditional knockout (CKO) of TGF- β 1 or TGF β RII specifically in myeloid cells (CD11b+) or astrocytes (GFAP+). In agreement with in situ hybridization results, which revealed that myeloid cells and not astrocytes are the main cellular source of TGF- β 1 in the injured mouse spinal cord, only inactivation of TGF- β 1 in myeloid cells resulted in changes in functional recovery after SCI. Mice with TGF- β 1-CKO from CD11b+ myeloid cells showed improved locomotor recovery compared with controls after SCI, while lack of TGF β RII signaling in either astrocytes or myeloid cells did not affect recovery of locomotor function. These findings suggest that TGF- β 1 released by myeloid cells contributes to spinal cord pathology after traumatic SCI.

Funding: Work supported by CIHR and the International Foundation of Research in Paraplegia.

Title: Retinoic Acid promotes neuronal regeneration and directed outgrowth in cultured spinal cord neurons from the frog, *Xenopus laevis*.

Authors: Chris Rand¹, Gaynor Spencer², Robert Carlone³

Affiliation: 1-3 Brock University

Abstract: "Retinoic acid (RA), an endogenous vitamin A metabolite, has been implicated in neuronal regeneration, in addition to its well-known functions in axial patterning and neurogenesis. Classically, RA is known to bind to two nuclear receptors, the retinoic acid receptor (RAR) and retinoid X receptor (RXR), which modify gene expression (though non-genomic effects of RA also occur). We recently showed for the first time that RA induces growth cone turning in a

non-genomic manner. Specifically, we used *Lymnaea* neurons to show that neuritically localized RXR mediates the chemoattractive response towards two different RA isomers, all-trans-RA and 9-cis-RA. Here, we demonstrate for the first time that the growth cone turning effects of RA are conserved in vertebrate neurons. Spinal neurons from stage 22 *Xenopus laevis* embryos were cultured and bath application of both all-trans-RA and 9-cis-RA was shown to induce trophic effects to promote neurite outgrowth. We next showed for the first time that focal application of the two RA isomers to the cultured spinal neurons produced positive growth cone turning toward the source of RA. Preliminary evidence suggests that the β subtype of the RAR may mediate this turning response in vitro, as the β antagonist, LE135, blocked the RA-mediated chemoattraction. These preliminary results indicate that RA-mediated chemoattraction is likely conserved between invertebrates and vertebrates, though the retinoid receptor mediating the effects may differ."

Funding: NSERC Discovery grants awarded to GES and RLC, Brock University Advancement Fund Seed Grant to RLC

Title: The Vitamin A metabolite, retinoic acid, causes dramatic changes in neuronal firing of identified molluscan neurons

Authors: Nicholas Vesprini¹, Gaynor Spencer², Taylor F. Dawson³, Doug Bruce⁴, Gaynor E. Spencer⁵

Affiliation: 1- 5 Brock University

Abstract: The vitamin A metabolite, retinoic acid (RA), is well-known to regulate gene expression during neuronal development and regeneration in vertebrates. We have also shown that RA can act as a trophic and chemotropic molecule to induce neurite outgrowth and positive growth cone turning in cultured molluscan neurons. While neuronal firing activity is known to play a role in neurite outgrowth and axon guidance, no work has previously examined whether RA can elicit electrophysiological changes. Utilizing identified neurons from the pond snail *Lymnaea stagnalis* we have shown that neurons acutely exposed to RA exhibited a dramatic change in firing pattern and showed significant changes in action potential waveform within minutes. We now extend these studies by investigating the involvement of the retinoic acid receptor (RAR) and the retinoid X receptor (RXR). Both of these receptors have been cloned from *Lymnaea* and the RXR is known to exert non-genomic effects. Furthermore, both receptors exhibit non-nuclear distributions in cultured *Lymnaea* neurons. Using both RAR agonists and antagonists, we first determined that the

RAR did not appear to play a role in producing the RA-induced changes in electrical activity. However, whilst application of an RXR agonist did not mimic the effects of RA, the application of an RXR antagonist partially impaired the RA-induced electrophysiological changes. This Lymnaea RXR has also been shown to mediate the non-genomic effects of RA in growth cone turning over the same time course, suggesting that there may be a causal link between the two responses.

Funding: "NSERC Discovery grant to GES; NSERC PGS-D to NV"

Title: Aging-related changes of glial cell excitability and synaptic plasticity

Authors: Alexandre St-Amour¹, Joanne Vallée², Richard Robitaille³

Affiliation: 1-3 GRSNC, département de Physiologie, Université de Montréal

Abstract: To be transmitted appropriately, neuronal information is modulated by a third element, the glial cell. Interestingly, during aging, synapses undergo functional and structural alterations. However, alteration of glial cells at synapses remains ill defined. Hence, we investigated functional neuron-glia interactions during aging to better understand this physiological phenomenon. We used the mouse neuromuscular junctions (NMJs), a well-known model to study neuron-glia interactions. We first examined the structural changes since glial cells at NMJs are known to regulate morphological stability of the synapse. Using immunohistochemical labeling of each synaptic elements of adult (90 days) and old (600 days) NMJs, we uncovered that the number of glial cells expressing S100B (specific glial protein) decreased during aging. Interestingly, glial processes were thinner and the glial coverage of the synapse was incomplete. We next investigated the excitability of glial cells by monitoring Ca²⁺ changes. This glial activity is known to regulate synaptic activity and plasticity. Ca²⁺ activation was induced by agonist applications (ATP, Muscarine) and by endogenous release of transmitters. Our results show that glial activity was altered and weakened in old animals. Consistent with these findings, we found that all forms of potentiation of transmitter release were decreased while synaptic depression was increased. This suggests that synaptic efficacy is weakened during aging. In conclusion, our work provides new evidence that neuron-glia perturbations during aging are associated with major functional alteration of synaptic function.

Funding: CIHR - NSERC - CFI - FRSQ

Title: Retinoic acid exposure reduces intracellular Ca²⁺ in cultured molluscan neurons

Authors: Taylor F. Dawson¹, Nicholas D. Vesprini², Gaynor E. Spencer³

Affiliation: 1-3 Brock University

Abstract: "The vitamin A metabolite, retinoic acid (RA), is known to play a role in the development and regeneration of the nervous system in vertebrates, influencing patterning, neuritogenesis and axonal pathfinding. Traditionally, RA has been thought to act in conjunction with its nuclear receptors, exerting its effects by altering gene expression; however recent evidence suggests a non-genomic role for RA in regenerating neurons. Our lab has shown that exposure to RA can influence neurite outgrowth and growth cone turning in cultured Lymnaea neurons, and that this behavior is dependent on Ca²⁺ influx. Recently, we have also shown that acute RA exposure elicits significant changes in the firing properties and action potential waveform in cultured Lymnaea neurons. Utilizing the ratiometric calcium indicator dye Indo-1 AM we now show that RA exposure results in a significant decrease in intracellular Ca²⁺ that correlates with the time course of the RA-induced electrophysiological changes. The RA-mediated effects on electrophysiological properties and intracellular Ca²⁺ also appear to be dose- and isomer-specific. We hypothesize that voltage-gated Ca²⁺ channels (VGCCs) are involved in the RA-induced effects, but there is no current evidence that RA can modulate the properties of VGCCs in mature neurons. As such we extend these studies, utilizing whole-cell patch clamp techniques, to characterize the effects of RA on the biophysical properties of VGCCs. This work demonstrates that RA can influence intracellular Ca²⁺ levels, which may underlie some of the physiological roles played by RA during nervous system development and regeneration."

Funding:

Funding: provided by an NSERC Discovery grant to GES, and NSERC PGS-D scholarships to NDV and TFD.

Title: Evidence of astrogliosis in the anterior cingulate white matter of depressed suicides

Authors: Susana Torres-Platas¹, Christa Hercher², Maria Antonietta Davoli³, Gilles Maussion⁴, Benoit Labonté⁵, Gustavo Turecki⁶, Naguib Mechawar⁷

Affiliation: 1-7 Douglas Mental Health University Institute

Abstract: Background: Increasing evidence suggests that cortical astrocytic function is disrupted in mood disorders and suicide. The fine neuroanatomy of astrocytes, however, remains to be

investigated in these psychiatric conditions. We performed detailed morphometric analyses of reconstructed gray and white matter astrocytes from depressed suicides and matched controls, and quantified GFAP expression in both cortical compartments to assess local astrocytic activation. Methods: Postmortem ACC samples (BA24) from 10 depressed suicides and 10 matched controls were obtained from the Quebec Suicide Brain Bank. Golgi-impregnated astrocytes from layer VI and underlying white matter were reconstructed and analyzed, and GFAP expression quantified by real time PCR. Results: Astrocytes in layer VI displayed no significant differences between groups for any of the quantified parameters. However, GFAP expression in this compartment presented a strong downregulation in depressed suicides, with an average value 25-fold lower than in controls. Astrocytes in the white matter had significantly larger and more ramified processes in depressed suicides, with values for these parameters being about twice as high as those measured in controls. GFAP expression in the white matter was higher in depressed suicides compared to control, but this increase did not reach significance. Conclusions: These results provide the first evidence of altered cortical astrocytic processes in mood disorders, and indicate a very strong downregulation of GFAP in BA24 gray (but not white) matter. The presence of hypertrophic astrocytes in white matter is consistent with reports suggesting white matter alterations in depression, and offers support to the neuroinflammatory theory of depression.

Funding: Supported by CIHR, FRSQ, and CONACYT

Title: Fine structural features of the mesostriatal dopamine innervation in the mouse

Authors: Noemie Berube-Carriere¹, Guillaume M. Fortin², Åsa Wallén-Mackenzie³, Louis-Eric Trudeau⁴, Laurent Descarries⁵

Affiliation: 1,2, 4, 5 Universite de Montreal, 3- Uppsala University

Abstract: "Conditional KO mice are increasingly used for experimental studies of the dopamine (DA) system. Yet, little is known about the ultrastructural features of the mesostriatal DA innervation in this species. Aiming to characterize Vglut2f/f;DAT-cre mice specifically lacking the Vglut2 gene in DA neurons, we analyzed the ultrastructural features of DA axon terminals (varicosities) in the core and shell of nucleus accumbens and the neostriatum of immature (P15) and mature (P70-90) KO mice, their control littermates, and a few wild-type mice, by means of immuno-electron microscopy with a specific antibody against tyrosine hydroxylase (TH). In the three regions, at both ages and irrespective of the genotype, the single section profiles of TH-

labeled varicosities were comparable in shape, size, vesicular content and proportion showing mitochondria. Moreover, in all subgroups, the proportion that displayed a synaptic membrane specialization (synaptic incidence) was exceedingly low, ranging from 0.5 - 5.1% and averaging $2.6 \pm 0.5\%$. Unless a large number of glutamatergic synapses is established by DA axon terminals which would not contain detectable levels of TH, a first conclusion is that the mesostriatal DA innervation in the mouse is mostly asynaptic. Knowing that the density of DA innervation is decreased by 20-30% in the nucleus accumbens (but not the NS) of adult *Vglut2^{f/f};DAT-cre* KO mice, a second conclusion is that specific deletion of the *Vglut2* gene in DA neurons might be more important for regulating the growth of these fibers than for their establishment and/or maintenance of a scarce synaptic connectivity."

Funding: CIHR grants MOP-3544 and MOP-106562 to L.D., MOP-49951 to L.-É.T., and a grant from NARSAD to L.-É.T.

Title: Selective plasticity enhancement in the trained hemisphere following monocular visual discrimination learning in rats

Authors: Audrey Hager¹, Hans Dringenberg²

Affiliation: 1-2 Queen's University

Abstract: The rat visual system is structured so that the large (>90%) majority of retinal ganglion axons reach the contralateral lateral geniculate nucleus (LGN) and visual cortex (V1). This structural design allows for relatively selective activation of one cerebral hemisphere under monocular viewing conditions. Here, we trained rats in a visual discrimination task using a Y-shaped water maze, with one eye occluded during the daily training session (10 trials). With training, rats learned to associate a specific visual cue with the location of a hidden platform in the maze under these monocular viewing conditions. Following task acquisition, rats were tested on their ability to perform the task with the 'trained' and 'untrained' eye ('probe trials', i.e., the eye occluded during training). Performance was impaired on probe trials, suggesting that training was partially confined to one cerebral hemisphere. Subsequent electrophysiological assessment of plasticity (long-term potentiation) in V1 elicited by electrical theta-burst stimulation of the LGN revealed greater LTP in the contralateral, 'trained' relative to the 'untrained' hemisphere (63 and 40% potentiation, respectively, $p < 0.05$). These experiments confirm previous work that visual experience (discrimination training) can result in an increase of the synaptic modification range of

V1 synapses, an effect we now show to display a degree of hemispheric lateralization. This observation may indicate that experience-induced plasticity enhancement is due to more direct, sensory-related activation of V1, rather than processes related to general brain activation and related neuromodulatory systems (supported by NSERC).

Funding: NSERC

Title: Impact of overexpressed vesicular acetylcholine transporter (VAcHT) on other components of the cholinergic system

Authors: Paul Nagy¹, Isabelle Aubert²

Affiliation: 1- Laboratory Medicine and Pathobiology, University of Toronto 2- Brain Sciences, Sunnybrook Research Institute

Abstract: Septohippocampal cholinergic neurons produce the neurotransmitter acetylcholine and are essential to critical brain functions, including memory and learning. Acetylcholine is synthesized by the enzyme choline acetyltransferase (ChAT) and is localized to synaptic vesicles by the vesicular acetylcholine transporter (VAcHT). Appropriate ChAT and VAcHT expression are required for adequate function of cholinergic neurons, however the detailed mechanisms that regulate cholinergic tone are not fully understood. Here we used transgenic mice modified to express the enhanced green fluorescent protein under control of the ChAT promoter. We show that this transgenic modification leads to a four-fold increase in the VAcHT gene copy number compared to non-transgenic controls and significant increases in VAcHT mRNA expression in both embryonic (29%) and adult (117%) septal brain tissue. In embryonic septal tissue, increased VAcHT expression elicits a significant reduction in ChAT and high affinity choline transporter (CHT1) mRNA expression. In contrast, we found that in adult septal tissue, cholinergic gene expression was not significantly different when compared to controls. Consistent with these findings, ChAT protein level and enzymatic activity were not significantly different in transgenic septal tissue compared to controls. These data suggest that in response to VAcHT overexpression, ChAT and CHT1 can be modified at the transcriptional level in the embryonic brain, perhaps as a mechanism to maintain normal cholinergic homeostasis. These particular regulatory events were not observed in the adult septum. Taken together, this study contributes to our understanding of cholinergic regulation during development, specifically in response to increased VAcHT

expression.

Funding: CIHR (PN, IA), NSERC (IA)

Title: NMDA receptor silencing during anoxia is mediated by calcium release from the mitochondria in anoxia-tolerant turtle neurons

Authors: Peter Hawrysh¹, Leslie Buck²

Affiliation: 1-2 University of Toronto

Abstract: Mammalian neurons are sensitive to anoxia and rapidly undergo excitotoxic cell death when deprived of oxygen. This is not observed in the western painted turtle (*Chrysemys picta bellii*), which can survive anoxia for several months. N-methyl-D-aspartate receptor (NMDAR) currents are reduced in anoxic turtle cortical neurons and this down-regulation occurs in response to a modest increase in cytosolic $[Ca^{2+}]$. Calcium chelation during anoxia abolishes the decrease in NMDAR currents, suggesting a critical role for calcium. The aim of this study was to investigate the mitochondria as an intracellular calcium source, and if mitochondrial calcium release can reduce NMDAR currents during normoxia. NMDAR currents were measured in pyramidal neurons in the turtle cortex using the whole-cell patch clamp configuration under normoxic and anoxic conditions. Relative changes in cytosolic calcium were determined by fluorometric analysis using Oregon Green 488 BAPTA-1 AM ester. During anoxia, NMDAR currents decreased by $46.8 \pm 5.7\%$ and cytosolic $[Ca^{2+}]$ increased by $9.3 \pm 0.3\%$. These changes in calcium and NMDAR currents were mimicked by atractyloside application, a mitochondrial permeability transition pore opener. Atractyloside application during normoxia resulted in a $34.1 \pm 0.1\%$ decrease in NMDAR currents and an $8.9 \pm 0.7\%$ increase in cytosolic calcium. Taken together, these data indicate that the anoxia-mediated rise in calcium is mitochondrially-mediated, and the modest increase in cytosolic $[Ca^{2+}]$ due to opening of the transition pore is sufficient to decrease NMDAR currents during normoxia.

Funding:

Title: Involvement of first and second order thalamic nuclei in the slow oscillation in anaesthetized mice

Authors: Maxim Sheroziya¹, Igor Timofeev²

Affiliation: 1-2 Robert-Giffard Laval University Research Center

Abstract: During the cortically generated slow oscillation both pyramidal and non-pyramidal neurons alternate between depolarized (active) and hyperpolarized (silent) states, which are reflected in local field potentials as depth-negative and depth-positive slow waves. Corticothalamic neurons entrain thalamocortical cells from a variety of nuclei into the slow oscillation. The role of first and second order thalamic nuclei in feedback synchronization of the slow oscillation was not investigated previously. We performed simultaneous cortical local field potential recordings with intracellular recordings in the thalamus of ketamine/xylazine anesthetized mice. All recorded thalamocortical cells (n=32) generated rebound low-threshold spikes in response to a hyperpolarizing current pulse, but they did not display spontaneous burst firing. First order thalamic VL and VPM cells (n=21) displayed unitary or spindle-like IPSPs correlated with cortical active states. Some cells (n=7) also revealed periods of low amplitude “saw-like” EPSPs and occasional spikes which were independent from cortical slow waves evidently originating from ascending pathways. Cells located in the higher order thalamic nucleus Po (n=10) revealed high amplitude EPSPs and firing correlated with cortical active states. We conclude that in anesthetized mice, due to a lack of efficient firing, the contribution of first order thalamic nuclei in synchronization of cortical active states is rather limited. By contrast, the second order nuclei likely play an important role in the synchronization of primary and secondary sensory areas.

Funding: Supported by NSERC, CHIR, NIH and FRSQ

Title: Acetylcholine is a fundamental requirement for afterdischarge generation in *Aplysia* neuroendocrine cells

Authors: Sean White¹, Neil Magoski²

Affiliation: 1-2 Queen's University

Abstract: The bag cell neurons of *Aplysia californica* translate brief synaptic input into a prolonged afterdischarge, resulting in peptide hormone release and the triggering of reproductive behaviour. While the input transmitter responsible for initiating the afterdischarge is unknown, strychnine can prevent the response (PNAS 75:5200). Since strychnine antagonizes excitatory cholinergic inputs in molluscan neurons, we explored the effects of acetylcholine both in cultured bag cell neurons and within the intact cluster of the abdominal ganglion. Acetylcholine elicited either a burst of accommodating action potentials or prolonged firing in cultured bag cell neurons.

This response was attributed to opening of an ionotropic cation channel. The acetylcholine-induced current was partially blocked by strychnine, and eliminated by a combination of the nicotinic antagonists, mecamylamine and alpha-conotoxin Iml. For neurons within the intact cluster, acetylcholine evoked depolarization and continuous spiking, particularly after the addition of the acetylcholinesterase inhibitor, neostigmine. Extracellular recording from bag cell neuron clusters revealed a genuine afterdischarge response to acetylcholine. In refractory clusters, subsequent acetylcholine application failed to induce excitatory responses. Furthermore, clusters previously exposed to acetylcholine became refractory to further stimulation. Finally, consistent with acetylcholine being an input transmitter, synaptically-induced afterdischarges were blocked by mecamylamine and alpha-conotoxin Iml. In conclusion, acetylcholine appears to act through an ionotropic receptor to increase the excitability of bag cell neurons, both in culture and in situ, sufficient to generate a long-lasting afterdischarge. This may represent a common mechanism underlying how a transient input produces a prolonged change in neuronal output.

Funding: CIHR MOP11211

Title: Electrical coupling ensures firing synchrony of neuroendocrine cells

Authors: Neil Magoski¹, Phillip Colmers²

Affiliation: 1-2 Queen's University

Abstract: A collective neuronal burst is often required for hormone release to elicit fundamental physiological responses. The firing of neurosecretory cells can be synchronized through gap junctions. For example, the bag cell neurons of the marine mollusc, *Aplysia californica*, are electrically-coupled neuroendocrine cells that initiate reproduction through a synchronous burst called the afterdischarge. However, the properties and role of electrical synapses in these neurons is poorly understood. Thus, we used whole-cell current- and voltage-clamp to study electrical coupling between paired bag cell neurons in culture. Junctional current was non-rectifying and independent of pre- and post-synaptic voltage (range -40 to -90 mV). Furthermore, junctional conductance remained linear across this voltage range, consistent with the gap junction being voltage-independent. The junctional conductance appeared to be a strong determinant of coupling coefficient, as the two parameters were linearly related. The electrical synapse also acted as a low-pass filter, such that identical hyperpolarizing pulses delivered at high frequencies pre-synaptically were attenuated post-synaptically. Nevertheless, electrotonic potentials evoked by

pre-synaptic action potentials did summate sufficiently to drive post-synaptic action potentials. When both coupled neurons were stimulated to spike simultaneously, there was a significantly higher degree of action potential synchrony compared to non-coupled neurons stimulated in the same fashion. Thus, Aplysia bag cell neurons are capable of forming electrical synapses in culture, which promote depolarization and facilitate firing synchrony. In vivo, these properties may contribute to afterdischarge generation and species propagation.

Funding: NSERC 386664

Title: Modulation of NMDARs by group II metabotropic glutamate receptors in hippocampal CA1 neurons

Authors: Catherine Trepanier¹, Michael F. Jackson², John F. MacDonald³

Affiliation: 1- University of Toronto 2-3 Robarts Research Institute

Abstract: Group II metabotropic glutamate receptors (mGluRs) have emerged as important targets for the treatment of schizophrenia. Although predominantly located presynaptically, there is some evidence for postsynaptic expression of these receptors where they couple to Gα_q/o protein and inhibition of adenylyl cyclase. Since hypofunction of postsynaptic N-methyl-D-aspartate receptor (NMDAR) function has also been implicated in the etiology of schizophrenia, we examined whether postsynaptic group II mGluRs (mGluR2/3) regulate NMDARs function in identified CA1 hippocampal neurons. Surprisingly, application of the selective group II mGluR agonist, LY 379 268 (10 nM) for 5 min, significantly enhanced the peak of NMDA-evoked currents. To establish which G-protein mediates the observed enhancement, we applied the Gα_q/o inhibitor pertussis toxin (5 ug/ml) inside the patch pipette and showed that it blocked the enhancement by LY 379 268. Similarly, application of recombinant RGS4 (1 ug/ml) also prevented the enhancement by group II mGluRs. Intracellular application of the Src inhibitory peptide, Src(40-58), blocked the mGluR2/3 effect on NMDAR currents whereas a peptide that selectively inhibits Fyn tyrosine kinase had no effect. Furthermore, the mGluR2/3-mediated potentiation did not discriminate between subtypes of NMDARs as the regulation was blocked by both the GluN2A-antagonist Zn²⁺ (0.5 uM) and the GluN2B-antagonist Ro 25-6981 (0.5 uM). Overall these results demonstrate that activation of mGluR2/3 recruits Src kinase to potentiate GluN2A- and GluN2B-containing NMDA currents in CA1 neurons. This may represent a novel mechanism to correct the

hypoglutamatergic state found in schizophrenia.

Funding: CIHR Grant 44008

Title: Contributions of basket cells to theta rhythms in model hippocampal CA1 networks

Authors: Katie A. Ferguson¹, Carey Y.L. Huh², Amilhon Benedicte³, Sylvain Williams⁴

Affiliation: 1- Department of Physiology, University of Toronto; Toronto Western Research Institute, University Health Network 2-4 Douglas Mental Health University Institute, McGill University

Abstract: Theta rhythms, 3-12 Hz oscillations recorded in the hippocampus of all mammals studied to date[1], are thought to play a lead role in spatial navigation and episodic memory. These rhythms are recorded from the hippocampus during R.E.M. sleep and spatial navigation, and play a critical role in the timing of place cell firing[1]. Although these oscillations have been heavily studied, the mechanism(s) responsible for the generation of these rhythms remains unknown. However, recent research using an intact hippocampus preparation in vitro suggests that the CA1 hippocampal region possesses the necessary circuitry to generate robust intrinsic theta rhythms[2]. To determine the mechanism(s) underlying the generation of these CA1 hippocampal theta rhythms, we created a mathematical network model. Our network model is composed of four types of cells: pyramidal cells, fast-spiking parvalbumin-positive basket cells (PV+BCs), slow-spiking cholecystokinin-positive basket cells (CCK+BCs), and oriens - lacunosum-moleculare (O-LM) interneurons. The network configuration is based on experimental data of known connectivities, representing the intrahippocampal connections among the chosen cell types, and each cell type is represented by a single-compartment conductance-based model. Our model produces robust theta rhythms in accordance with the experimental data. Interestingly, we find that inhibitory input imposed on pyramidal cells from the PV+BCs are key in producing theta rhythms. This finding is surprising, as research has focused on the role of PV+BCs in faster gamma rhythms (20-100 Hz). Synaptic constraints from the experimental data applied to the network model will determine the applicability of the underlying network model mechanism to the biological system.

Funding: This study was funded by the Canadian Institutes of Health Research

Title: BDNF and Painful Oscillations in the Rat Spinal Cord

Authors: Sascha Alles¹, Van Lu², James Biggs³, Klaus Ballanyi⁴, Peter Smith⁵

Affiliation: 1,3-5 University of Alberta 2- NIH/NIAAA

Abstract: "Peripheral nerve injury promotes the release of brain derived neurotrophic factor (BDNF) from primary afferents and spinal microglia. This induces changes in the properties of dorsal horn neurons that lead to the 'central sensitization' that underlies neuropathic pain (Coull et al., Nature 435, 7070, 2005). Whilst analyzing the long-term actions of BDNF on organotypic spinal cord slices, we noticed that groups of cells in neurotrophin-treated cultures exhibited synchronous oscillations in intracellular Ca²⁺ (Cai) as monitored using Fluo-4AM using confocal calcium imaging. 5-6 days treatment of cultures with 200ng/ml BDNF induced Cai oscillations at 0.14±0.01Hz (n=7) that were synchronous with extracellularly recorded field potentials. Oscillations were eliminated in Ca²⁺ free medium or by 200 µM Cd²⁺, 0.1µM TTX or 10 µM NBQX. This indicates involvement of voltage-gated Ca²⁺ and Na⁺ channels and AMPA-type receptors. The NMDA receptor blocker, AP-5 (50µM) reduced the amplitude but not the frequency of oscillations. The possible contribution of gap junctions was inferred from the increased inter-event interval between Cai oscillations in 7 out of 24 neurons in the presence of carbenoxolone [100µM]. We also found that Cai rises produced by acute application of 10⁻⁷µM AMPA or 50µM NMDA were augmented in BDNF treated cultures. These findings suggest that BDNF-evoked Cai oscillations are primarily neuronal in origin and may result, at least in part, from increased neuronal sensitivity to glutamate. Since Cai oscillations were also observed in cultures to exposed to activated microglial conditioned medium, this type of activity may also contribute to the etiology of neuropathic pain. "

Funding: CIHR Pfizer Neuropathic Pain Research Award

Title: Noise-induce intrinsic plasticity of neocortical pyramidal neurons: Role of active and silent states

Authors: Mathieu D'Amours¹, Igor Timofeev²

Affiliation: 1-2 Centre de Recherche de l'Université Laval à Robert-Giffard

Abstract: During slow wave sleep (SWS), cortical pyramidal neurons alternate between a silent, low-conductance and hyperpolarized (down) state, and a highly fluctuating, high-conductance and depolarized (up) state. Thalamocortical mechanisms are known to be involved in sleep-dependent memory consolidation, but studies on the cellular basis of these mechanisms have focused mainly

on the synaptic plasticity involved. Whether intrinsic plasticity takes part in these mechanisms is not known. Using a brain slice preparation of rats, cortical pyramidal neurons were injected noisy stimuli in a current-clamp mode. The parameters of this noise were adjusted to mimic neuronal activities observed during natural slow-wave sleep and waking states. Some parts of noisy stimuli were periodically repeated to probe the variability of spike timing. Action potentials triggered by noisy stimuli occurred with either moderate (millisecond scale) or low (tens of milliseconds scale) precision during wake-like stimuli. The precision of spikes in millisecond scale was increased during SWS-like stimuli and it was further increase during wake-like stimuli that followed SWS-like stimulation protocol. We propose that hyperpolarized periods of slow oscillation affect expression of intrinsic currents that increase precision of neuronal firing during consecutive waking states.

Funding: Supported by CHIR, NSERC, NIH and FRSQ.

Title: Fast and slow gamma rhythms are intrinsically and independently generated in the subiculum

Authors: Jesse Jackson¹, Romain Goutangy², Sylvain Williams³

Affiliation: 1-3 McGill University

Abstract: Gamma rhythms are essential for memory encoding and retrieval. Despite extensive study of these rhythms in the entorhinal cortex, dentate gyrus, CA3 and CA1, almost nothing is known regarding their generation and organization in the most prominent hippocampal output: the subiculum. Here we show using the complete rat hippocampus in vitro, that the subiculum intrinsically and independently generates spontaneous slow (30-50Hz) and fast (100-150Hz) gamma rhythms during the rising phase and peak of persistent theta rhythm. These two gamma frequencies are phase modulated by theta rhythms without any form of afferent inputs from the entorhinal cortex or CA1. Both subicular principle cells and interneurons phase lock to fast and slow gamma and single cells are independently phase modulated by each form of gamma rhythm, enabling selective participation in neural synchrony at both gamma frequencies at different times. Fast GABAergic inhibition was required for the generation of fast gamma whereas slow gamma is generated by excitatory and inhibitory mechanisms. In addition, the transverse subicular axis exhibits gamma rhythm topography with faster gamma coupling arising in the distal subiculum region. The subiculum therefore possesses a unique intrinsic circuit organization which can

autonomously regulate the timing and topography of hippocampal output synchronization. These results suggest the subiculum is a third spontaneous gamma generator in the hippocampal formation and these gamma rhythms likely play an active role in mediating the flow of information between the hippocampus and multiple cortical and subcortical brain regions.

Funding: CIHR, NSERC and FRSQ

Title: The direction of long-term synaptic plasticity is reversed at corticostriatal synapses of the indirect pathway in a mouse model of L-DOPA induced dyskinesia

Authors: Sherri Thiele¹, Joanne Nash², Jonathan Brotchie³

Affiliation: 1-2 University of Toronto at Scarborough 3- Toronto Western Research Institute

Abstract: "L-DOPA is the most effective treatment for symptoms of Parkinson's disease (PD). Within 5-7 years of treatment, patients experience side-effects of uncontrollable, involuntary movements termed L-DOPA induced dyskinesia (LID), limiting the therapeutic value of L-DOPA. In rodent models of LID, synaptic activity of the direct striatal output pathways is altered, which is likely to contribute to the mechanisms of LID. We hypothesize that dysfunctional synaptic plasticity also occurs at synapses of the indirect pathway, and contributes to symptoms of LID. We generated a model of LID using 6-OHDA-lesioned, A2A-eGFP BAC transgenic mice to study the indirect pathway in isolation. Long-term changes in synaptic plasticity were assessed. In slices prepared from LID mice, the ON-L-DOPA state, (i.e when dyskinesia is exhibited), was mimicked by the inclusion L-DOPA (250nM), while the OFF-L-DOPA state was mimicked by the exclusion of L-DOPA from the recording chamber. In both sham-operated and parkinsonian mice, the positive and negative timing STDP paradigms led to a 140% \pm 1.3% increase (LTP) and 67% \pm 1.1% decrease (LTD) in synaptic strength respectively compared to baseline. In the LID-OFF L-DOPA group, both the positive and negative timing protocols induced enhanced LTP (189% \pm 1.3% and 221% \pm 3.0% respectively). Whereas, in the LID-ON L-DOPA group, LTD was observed in response to both positive (79% \pm 2.0%) and negative (64.3% \pm 2.7%) STDP protocols. In LID, the indirect striatal pathway is underactive, as demonstrated in the current study by the inability of synapses to express LTP. We are currently investigating the pharmacological mechanisms that restore LTP on the indirect pathway. "

Funding: NSERC

Title: Vacuolar H⁺-ATPase subunit V0a1 and V0a2 cooperatively regulate secretory vesicle acidification, transmitter uptake and storage

Authors: Ner Mu Nar Saw¹, Soo-Young Ann Kang², Leon Parsaud³, Ga-young Anna Han⁴, Tiandan Jiang⁵, Krzysztof Grzegorzczak⁶, Michael Surkont⁷, Lijun Li⁸, Shuzo Sugita⁹,

Affiliation: 1-9 University of Toronto

Abstract: The V0 sector of the vacuolar H⁺-ATPase (V-ATPase) is a multi-subunit complex that forms a proteolipid pore. In mammalian cells the V0 complex is composed of at least four subunits - a, c, c', and d - the largest of which is V0a (~110 kDa). Among the four isoforms of V0a (a1-a4), the isoform(s) critical for secretory vesicle acidification has yet to be identified. In this study we used PC12 cells, derived from rat pheochromocytoma cells, to elucidate the roles played by V0a1 and V0a2. We engineered PC12 cells in which V0a1, V0a2 or both are down-regulated using lentivirus-mediated short hairpin RNA (shRNA). Our results show that while down-regulating V0a1 (a1KD) show significant but small reductions of dense core vesicle (DCV) acidification and knocking down V0a2 (a2KD) show no significant acidification defects knocking down both V0a1 and V0a2 (a1a2DKD) show severe reductions of DCV acidification. Consequently, a1a2DKD cells also show severe defects in transmitter uptake (radioactively-labelled norepinephrine) and storage (dopamine). The importance of V0a1 in DCV acidification is strengthened by the fact that the expression of knockdown-resistant V0a1 suppressed the acidification defects caused by down-regulating both endogenous V0a1 and V0a2. Furthermore our immunofluorescence analyses of fluorescent protein-fused V0a1, V0a2 or V0a3 suggest that V0a1 is enriched in secretory vesicles whereas V0a2 and V0a3 are enriched on the Golgi and early endosomes, respectively. Overall, our data suggest that V0a1, and to a lesser extent V0a2, cooperatively regulate the acidification as well as transmitter uptake and storage of dense-core vesicles.

Funding: Heart and Stroke Foundation (NA6217, T6700) Canadian Institute of Health Research (MOP-57825 and MOP-93665)

Title: "Interactions between Intercellular Adhesion Molecule-5 (ICAM-5) and β 1 integrins regulate neuronal synapse formation"

Authors: Lin Ning¹, Li Tian², Carl Gahmberg³

Affiliation: 1-3 University of Helsinki, Finland

Abstract: "ICAM-5 is a dendrite-specific adhesion molecule, which functions in both the immune

and nervous systems. It binds to the $\beta 2$ integrin Lymphocyte function-associated antigen 1 (LFA-1), whereby it mediates an interaction of immune cells to neurons. Upon N-methyl-D-aspartic acid (NMDA) receptor activation, matrix metalloproteases are up-regulated, resulting in ICAM-5 cleavage, and release of the soluble ICAM-5 fragment promotes dendritic spine maturation. However, the mechanisms by which ICAM-5 regulates spine development have remains poorly understood. In this study, we describe an interaction between dendritic ICAM-5 and $\beta 1$ integrins, located in the axonal terminals. $\beta 1$ integrins were immune precipitated with ICAM-5 from mouse brain and the binding region in ICAM-5 was localized to the two first Ig-domains. $\beta 1$ integrins were juxtaposed to filopodia tips at the early stage of synaptic formation, but as synapses matured, integrins $\beta 1$ covered the mushroom spines. When the interaction between ICAM-5 and $\beta 1$ integrins was up-regulated or down-regulated by antibody treatments, ICAM-5 ectodomain cleavage decreased or increased, respectively, resulting in altered spine maturation. These results suggest that the interaction between ICAM-5 and $\beta 1$ integrins is important for synapse formation." **Funding:** Academy of Finland, Sigrid Jusélius Foundation, Magnus Ehrnrooth Foundation, Finnish Medical Association and Liv och H \ddot{u} lsa Foundation.

Title: Aging-related changes of glial cell excitability and synaptic plasticity

Authors: Richard Robitaille¹, Alexandre St-Amour², Joanne Vallée³

Affiliation: 1-3 Université de Montréal

Abstract: To be transmitted appropriately, neuronal information is modulated by a third element, the glial cell. Interestingly, during aging, synapses undergo functional and structural alterations. However, alteration of glial cells at synapses remains ill defined. Hence, we investigated functional neuron-glia interactions during aging to better understand this physiological phenomenon. We used the mouse neuromuscular junctions (NMJs), a well-known model to study neuron-glia interactions. We first examined the structural changes since glial cells at NMJs are known to regulate its morphological stability. Using immunohistochemical labeling of each synaptic elements of adult (90 days) and old (600 days) NMJs, we uncovered that the number of glial cells expressing S100B (specific glial protein) decreased during aging. Interestingly, glial processes were thinner and the glial coverage of the synapse was incomplete. We next investigated the excitability of glial cells by monitoring Ca²⁺ changes. This glial activity is known to regulate

synaptic activity and plasticity. Ca²⁺ activation was induced by agonist applications (ATP, Muscarine) and by endogenous release of transmitters. Our results show that glial activity was altered and weakened in old animals. Consistent with these findings, we found that all forms of potentiation of transmitter release were decreased while synaptic depression was increased. This suggests that synaptic efficacy is weakened during aging. In conclusion, our work provides new evidence that neuron-glia perturbations during aging are associated with major functional alteration of synaptic function.

Funding: CIHR, NSERC, FRSQ, CFI

Title: Dendritic calcium transients evoked by backpropagating action potentials control the induction of long-term potentiation at inhibitory synapses onto hippocampal interneurons

Authors: Simon Chamberland¹, Lisa Topolnik²

Affiliation: 1-2 Universite Laval

Abstract: "Dendritic calcium signaling is essential for different forms of synaptic plasticity. The functional organization of calcium signaling in dendrites of different sub-types of GABAergic interneurons (INs) remains largely unknown. Here we combined two-photon calcium imaging and whole-cell patch-clamp recordings in mouse hippocampal slices to investigate the mechanisms and significance of calcium transients evoked by backpropagating action potentials (bAP-CaTs) in local circuit and long-range projecting hippocampal INs. Long-term potentiation (LTP) at inhibitory synapses onto INs was induced by theta-burst firing (TBF, 3APs at 100-Hz, repeated 10 times at 5-Hz). In dendrites of all INs examined, the amplitude of bAP-CaTs decreased significantly with distance from the soma. Moreover, bAP-CaTs demonstrated the cell type-specific properties, with the long-range projecting cells having the highest calcium elevations among INs examined. In addition, L-, P/Q- and T-types voltage-gated calcium channels (VGCCs) contributed to bAP-CaTs in a cell-type-specific manner. Furthermore, TBF alone was sufficient to induce LTP at inhibitory synapses onto INs. LTP was expressed presynaptically; it was dependent on the intracellular calcium elevation and required the activation of T- but not of L-type VGCCs. Our data indicate that in inhibitory INs bAP-CaTs are spatially restricted to proximal dendritic sites, where they control the efficacy of transmission at inhibitory synapses. The pathway by which this happens appears to constitute a negative feedback loop - increased firing activity of INs potentiates the inhibitory drive they receive, thus decreasing the activity of INs further. T-type VGCCs may play a central role

in controlling this form of synaptic plasticity. "

Funding:

Title: Schizophrenia susceptibility pathway neuregulin1-ErbB4 suppresses the Src kinase upregulation of NMDAR responses during theta-patterned induction of lasting synaptic potentiation

Authors: Graham Pitcher¹, Lorraine Kalia², David Ng³, Nathalie Goodfellow⁴, Kathleen Yee⁵, Evelyn Lambe⁶, Michael Salter⁷

Affiliation: 1-3,7 The Hospital for Sick Children 4,6 University of Toronto 5- Tufts University School of Medicine

Abstract: A prominent theory is that the primary causal mechanism underlying the cognitive dysfunction and core behavioral manifestations in schizophrenia is 'hypofunction of the NMDA receptor'. We investigated this possibility by taking advantage of the candidate schizophrenia genes, Nrg1 and Erbb4. We examined the effects of NRG1-ErbB4 signaling on basal NMDA receptor (NMDAR) function and on the upregulation of NMDAR function, and subsequent synaptic potentiation, by the non-receptor tyrosine kinase Src. We made whole cell-recordings from neurons in acute brain slices from rodents and studied excitatory post-synaptic responses evoked by stimulating inputs to neurons in CA1 hippocampus or in prefrontal cortex. For the hippocampus we also studied long-term potentiation (LTP) evoked by electrical stimulation patterned on theta rhythm. We found, contrary to prediction of the NMDAR hypofunction theory, that activating the NRG1-ErbB4 pathway had no effect on NMDAR function, yet blocked LTP in CA1, the dominant form of synaptic plasticity arising from NMDAR activation. We resolved this apparent paradox by discovering that NRG1-ErbB4 signaling blocks the enhancement of NMDAR function by Src in the hippocampus and the prefrontal cortex. In addition, we discovered that NRG1-ErbB4 signaling, by inhibiting Src, dramatically suppressed the neuronal membrane response to brief theta rhythm-patterned stimulation. Our findings suggest that suppression of the Src-mediated enhancement of NMDARs may be a common neural mechanism in schizophrenia. That NRG1-ErbB4 signaling suppresses responses during theta rhythm but is without effect on responses to individual, isolated stimuli demonstrates a previously unknown critical timing period during which neuronal activity is exquisitely vulnerable.

Funding: Acknowledgments: Canadian Institutes of Health Research

Title: The neocortical synchrony of the slow oscillation silent state depends on active inhibition and thalamocortical functional afferents

Authors: Maxime Lemieux¹, Igor Timofeev²

Affiliation: 1-2 Centre de recherche Université Laval Robert-Giffard

Abstract: The slow oscillation (<1 Hz) of NREM sleep is an alternation of active and silent states of cortical and thalamic neurons. Previous studies have reported that active state onset propagates and that silent state onset occurs synchronously. Our goal was to study mechanisms underlying this synchrony. We studied slow oscillation induced by ketamine-xylazine anesthesia in the cat neocortex with multisite local field potentials and intracellular recordings. In the intact neocortex, the silent state onset occurs within 10-30 ms in average for cortical sites distant of 10 mm. In slab, a portion of neocortex devoid of thalamocortical and long range corticocortical projections, silent state onset lags by hundreds of milliseconds. Extracellular stimulations often elicited a transition to silent state with a maximal likelihood 500 ms after active state onset. Pharmacological inactivation of thalamic functional afferents affected neocortical silent state onset synchronization. Dual intracellular recordings in the affected cortical territory showed that silent state onset was asynchronous. To investigate the contribution of active inhibition in triggering silent state transition, we recorded cells with pipettes filled with potassium chloride (KCl 2M) to reverse chloride concentration gradient simultaneously with closely located cells recorded with potassium acetate 2M (control). All cells recorded with KCl pipettes lagged behind control cells. Our results showed that active inhibition contributes to onset of silent state and that its synchronous onset requires functional thalamic afferents.

Funding: FRSQ, CIHR, NSERC, NIH.

Title: Retinal CB1 receptors control glycinergic modulation of visual responses by regulating intracellular chloride in the inner retina

Authors: Lois Miraucourt¹, Jenny Tsui², Annie Castonguay³, Yves De Koninck⁴, Edward S. Ruthazer⁵

Affiliation: 1,2,5 McGill University 3,4- Centre de Recherche Université Laval Robert-Giffard

Abstract: We studied the influence of endocannabinoid signaling on visual function using the retinotectal system of *Xenopus laevis* tadpoles. In in vivo patch clamp recordings from tectal neurons, we observed that application of the type 1 cannabinoid receptors (CB1R) agonist WIN 55,212-2 unexpectedly enhanced the excitatory postsynaptic currents evoked by direct electrical stimulation of retinal ganglion cells (RGCs) in the eye. This enhancement was not only blocked by the CB1R antagonist AM-251, but also by the glycine receptor antagonist strychnine and the NKCC1 chloride transporter blocker bumetanide. However, responses evoked by direct optic tract stimulation were unaffected by bath application of WIN, implicating the retina rather than the tectum as the site of relevant CB1R activation. Extracellular RGC recordings showed that WIN preferentially increased the spike frequency evoked by OFF visual stimuli, an effect that was prevented by pretreatment with AM-251. However, electroretinograms showed no effect of WIN on light-evoked responses in the outer retina, implicating the inner retina as the main site of action. Similarly, in vivo 2-photon imaging of retinal neurons transfected to express the fluorescent ratiometric chloride indicator clomeleon, revealed that application of WIN induced no change in bipolar cells intracellular chloride, but rapidly increased it in a subset of amacrine cells and RGCs. To test for functional consequences on vision we have begun mapping CB1R-mediated changes in visual receptive field properties. Taken together, these results present a model where endocannabinoid signaling in the retina impacts vision by modulating retinal excitability through regulation of chloride currents.

Funding: Funded by Jeanne-Timmins Costello Fellowship (LSM, JT), FRSQ (JT), CIHR (ESR), Epilepsy Canada (LSM) and EJLB Foundation (ESR).

Title: Intersectin1 (Itsn1) modulates Ca²⁺-dependent replenishment of the readily-releasable pool of synaptic vesicles at a central synapse

Authors: Yi-Mei Yang¹, Ameet Sengar², Jamila Aitoubah³, Giovanbattista Grande⁴, Michael Salter⁵, Lu-Yang Wang⁶

Affiliation: 1-6 The Hospital for Sick Children and University of Toronto

Abstract: Itsn1 is an evolutionarily conserved protein and abundantly expressed in neurons. Functional studies from invertebrate preparations suggest that Itsn1 regulates dynamin-dependent endocytosis, but its effects at mammalian central synapses are largely unknown. Using the calyx of Held synapse in the brainstem taken from wild-type (WT) and Itsn1 knockout (KO)

mice, we labeled Itsn1 with its specific antibody and showed that Itsn1 localizes to both pre- and postsynaptic elements. Patch-clamp recordings demonstrated deletion of Itsn1 has little effect on evoked or spontaneous synaptic responses or short-term plasticity at various frequencies. By applying two high-frequency trains at different intervals, with the first train to deplete the readily-releasable pool (RRP) of synaptic vesicles and the second train to measure replenishment of the RRP, we revealed that the recovery kinetics from depletion can be fitted by a double-exponential function and knock-out of Itsn1 selectively reduces the fast component of recovery. Application of TEA, which broadens action potentials and increases presynaptic Ca²⁺ concentration, dramatically augments the fast component in WT but not in KO synapses. This suggests that Itsn1 is specifically required for Ca²⁺-dependent fast replenishment. Furthermore, we measured the recovery time course in the presence of dynamin inhibitors at WT synapses. Surprisingly, we found that blocking the function of dynamin did not attenuate the fast component of recovery, indicating Itsn1-mediated replenishment is independent of dynamin. Collectively, we conclude that Itsn1 is a core mediator necessary for Ca²⁺-dependent fast refilling of the RRP without interfering exocytosis or dynamin-dependent endocytosis at this central synapse.

Funding: CIHR

Title: HCN Channels Modulate Adrenergic and Cholinergic Persistent Activity in Medial Prefrontal Cortex

Authors: Steven Cordeiro Matos¹, Zizhen Zhang², Philippe Séguéla³

Affiliation: 1-3 Montreal Neurological Institute, McGill University

Abstract: "Hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels are involved in dendritic integration and play a key role in pathological hyperexcitability associated with neuropathic pain and epilepsy. HCN channels are widely distributed throughout the central nervous system and have been shown to colocalize with $\alpha 2$ adrenoceptors on dendritic spines of prefrontal neurons. Through activation of $\alpha 2$ adrenoceptors, norepinephrine (NE) enhances working memory performance and delay-related neuronal activity in the medial prefrontal cortex (mPFC). This NE-evoked increase in cell firing is believed to reflect a recurrent network mechanism associated with HCN channel inhibition suggesting a novel role for HCN channels in prefrontal functions including pain-related learning and memory. Recently, intrinsic neuronal persistent firing (PF) induced by metabotropic glutamate and acetylcholine receptor activation

has been reported in the mPFC and proposed to be a non-synaptic substrate for working memory. However, the role of NE and HCN channels in modulating intrinsic properties of pyramidal neurons in the mPFC has not been addressed. Here we report, using whole-cell patch-clamp in synaptically isolated pyramidal neurons in layers II/III of the anterior cingulate subdivision of the mPFC, that NE-induced PF involves presynaptic $\alpha 1$ adrenoceptor-mediated glutamate release. Blocking HCN channels with ZD7288 not only increased membrane input resistance but also facilitated NE-induced PF. Furthermore, both activation of $\alpha 2$ adrenoceptors with clonidine and blockade of HCN channels with ZD7288 enhanced PF induced by the cholinergic agonist carbachol. Thus, our results suggest that HCN channels provide a molecular link for interactions between adrenergic and cholinergic receptor inputs in prefrontal mnemonic processes."

Funding: This work was supported by an operating grant from CIHR.

Title: Differential implication of parvalbumin- vs. somatostatin-expressing CA1 interneurons during hippocampal theta rhythm: an in vitro approach using an intact hippocampal preparation

Authors: Carey You Lim Huh¹, Bénédicte Amilhon², Sylvain Williams³

Affiliation: 1-3 Douglas Institute & McGill University

Abstract: Hippocampal interneurons are thought to be important for theta rhythm generation but the exact role of each interneuron subtype remains elusive. Here, we investigated how two particular classes of CA1 interneurons, parvalbumin (PV)-positive basket cells and somatostatin (SOM)-positive O-LM cells, fire during an intrinsically generated hippocampal theta rhythm. We used an acute, intact hippocampal preparation and simultaneously recorded CA1 oscillations (3-12Hz) and activity of PV neurons in CA1 stratum pyramidale/oriens or SOM neurons in CA1 stratum oriens in whole-cell mode. For visualization of specific interneuron subtypes, transgenic mice with Cre-driven expression of tdTomato under the control of PV or SOM promoter were used. We found that 4 of 7 PV neurons and 5 of 9 SOM neurons displayed spontaneous burst-firing that was robustly phase-locked to the peaks of CA1 theta while the remaining PV and SOM cells fired tonically or showed little spontaneous firing. Next, EPSCs and IPSCs were examined in voltage clamp at -70 and +15mV, respectively. We found that PV neurons received EPSCs that were 2.5-fold greater in amplitude compared to SOM neurons (773pA vs. 304pA, $p < 0.05$). In contrast, IPSC amplitude was not significantly different between groups (339pA vs. 228pA, $p > 0.05$). These results demonstrate that given an intrinsically generated hippocampal oscillation,

both PV- and SOM-expressing CA1 interneurons prefer to fire at the peaks of theta cycles.

However, PV neurons appear to receive a stronger excitatory input from the network, suggesting that they may contribute more to the network rhythm. We are currently testing this hypothesis using optogenetic methods.

Funding: CIHR, NSERC

Title: Exploring macrophage phenotypes in injured trigeminal nerves

Authors: SeungHwan Lee¹, Ji Zhang²

Affiliation: 1-2 McGill University

Abstract: Macrophages are important immune effector cells. Injury of peripheral nerves triggers infiltration of macrophages into damaged nerves where they play critical roles in pain pathogenesis. We used a well established animal model of trauma associated oro-facial pain (loose ligation on mental nerves) which induced mechanical hypersensitivity in lower lip of rats for at least a month. A burst of Iba-1+ macrophages were found around and within the damaged nerves from day 3-day 28 post-injury. From day 3-day 14, Iba-1+ macrophages expressed high levels of ED1(CD68), a marker for phagocytic cells. They displayed a large, irregular cell shape, containing many vacuoles found within damaged nerve fibers. However, another Iba-1+ population around the lesion site and perineurium displayed a low expression profile of ED-1, but high level of MAC-1(CD 11b). Interestingly, MAC-1 high ED-1 low macrophages expressed both pro- (IL-6, MIP-1alpha) and anti-inflammatory (TGF-beta1) mediators. In contrast, ED-1highMAC-1low macrophages did not express either pro- or anti-inflammatory mediators at all examined time points. At day28 post-injury, while the number of MAC+ cells was reduced, ED-1+ macrophages were found essentially distal to the injury site with almost complete vacant in the zone of demyelination. In parallel with that, the expression of pro- and anti-inflammatory mediators was significantly reduced. As macrophages display remarkable plasticity and distinct functions, exploring the full spectrum of macrophage phenotypes in injured peripheral nerve will allow us to further understand their involvement in chronic pain. Individual macrophage population may be selectively targeted by cell-specific therapeutics for an effective treatment.

Funding: Neuroinflammation CIHR Strategic Training Program

Title: Role of neural activity in the maturation of perisomatic GABAergic innervation in the post-natal cortex

Authors: Elie Baho¹, Graziella Di Cristo²

Affiliation: 1-2 CHU Sainte-Justine/Université de Montréal

Abstract: GABAergic interneurons play an important role in cortical function and plasticity. Alterations in GABAergic circuits are implicated in various neurodevelopmental disorders. The GABAergic network comprises of diverse interneuron subtypes that have different morphological and physiological characteristics, and localize their synapses onto distinct subcellular locations on the postsynaptic targets. Precisely how activity and molecular driven mechanisms conspire to achieve the remarkable specificity of GABAergic synapse localization and formation is unknown. Here we focus our study on a subtype of GABAergic neurons - the basket interneurons that localize their synapses onto the soma and proximal dendrites of the postsynaptic targets, and tightly regulate their firing patterns. Although recent studies have shown the activity dependence of basket synapse formation, the cellular and molecular mechanisms underling the effect of neural activity on the development of GABAergic synapses, axonal arborisations and innervation patterns in the developing brain are yet unclear. Using the *Drosophila* allatostatin receptor and the tetanotoxin light-chain, we respectively reduced and suppressed neural activity to characterize its role during perisomatic synapse maturation and maintenance. Here we show that, surprisingly, reducing and completely blocking neural activity of a single GABAergic cell have opposite effects on the maturation of its innervation field and synaptic density in organotypic slices. On the other hand, both treatments severely affect innervation maintenance at later ages. Future experiments will allow us to clarify the molecular mediators of the role of activity in GABAergic synapse development in the brain.

Funding: NSERC CIHR Scottish Rite

Title: Regulation of dendritic spine formation by dopamine and glutamate in striatal medium spiny neurons

Authors: Dominic Thibault¹, Louis-Eric Trudeau²

Affiliation: 1-2 University of Montreal

Abstract: The striatum is the main input into the basal ganglia circuit, a pathway that is crucial in the regulation of voluntary movement and certain aspects of motivation and cognition. The vast

majority of striatal neurons are called Medium Spiny Neurons (MSNs) because they express a high density of dendritic spines, which are postsynaptic specializations where glutamate afferents from the cortex and thalamus make excitatory synapses. The striatum also receives dopaminergic (DAergic) inputs from the substantia nigra. MSNs are mainly segregated into 2 subpopulations based on their expression of either D1 or D2 dopamine (DA) receptors. In Parkinson's disease, loss of DAergic afferents into the striatum leads to a massive reduction in MSN dendritic spine density, mainly in the D2-expressing population. As of yet, the specific role of DA in the initial formation of spines during development remains poorly understood. Based on recent findings by our laboratory, we hypothesize that DA is a key modulator of both spinogenesis on immature MSNs and of the maintenance of spines on mature MSNs. We will describe the use of a new postnatal primary neuron culture model including MSNs, DAergic and cortical neurons. We are currently in the process of evaluating how DAergic and glutamatergic inputs interact in the regulation of dendritic spine formation, maturation and maintenance. Our preliminary data suggest that DA and glutamate indeed drive the formation of more dendritic spines in this model. We are currently investigating which receptors contribute the most to this regulatory effect. Work funded by the CIHR.

Funding: Work funded by the CIHR.

Title: Role of microtubules in osmosensory transduction in supraoptic nucleus neurons

Authors: Masha Prager-Khoutorsky Prager-Khoutorsky¹

Affiliation: 1- Centre for Research in Neuroscience

Abstract: Maintenance of constant plasma osmolality in mammals is vital, as changes in cell volume caused by severe acute hyperosmolality or hypoosmolality can irreversibly damage organs and cause lethal neurological trauma. The release of vasopressin during hypertonicity plays a key role in osmoregulation because it promotes water reabsorption by the kidney. Magnocellular neurosecretory cells (MNCs) in the hypothalamic supraoptic nucleus are intrinsically osmosensitive. MNCs transduce hypertonicity into spiking, which stimulates vasopressin release from axon terminals located in the neurohypophysis. This transduction is mediated by stretch-inhibited nonselective cation channels which are activated during hypertonicity-evoked shrinking, and inhibited during hypotonicity-evoked swelling. Recent studies imply that osmosensation is a mechanical process, and sensitivity of transduction

increases in proportion to actin filament density. Here, we investigated the role of microtubules in this process using confocal imaging and patch clamp recordings from supraoptic nucleus MNCs acutely isolated from adult rats. Images of brain sections and isolated MNCs demonstrated that these neurons feature a prominent microtubule network compared to other neurons. Using whole-cell current-clamp and voltage-clamp recordings from acutely isolated MNCs, we tested the hypothesis that this unique microtubule structure is important for intrinsic osmo- and mechanosensation. We found that disruption of microtubules in MNCs with 0.5 μ M nocodazole abolishes suction- and hypertonicity-evoked activation of cation channels. Conversely, stabilization of microtubules with 5 μ M taxol enhances responses to suction- and hypertonicity. We conclude that MNCs from supraoptic nucleus exhibit a unique microtubule network that is an essential component for mechano- and osmotransduction.

Funding: Work in the Authors' laboratory is supported by CIHR, James McGill Research Chair to C.W.B. and HSFC Fellowship to M.P.K.

Title: TRPM2 functions in ischemic cell death by regulating NMDA receptor expression

Authors: Ishraq Alim¹, Lucy Teves², Rongwen Li³, Kinga Szdlowska⁴, Michael Tymianski⁵

Affiliation: 1- University of Toronto 2-5 Toronto Western Hospital Research Institute

Abstract: Ischemia is associated with a shortage of energy which influences neuronal membrane potential and leads to excessive neurotransmitter release. This in turn causes over-activation of various postsynaptic glutamate receptors (NMDA, AMPA) and other cation channels (ASICs, NCX, hemichannels, and TRPM channels). As a result there is an excessive influx of calcium into neurons, which activates several signaling cascades leading to neuronal death. TRPM2 channels have been suggested to play a critical role in Ca²⁺ influx during ischemia and cell death. Our study used TRPM2 knockout (KO) mice to determine the role of TRPM2 in ischemic cell death. First, we used an in vivo stroke model, middle cerebral artery occlusion (MCAO), and observed that in 1hr occlusion followed by 48 hr reperfusion, TRPM2 KO mice had a 26% decrease ($p < 0.05$) in infarct volume when compared to wild-types (WT). Next, we used field potential recordings to determine if TRPM2 modulates synaptic activity in hippocampal slices during oxidative stress. Bath application of 200 μ M of H₂O₂ (ROS) caused a 50% increase ($p < 0.01$) in evoked fEPSP slope in KO CA1 region and had no effect in WT CA1 region. Finally, we used western blots to determine if TRPM2 KO has an effect on NMDA receptor expression in the hippocampus. NR2B expression was

reduced, while NR2A expression was increased in TRPM2 KO when compared to WT. These findings suggest that in the absence of TRPM2, extrasynaptic NMDA receptors involved in cell death (NR2B) are reduced, while synaptic NMDA receptors involved in excitability (NR2A) are increased.

Funding:

Title: Modulation of postsynaptic nmdars by endocannabinoids via non-CB1 receptor-dependent actions

Authors: KAI YANG¹, GANG LEI², MICHAEL JACKSON³

Affiliation: 1-3 Robarts Research Institute

Abstract: At many excitatory and inhibitory synapses, endocannabinoids released by postsynaptic cells act retrogradely on presynaptic G-protein-coupled cannabinoid (CB1) receptors to inhibit neurotransmitter release. Here, we demonstrate that cannabinoids may also modulate the function of NMDARs at postsynaptic sites. In isolated hippocampal pyramidal neurons, application of the endocannabinoids anandamide or 2-arachidonylglycerol (2-AG), both applied at physiological concentrations, potentiated NMDAR-mediated currents. This effect could be observed in the presence of either a CB1 antagonist or vanilloid receptor 1 antagonist. In addition, the application of bisindolylmaleimide, a PKC inhibitor, abolished the enhancement of NMDAR function induced by anandamide and 2-AG. In summary, our study has identified NMDARs as a target for postsynaptic, non-CB1 actions of cannabinoids.

Funding: Supported by CIHR and Banting Research Foundation.

Title: The role of CaMKII-beta in structural plasticity of dendritic spines

Authors: Gurpreet Lakhanpal¹, Karam Kim², Yasunori Hayashi³, Kenichi Okamoto⁴

Affiliation: 1,4 Samuel Lunenfeld Research Institute, Mount Sinai Hospital 2,3 RIKEN Brain Science Institute

Abstract: Understanding how synaptic structure is maintained and modulated by activity is essential for elucidating the mechanisms of learning and memory. Here, we look at how CaMKII-beta (calcium/calmodulin-dependent protein kinase type II beta), an abundant F-actin binding Ser/Thr kinase, is involved in the structural plasticity of dendritic spines. Synaptic plasticity, such as LTP (long-term potentiation), has been considered the cellular basis for the process of learning

and memory. Recent advances in cellular imaging have revealed another aspect of synaptic plasticity, called “structural plasticity”, which involves activity-dependent structural changes in dendritic spines. Dendritic spines are tiny protrusions ($\sim 1 \mu\text{m}$) present on dendrites in excitatory neurons. Live imaging has enabled us to monitor structural changes in dendritic spines in living tissue. However, despite advances in imaging techniques, the molecular mechanisms governing structural plasticity are not yet known. We previously found that CaMKII-beta bundles F-actin and stabilizes actin dynamics to maintain dendritic spine structure. Moreover, when CaMKII-beta is activated it unbundles actin filaments in in vitro (Okamoto et al., PNAS 2007). These results suggest that its kinase activation status may regulate activity-dependent reorganization of the actin cytoskeleton in dendritic spines during LTP. In this study, we found that autophosphorylation of the CaMKII-beta F-actin binding domain was crucial for bundling actin filaments. Furthermore, mimicking autophosphorylation of the F-actin binding domain altered localization of CaMKII-beta in dendritic spines, suggesting activity-dependent regulation of the actin cytoskeleton. We will discuss in further detail how CaMKII-beta regulates dendritic spine structure during LTP by the interaction of CaMKII-beta and actin.

Funding: CIHR

Title: Oligodendroglial Expression of DCC is Required for the Organization of Paranodal Junctions in vivo

Authors: Sarah-Jane Bull¹, Jenea M Bin², Alexandre Boutet³, Abbas F. Sadikot⁴, Timothy E. Kennedy⁵

Affiliation: 1-5 McGill University

Abstract: Netrin-1 and the netrin receptor DCC are expressed by mature myelinating oligodendrocytes (OLs) in the adult central nervous system (CNS). In the developing CNS, these proteins direct the migration of oligodendrocyte precursor cells and promote OL process branching during maturation. Furthermore, in vitro studies of mature myelinating oligodendrocytes have implicated netrin-1 and DCC in the maintenance of paranodal axoglial junctions, which segregate myelinated axons into distinct domains that are essential for efficient saltatory conduction. DCC is expressed by both neurons and OLs, however, it is not possible to study DCC function in the adult CNS in vivo using conventional knockout mice, as DCC nulls pups die shortly after birth. To investigate the cell-autonomous function of DCC expressed by mature

myelinating oligodendrocytes *in vivo*, we adopted a conditional gene knockout strategy to selectively delete DCC expression from OLs. We demonstrate that conditional knockout mice develop balance and coordination deficits, and that DCC is required to maintain the organization of oligodendroglial paranodal junctions, myelin ultrastructure, and myelin protein composition. We conclude that DCC expression by mature myelinating OLs is essential for myelin maintenance *in vivo*.

Funding: This work was supported by grants from from the Multiple Sclerosis Society of Canada.

Title: LITAF expression influences the activity and intracellular localization of the ubiquitin ligase Itch

Authors: Guillaume Desrochers¹, Heather Eaton², Samuel Drory³, Julie Metcalf⁴, Craig Brunetti⁵, Annie Angers⁶

Affiliation: 1,3,6 University of Montreal 2,4, 5 Trent University

Abstract: Charcot-Marie-Tooth (CMT) is the most common inherited neurological disease caused by mutations in neuronal proteins and affecting nervous conductivity. LITAF is one of the genes associated with a severe demyelinating form of the syndrome. This gene encodes LITAF/SIMPLE, a small protein characterized by a Zinc-finger domain, termed the SIMPLE-like domain (SLD), which resembles the ubiquitin ligases RING-finger domain interrupted by a trans-membrane domain. To date, no known function has been attributed to the SLD. Another feature of LITAF is the presence of two PPXY motifs that mediate recognition by WW-domain containing proteins. LITAF also harbours a lysosomal targeting sequence, and has been observed in the lysosome, late endosome and plasma membrane. CMT is also characterized by protein aggregates, suggested to induce Schwann cell apoptosis conducting to demyelination. LITAF function has thus been suggested to be related to protein degradation. Here, we demonstrate that LITAF is localized to the lysosomal compartment in a variety of cell lines. We also show that the ubiquitin ligase Itch and LITAF strongly interact via the two PPXY motifs on LITAF. Interestingly, co-expression of LITAF with Itch induces major changes in Itch intracellular localization, by binding it and bringing Itch to the lysosome. Preliminary results also indicate that LITAF expression induces strong degradation of Itch, possibly by stimulating its autocatalytic activity. Determining the physiological parameters inducing this interaction and Itch downregulation could help shed light onto LITAF function and

its role in CMT.

Funding: NSERC

Title: Protein kinase C regulates secretion in neuroendocrine cells

Authors: Chris Groten¹, Neil Magoski²

Affiliation: 1-2 Queen's University

Abstract: Second messenger systems such as protein kinase C (PKC), Ca²⁺, and cyclic adenosine monophosphate influence neuronal secretion by regulating membrane permeability and vesicle trafficking. The bag cell neurons of *Aplysia californica* have been used extensively to examine the regulation of secretion. Upon stimulation, these neuroendocrine cells undergo a long-lasting afterdischarge during which hormones are released to initiate reproduction. Secretion in these cells is due to both Ca²⁺ influx and release from intracellular stores, such as the mitochondria. PKC is activated during the afterdischarge and may serve to amplify secretion. Here, we test the hypothesis that PKC regulates secretion from bag cell neurons. Experiments were performed under whole-cell recording in cultured bag cell neurons loaded with fura, allowing for monitoring of intracellular Ca²⁺ in voltage-clamp. Secretory output was determined by measuring the change in capacitance following a 1 minute train of depolarizing voltage-steps. The PKC signalling pathway was activated with the phorbol ester, phorbol-12-myristate-13-acetate (PMA). Mitochondrial Ca²⁺ was liberated using carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP). A train elicited a large rise in capacitance that was enhanced in PMA-treated neurons. PMA did not influence Ca²⁺ channel activity, as measured by intracellular Ca²⁺ imaging and Ca²⁺ current recordings. Moreover, PMA augmented secretion induced by Ca²⁺ released from mitochondria. PKC appears to alter secretory output through the regulation of secretory processes, such as vesicle trafficking or priming, rather than channel modulation. This implicates PKC as a vital regulatory component that dictates hormone release and species propagation.

Funding: CIHR MOP 111211

Title: Neuropeptide Y Inhibits Dendritic Ca²⁺ Electrogenesis and Long-Term Depression in Layer 5 Pyramidal Neurons

Authors: William Colmers¹, Trevor Hamilton², Matthew Larkum³

Affiliation: 1- University of Alberta 2- Grant MacEwan University 3- University of Bern

Abstract: "The dendritic structure of neocortical layer 5 pyramidal neurons is tuned to integrate synaptic input and action potentials. Above a critical frequency (CF), a train of backpropagating action potentials (bAPs) can induce regenerative Ca^{2+} currents, giving rise to distal dendritic spikes. Such dendritic spikes not only propagate to the soma and influence neuronal output but also contribute to synaptic plasticity. Neuropeptide Y (NPY) is released by neocortical interneurons and NPY1 receptors are expressed on layer 5 pyramidal dendrites. We made simultaneous somatic and dendritic whole-cell patch clamp recordings of these cells in slices of rat somatosensory cortex. Trains of 4 bAPs were elicited at the soma. NPY (1 μM) significantly, and reversibly, increased the CF and decreased the afterdepolarization at both distal and somatic electrodes in all neurons tested. Local application of NPY at the distal dendritic tuft, but not the soma, significantly and reversibly increased CFs recorded in the distal dendrite (Ca^{2+} transients) and at the soma (Vm). NPY had no presynaptic effects at inputs to layer 5 pyramidal neurons. Long-term depression (LTD) was induced via 100 pairings of an EPSP generated in L1 with a supra-CF burst of 2 bAPs. Bath application of NPY prevented LTD, though it could be reliably induced after NPY washout. Our results provide evidence that NPY acts on distal dendrites to inhibit Ca^{2+} electrogenesis, which in turn prevents LTD. NPY may thus prevent the resetting of synaptic dynamic range thought to be a major role for LTD."

Funding: Supported by CIHR support to WFC (MT10250). WFC is an AHFMR Medical Scientist

Title: Quantal transmission at synapses originating from hippocampal interneuron-specific interneurons

Authors: Christopher Lacharité Mueller¹, Lisa Topolnik², Simon Chamberland³

Affiliation: 1-3 Université Laval

Abstract: In the hippocampus, there is a unique population of inhibitory interneurons (INs) that innervate exclusively other GABAergic cells. The majority of these interneuron-specific interneurons (ISIs) co-express vasoactive intestinal polypeptide (VIP) and calretinin. Despite their potential ability to control and synchronise the entire hippocampal circuitry, the properties of these cells and the synapses they form remain largely unknown. Using a combination of single and paired whole-cell patch-clamp recordings between type III ISIs (ISI-IIIs) and oriens-alveus (O/A) INs, glutamate uncaging-based two-photon laser scanning photostimulation (GU-LSPS) and immunohistochemistry, we mapped and characterised the connections between putative ISIs and

O/A INs in hippocampal slices of VIP-eGFP mice. GU-LSPS-based mapping and paired recordings showed that putative ISI-IIIs were connected to O/A INs, including oriens-lacunosum-moleculare (O-LM) cells. Unitary inhibitory postsynaptic currents (uIPSCs) at ISI-III - O-LM synapses had a small amplitude and relatively slow kinetics, consistent with a dendritic location of synapses. Variance-mean analysis revealed the existence of multiple release sites with low initial release probability. Furthermore, uIPSCs summated efficiently during high frequency (100-200 Hz) firing of ISI-IIIs. In addition, when slightly depolarized from resting membrane potential, ISI-IIIs showed intrinsic theta oscillations that were associated with rhythmic uIPSCs in O-LMs. Our data indicate that ISI-IIIs contact O-LM INs with multiple release sites and low initial release probability synapses. Furthermore, repetitive firing of ISI-IIIs at physiological frequencies is translated into efficient inhibition of O-LM INs providing a means of controlling their dendritic integration and spike initiation.

Funding: CIHR, NSERC, Savoy foundation

Title: Role of cofilin1 in dendritic spine structural plasticity

Authors: Mustafa Khan¹, Gurpreet Lakhanpal², Kenichi Okamoto³

Affiliation: 1- Samuel Lunenfeld Research Institute

Abstract: "Understanding how synaptic structure and function is modulated by activity is essential for elucidating the mechanisms of learning and memory. Here we study the role of cofilin1, an actin binding protein, in structural plasticity of dendritic spines. Dendritic spines are tiny protrusions found on dendrites that are the sites of excitatory synaptic transmission in the central nervous system. Actin is found in high concentrations in dendritic spines where it serves as the major cytoskeletal protein. During LTP (long-term potentiation), actin polymerizes rapidly and persistently in dendritic spines and causes enlargement of the spine. This is known as structural plasticity; however, the precise mechanism is still unclear. Since the regulation mechanism of the actin cytoskeleton is a key locus in spine structural change, we initiated a study of a major actin regulating protein called cofilin1. Cofilin1 functions to directly depolymerize or sever actin filaments to promote actin disassembly in cells. In neurons, Cofilin1 localizes in dendritic spines, suggesting its involvement in dendritic spine structure regulation by regulating actin dynamics. Cofilin1 activity is regulated through phosphorylation of its Ser-3 residue, and in dendritic spines, cofilin1 is strongly phosphorylated following LTP induction. This suggests that

cofilin1 activity may play an important role in structural plasticity of dendritic spines during LTP. Using caged glutamate and 2-photon laser scanning microscopy, we have initiated a study of how cofilin1 regulates dendritic spine structure during single-synapse LTP induction. We will discuss the importance of cofilin1 function in spine structural plasticity."

Funding: CIHR

Title: Astrocytes induce AMPA-mediated plasticity through the activation of pannexin-1 in hypothalamic synapses

Authors: R. Carolina Gutierrez Herrera¹, Roger J. Thompson², Jaideep S. Bains³

Affiliation: 1-3 Hotchkiss Brain Institute, Department of Physiology and Pharmacology

Abstract: "The magnocellular neurosecretory cells (MNCS) of the paraventricular nucleus (PVN) of the hypothalamus integrate incoming signals to generate a distinct output: secretion of hormones such as oxytocin (OT) from their terminals into the posterior pituitary. Using electrophysiology and 2-photon imaging in acute hypothalamic slices, our laboratory has made two key discoveries: 1) Astrocytes release ATP in response to noradrenaline or glutamate, potentiating glutamate synapses through the activation of postsynaptic P2X7 receptors; 2) potentiation requires AMPAR insertion. We have now observed that OT cells specifically express the channel pannexin 1 (Panx1). Based on reports of a P2X7R- Panx1 complex, we hypothesized that the ATP-mediated long-term plasticity (LTPATP) requires activation of Panx1 in OT neurons. Using electrophysiological and pharmacological tools we show that glutamate synapses exhibit LTP in response to the non-hydrolyzable ATP analogue, BzATP (30 uM, 5 min). LTP was blocked either by botulinum toxin-C or the calcium-calmodulin dependent protein kinase II (CaMKII) auto inhibitory peptide, indicating CaMKII and AMPA receptor insertion dependency. Blocking Panx1 with probenecid or the 10panx peptide prevented LTPATP. To determine the functional consequences of LTPATP, we assessed the impact of afferent stimulation on spike output in control and after 30 minutes of BzATP in the absence and presence of 10panx. BzATP increased spike probability in response to the same current injection. This effect was abolished when Panx1 was blocked. These findings describe a new form of synaptic plasticity induced by ATP and gated by Panx1 channels, that is sufficient to enhance spike output in OT neurons. "

Funding: CIHR

Title: Functional interaction between α CaMKII and GluN2B controls ERK-dependent structural plasticity.

Authors: Farida El gaamouch¹, Olivier Moustie², Mado Lemieux³, Paul De Koninck⁴, Alain Buisson⁵, Olivier Nicole⁶

Affiliation: 1,3,4 Centre de recherche Université Laval Robert-Giffard 2- CNRS UMR 6232, CINAPS 5- Centre Inserm U836 Institut des Neurosciences de Grenoble 6- CNRS UMR 5293 - Institut des Maladies Neurodégénératives

Abstract: "One key neurobiological mechanism underlying memory formation and storage resides in activity-driven modifications of synaptic strength and structural remodeling of synapses. Over the past decade, the ERK/MAPK pathway has emerged as a central player in signaling mechanisms involved in the activity-driven synaptic changes. One of the signaling events that activates ERK cascade is a rise in free intracellular Ca²⁺ concentration through the NMDA receptors. Despite the key role of prolonged ERK activation in synaptic plasticity, the role of NMDAR subunit composition in ERK signaling cascade is still under debate and the mechanisms by which a short elevated Ca²⁺ concentration induces a long lasting ERK activation remains unknown. We demonstrate that only GluN2B-containing NMDAR activation leads to ERK phosphorylation. In addition, we show that α CaMKII, but not β CaMKII or CaMKI, is involved in synaptic NMDAR-induced sustained ERK activation. To determine if the binding of α CaMKII to GluN2B is required for synaptic NMDAR-induced ERK activation, we used an α CaMKII mutant unable to interact with GluN2B, our results indicate that 1) a direct interaction between GluN2B and α CaMKII is essential to induce a prolonged ERK phosphorylation, 2) blocking of GluN2B- α CaMKII interaction as well as applying ERK inhibitors or a selective GluN2B antagonist alters the increase of dendritic spine size induced by synaptic activity. Thus the interaction between GluN2B and α CaMKII may serve to support long-lasting changes in synaptic plasticity, via ERK signaling. Altogether these functions suggest that the GluN2B/ α CaMKII/ERK triad may be considered as a major contributor for the formation of new memories."

Funding:

Title: Role of the Synaptotagmin-Dynamin interaction in synaptic vesicle recycling

Authors: Robyn McAdam¹, Fiona Young², Vanessa Blandford³, Sebastien Thomas⁴, Peter McPherson⁵

Affiliation: 1-5 Montreal Neurological Institute

Abstract: Synaptic vesicle protein Synaptotagmin I (Syt I) is a well-studied calcium sensor critical for synchronized neurotransmission. However, the role of the highly conserved Syt I juxtamembrane domain has yet to be investigated. My lab has shown that the Syt I juxtamembrane region interacts with the endocytic protein Dynamin. Using pulldown assays with GST-Syt I fusion proteins, we have shown that this interaction is modified by both conserved Syt I alternative splicing and phosphorylation. We propose that this interaction promotes rapid recycling of synaptic vesicles, and thus may influence short-term synaptic plasticity. Demonstrating its role in vesicle recycling, presented here are data from FM dye uptake experiments with mature hippocampal cultures in which the Syt I-Dynamin interaction has been disrupted.

Funding: Dr. Sossin's lab receives funds from NSERC for this project.

Title: Synaptopodin, an actin-associated protein, is involved in mature dendritic spine plasticity in the hippocampus

Authors: David Verbich¹, Denise Becker², Andreas Vlachos³, Thomas Deller⁴, R. Anne McKinney⁵

Affiliation: 1,5 McGill University 2-4 Goethe-University

Abstract: "Synapses are the fundamental units of brain computation and unravelling how they operate is key for understanding normal brain function and dysfunction. Most central excitatory synapses reside on motile dendritic spines. Recently, we demonstrated that subsets of spines on CA1 hippocampal neurons can form spine head protrusions (SHPs) in response to glutamate spillover controlled by astrocytic uptake. SHPs form on innervated spines and contact neighbouring presynaptic boutons, rapidly modifying microcircuitry. Interestingly, SHPs form preferentially on subsets of larger spines; why this is so is unknown. We hypothesize that the presence of a spine apparatus (SA), a smooth endoplasmic reticular-type organelle found in ~20% of CA1 spines, is required for this remodelling. To test this, we focused on synaptopodin (SP), a protein necessary for SA formation and certain forms of hippocampal plasticity and learning. Immunohistochemistry revealed that the majority of SHPs contained SP puncta. Using SP-null hippocampal slices, which lack all SA but have spine density and morphologies similar to wild type slices, we found that SHP formation was almost completely abolished. It thus seems likely that the SA is central for mature spine remodelling. We have begun to study the mechanisms of this SA-dependent remodelling. As a probable internal Ca store, we found that blocking Ca-induced Ca-

release inhibits new SHPs. Moreover, SHPs require both protein synthesis and intact actin dynamics. We posit that by acting as an integrator of cytoskeletal and Ca dynamics, the SA controls the intracellular signalling required for SHPs and thus the mature rewiring of dendritic spines."

Funding: CFI, CIHR, DAAD, NSERC

Title: Long-term interleukin-1beta exposure increases small IB4-positive and medium sensory neuron excitability by differential actions on ionic conductances

Authors: Patrick Stemkowski¹, Kelvin Jones², Peter Smith³

Affiliation: 1-3 University of Alberta

"We have shown that long-term exposure of rat dorsal root ganglion (DRG) neurons to interleukin-1 β (IL-1 β) selectively promotes increased excitability of small isolectin B4 (IB4)-positive and medium sized neurons (Stemkowski and Smith, IASP

Abstract: PM145, 2010). This finding may have relevance to the establishment of nerve injury induced neuropathic pain. Alterations in several ionic mechanisms have been proposed to underlie hyperexcitability in sensory neurons after nerve injury, including increased availability of sodium currents (INa) (Abdulla and Smith, J Neurophysiol., 88:2518, 2002) and hyperpolarization-activated current (IH) (Yao et al., J Neurosci., 23:2069, 2003), as well as decreased availability of potassium currents (IK) (Abdulla and Smith, J Neurophysiol., 85:644, 2001). We used whole-cell recording to examine the mechanism(s) underlying increased excitability of small IB4-positive and medium sized DRG cell bodies after 5-6 days exposure to 100pM IL-1 β . In medium neurons, IL-1 β significantly increased rates of IH activation (p<0.05). However, preliminary computer modeling studies suggest that changes in IH, alone, are not sufficient to drive increased excitability. Other possibilities include a leftward shift in the voltage dependency for activation of tetrodotoxin-sensitive (TTX-S) INa and reductions in various IK densities, such as Ca²⁺-dependent (IK,Ca) and A-type components. In small IB4-positive neurons, IL-1 β significantly slowed the rate of TTX-S INa inactivation and reduced IK,Ca density. These findings show that long-term IL-1 β exposure promotes cell specific changes to several ionic conductances. We are continuing to use computer models in the determination of the relative contribution each conductance makes to increased DRG neuron excitability. "

Funding: We would like to acknowledge CIHR, AHFMR and the Centre for Neuroscience for their and support.

Title: Impaired Chloride homeostasis gives rise to Cl mediated ionic memory and disturbs information processing.

Authors: Nicolas Doyon¹, Yves De Koninck², Steve Prescott³

Affiliation: 1-2 Université Laval 3- Pittsburgh University

Abstract: Impaired Cl⁻ homeostasis caused by KCC2 downregulation is known to be an important factor in various diseases such as pathological pain. While the role of transmembrane Cl⁻ gradient collapse on disinhibition is straightforward, its impact on cell dynamics as well as on information processing is much more subtle. In such instance, intracellular Cl⁻ becomes unstable and its slow dynamics can alter many properties of a cell. Using an extended Morris-Lecar model, we show how KCC2 downregulation gives rise to a Cl⁻ related ionic memory about past inhibitory synaptic input. This ionic memory however comes at the cost of impaired information transmission about current synaptic activity especially for low frequency events. Moreover, the slow changes in intracellular Cl⁻ concentration alter the dynamic properties of a neuron which may introduce bistability thus making the cell switch from type I to type II firing. This bistability may increase the extent and time constant of Cl⁻ related ionic memory making it theoretically permanent. We also investigated the impact of cell geometry, mainly the surface to volume ratio. We found that the time scope of ionic memory increases with the radius of the soma. Moreover the high density of spike generating voltage gated channels as well as GABA_A synapse in the AIS exacerbated the Cl⁻ related bistability. Bifurcation analysis revealed the possibility of several spiking pattern occurring only under fluctuations of Cl⁻ concentration.

Funding:

Title: Forward and backward propagation of electrical signals within dendritic trees of thalamocortical neuron is strongly modulated by morphological properties

Authors: Reza Zomorodi Moghaddam¹, Helmut Kroger², Igor Timofeev³

Affiliation: 1,2 Laval University, Department of Physics 3- Laval university, Dept of Psychiatry and Neuroscience

Abstract: Complex arborization as well as voltage-dependent conductances of dendrites plays a

determinant role in the process of synaptic integration and in the generation of firing pattern of a neuron. In this study, we examined the impact of the morphological properties of thalamocortical (TC) neurons on the efficacy of forward propagation (FP) of EPSPs arriving at the distal dendritic branches and the extent of back propagating (BP) electrical signals. We also investigated the effect of interaction between BP and FP signals in responsiveness of the cell. The analysis of geometrical (length, diameter, geometrical ratio, distribution of membrane area) and topological (mean path length and asymmetry index) properties of TC neuron from VPL nucleus revealed significant diversity in geometrical and topological properties between dendritic arborization of single TC neurons. These varieties led to different efficacy of BP action potentials and FP EPSPs, which depends primarily on the geometrical and secondly topological properties. In a multicompartment model of reconstructed TC neurons, we demonstrated that low-threshold spikes propagating from the soma to distant dendrites induces a strong shunting effect that prevents distally generated EPSPs from propagating toward the soma. We conclude that due to distinct morphological features, each dendritic tree of a single TC neuron has a specific ability for FP of distal synaptic inputs to reach the soma, which is controlled by back-propagating electrogenic signals.

Funding: Supported by NSERC, CHIR, NIH and FRSQ

Title: Subunit specific regulation of GABAA receptor diffusion

Authors: Kim Gerrow¹, Antoine Triller²

Affiliation: 1- l'Institute de biologie de l'ENS

Abstract: GABAergic phasic (synaptic) and tonic (extrasynaptic) inhibition in the hippocampus are due to distinct GABAA receptor subunits. Here we describe for the first time a mechanism by which this balance of phasic and tonic inhibition can be altered. Using single particle tracking with quantum dots, we found that the surface diffusion of synaptic Alpha-2 containing receptors can be modulated by the activity of metabotropic GABAB receptors in an anti-homeostatic manner. Interestingly, GABAB signalling had no effect on the diffusion of synaptic Alpha-1 receptors, and showed the opposite effect on Alpha-5 receptors. This change in diffusion of Alpha-2 coincides with a change in the amount of Alpha-2 receptors at synapses, but not the amount of the scaffolding protein gephyrin, suggesting a change in the affinity between receptor and scaffold as the underlying mechanism. Interestingly, GABAB signalling had no effect on the diffusion of synaptic Alpha-1 receptors, and showed the opposite effect on Alpha-5 receptors. Using constructs

that contain just the gephyrin binding loop of Alpha-2 we have been able to show a similar change in diffusion in response to GABA_B signalling. This construct offers us an excellent tool in determining the molecular mechanisms behind these changes in diffusion.

Funding: INSERM, CNRS, EMBO

Title: Role of exon 11 in regulator of G-protein signaling 7 (RGS7) regulation of specific Gi/o-coupled pathways.

Authors: Van Lu¹, Henry Puhl², Jianhua Zhang³, William Simonds⁴, Stephen Ikeda⁵

Affiliation: 1,2,5 NIAAA/NIH 3,4 NIDDK/NIH

Abstract: "The regulator of G-protein signaling 7 (RGS7) binds and is co-expressed with the G-protein $\beta 5$ subunit ($G\beta 5$) throughout the nervous system. A mutant mouse, *Rgs7^{tm1Lex}*, has an in-frame deletion mutation of exon 11 and was utilized to study the role of this complex in G-protein signaling. Since the deleted region includes part of the $G\beta 5$ -binding domain, the interaction between these two proteins was studied by measuring the efficiency of Förster resonance energy transfer (E-FRET) between Cerulean-tagged RGS7 constructs (wild-type and a construct with an equivalent deletion mutation, RGS7 Δ) and Venus-tagged $G\beta 5$ in transfected HeLa cells. The RGS7 Δ construct produced an E-FRET value of 0.13 ± 0.006 , comparable to wild-type RGS7, 0.17 ± 0.005 , and well above negative control values. Co-immunoprecipitation studies confirmed E-FRET results. Therefore, association of the RGS7 Δ mutant protein with $G\beta 5$ is maintained. The functional consequences of the deletion mutation were studied by measuring Ca^{2+} channel modulation by G-protein coupled receptor agonists in isolated superior cervical ganglion neurons using whole-cell voltage-clamp. The potency of the muscarinic agonist oxotremorine-M was significantly greater in homozygous *Rgs7^{tm1Lex}* mice compared to wild-type littermates (wild-type $EC_{50} = 103 \pm 18$ nM, *Rgs7^{tm1Lex}* $EC_{50} = 41 \pm 9$ nM; t-test, $P < 0.05$), and was rescued following cDNA injection of RGS7 ($EC_{50} = 119 \pm 2$ nM), but not cDNA for RGS7 Δ or RGS7 N398A, a GTPase-null mutant. The mechanism of altered responses is under further investigation; nevertheless, this *Rgs7^{tm1Lex}* mouse has provided new insights into the role of RGS7 in regulating G-protein signaling. "

Funding: NIH Intramural program (NIAAA).

Title: PKC and calcineurin-mediated regulation of KCC2 and synaptic inhibition

Authors: Jessica C Pressey¹, Trevor Balena², Melanie A Woodin³

Affiliation: 1-3 University of Toronto

Abstract: "Classic synaptic inhibition in the CNS results from GABA binding to postsynaptic GABAARs, which are Cl⁻-permeable ionotropic receptors. Thus, GABAergic transmission is critically dependent on neuronal Cl⁻ regulation. The neuronal Cl⁻ gradient is largely established by the neuron-specific K⁺-Cl⁻ cotransporter KCC2, which maintains a low intracellular concentration of neuronal Cl⁻ ([Cl⁻]_i). Our lab is currently investigating the cellular mechanisms that regulate KCC2-mediated Cl⁻ extrusion. Because KCC2 contains a PKC phosphorylation site in its C-terminus that has been previously implicated in KCC2 regulation, we have focused our attention on the PKC-mediated phosphorylation and calcineurin-mediated dephosphorylation of this membrane protein. First, we inhibited either PKC or calcineurin pharmacologically and then performed electrophysiological experiments on low-density dissociated cultured hippocampal neurons. The strength of synaptic inhibition was determined by the reversal potential for GABA (E_{GABA}); we found that PKC inhibition (with PKC Inhibitor Peptide 19-36 in the intracellular pipette) significantly strengthened inhibition with a hyperpolarization of E_{GABA}, while the extracellular application of the calcineurin-specific inhibitor FK-506 (5 μM) significantly weakened inhibition with a depolarization of E_{GABA}. Next, we performed fluorescence imaging of neuronal Cl⁻ using the genetically encoded Cl⁻ indicator termed Clomeleon, and found that PKC inhibition significantly reduced the concentration of neuronal Cl⁻, in support of our electrophysiological results. Taken together our combined electrophysiological and imaging data suggest that neuronal Cl⁻ regulation is mediated by both PKC and calcineurin. We are currently investigating the role of these posttranslational modifications in the induction of inhibitory synaptic plasticity."

Funding: Funded by an NSERC Discovery Grant and Accelerator Supplement to MAW.

Title: Serotonin Modulates the Slow Oscillation in Prefrontal Cortical Neurons of Anesthetised Mice

Authors: Courtney Pinard¹, Martin Beaulieu², Igor Timofeev³

Affiliation: 1-3 Centre de Recherche Université Laval Robert-Giffard

Abstract: Slow-wave sleep is characterized by spontaneous alterations of activity and silence in corticothalamic networks. In addition, depolarizing and hyperpolarizing fluctuation in the membrane potential is one parameter that is usually seen in intracellular neuronal recordings in the cortex. Neuromodulators (serotonin, dopamine, norepinephrine, acetylcholine) regulate states

of vigilance. Norepinephrine and serotonin neurons discharge during behavioural arousal and EMG activity. The role of serotonin in mediating cortical active and silent states is less clear. Although serotonin neurons are most active during waking state, they also demonstrate slow firing during slow-wave sleep. We used a naturalized model of serotonin deficit in mice with an 80% reduction in serotonin synthesis due to a functional (C1473G) single-nucleotide polymorphism, to examine the role of serotonin in the slow oscillation. In vivo intracellular and local field potential recordings were conducted in the prefrontal cortex of CD1 and tryptophan hydroxylase-2 (tph2) mice anesthetised with ketamine-xylazine anesthesia. Intracellular recordings from control and tph2 mice indicate that prefrontal cortical neurons from both strains of mice demonstrate spontaneous alterations of activity and silence. However, most prefrontal cortical neurons from tph2 mice did not show profound hyperpolarization in the membrane potential during silent state. These results suggest that the action of serotonin on voltage-dependent K⁺ channels may play an important role in the generation of the hyperpolarizing (inactive) state of slow-wave sleep.

Funding: This work was supported by the Savoy Foundation, FRSQ, NSERC, NIH, and CIHR.

Title: Metabotropic glutamate type 5 receptors mediate dopamine D2 antagonist-induced striatal gene expression

Authors: Jerome Maheux¹, Emanuele Tirota², Emiliana Borrelli³, Claude Rouillard⁴, Pierre-Paul Rompré⁵, Daniel Levesque⁶

Affiliation: 1,5,6 Université de Montréal 2,3 University of California Irvine 4- Laval University

Abstract: "Antipsychotic drugs are used to alleviate schizophrenia symptoms. Despite the fact that all antipsychotic drugs interact with dopamine D2 receptors, the exact mechanism that explains their activity still remains elusive. Here we explored the relationship between glutamate and dopamine receptors in the modulation of the transcription factor Nur77 (NGFI-B, NR4A1) in the striatum, a brain structure involved in antipsychotic drug effects. First we performed eticlopride (ETI; D2 antagonist) treatments in post-synaptic dopamine D2L receptor isoform knockout mice, as well as in rats bearing an ibotenic acid-induced cortical lesion. Additionally, groups of mice received an acute injection of vehicle, MPEP (metabotropic glutamate type 5; mGluR5 receptor antagonist), ETI or both compound. Organotypic cultures of striatal slices were also used. ETI was still able to strongly induced Nur77 mRNA levels in the striatum of D2L receptor knockout mice,

while cortical lesions strongly attenuated ETI-induced Nur77 expression in the striatum. On the other hand, blockade of mGluR5 receptors strongly reduced ETI-induced Nur77 mRNA levels in the striatum, whereas MPEP alone remained inactive. Furthermore, blockade of glutamate reuptake in organotypic slices strongly activated Nur77 transcription that can be abolished by a mGluR5 antagonist or a D2 agonist. This suggests that modulation of Nur77 in striatal cells following dopamine D2 antagonists is mediated by pre-synaptic dopamine D2S receptors and depend on the corticostriatal pathway activity. Thus, modulation of striatal cell activity following a D2 antagonist might not involve a direct blockade of post-synaptic D2 receptors, but instead relies on D2S-mediated pre-synaptic modulation of glutamate neurotransmission."

Funding: Work supported by the Stanley Medical Research Institute. JM holds a studentship from the Canadian Institute for Health Research (CIHR).

Title: Nesca, A novel adaptor protein that modulates neurite dynamics and syntaxin-1 vesicle transport in neurons

Authors: Alfonso Dietrich¹, James MacDonald², Todd Hryciw³, Robert Grant⁴, Susan Meakin⁵

Affiliation: 1-5 The University of Western Ontario

Abstract: "Molecular transport and neurite outgrowth are two processes critical to proper neuron function and survival. Nesca is a novel RUN and SH3 domain containing protein that we previously described to play a role in nerve growth factor mediated neurite outgrowth. Here, we demonstrate that Nesca interacts with the microtubule subunits β III and acetylated α -tubulin and that it binds directly to microtubules and F-actin through in vitro reconstitution assays. Furthermore, we find that Nesca co-immunoprecipitates with the molecular motors kinesin-1 (KIF5B) and cytoplasmic dynein as well as with the synaptic membrane precursor protein, syntaxin-1, in primary brain lysates. Through confocal microscopy and analysis of purified post synaptic densities, we also find that Nesca is a component of dendritic spines. Moreover, immunocytochemistry of Nesca expression in E18 hippocampal neurons indicates that it is expressed in a punctate pattern and that it co-localizes with syntaxin-1 positive vesicles suggesting that it is an adaptor involved in the transport of t-SNARE subunits, synaptic vesicle membrane fusion and exocytosis. It is hypothesized that Nesca modulates neurite outgrowth and is an adaptor protein in syntaxin-1 mediated molecular transport in neurons. To address structure: function relationships between Nesca and its role(s) in neuronal differentiation and association with intracellular vesicles, deletion

mutants were assayed in the human SN56 cell line (septal neurons fused with neuroblastomas) following differentiation. Preliminary studies indicate that carboxy terminal deletions alter soma size, neurite outgrowth and vesicle formation. Collectively, these data suggest that Nesca maintains multiple roles in both molecular transport and neurite outgrowth. "

Funding: Ontario Graduate Scholarship and the Neuromuscular Research Partnership between the CIHR, Muscular Dystrophy Canada and the ALS Society of Canada.

Title: Assembly and Gating of Heteromeric Kainate Receptors

Authors: Patricia Brown¹, Mark Aurousseau², Hugo McGuire³, Rikard Blunk⁴, Derek Bowie⁵

Affiliation: 1- Pharmacology & Therapeutics and Integrated Program in Neurosciences, McGill University 2,5 Pharmacology & Therapeutics, McGill University 3,4 Department of Physics and GEPRM, Universite de Montreal

Abstract: Evidence suggests that kainate-type ionotropic glutamate receptors (KARs) exist primarily as heteromeric complexes in native systems where the KAR responses exhibit slow and variable deactivation kinetics. This contrasts sharply to the fast kinetics typically obtained from recombinant homomeric GluK2 receptors. The finding that recombinantly-expressed heteromeric KARs also display slow deactivation kinetics has served as an attractive starting point to explaining the behaviour of native KARs. Here, we investigate the biophysical, structural and stoichiometric properties of recombinant heteromeric GluK2/GluK5 KARs using a combination of patch-clamp electrophysiology and fluorescent subunit counting. Ultra-fast agonist application onto outside-out patches containing GluK2/GluK5 KARs reveals a correlation between the slow deactivation kinetics and the degree of receptor heteromerization, the interpretation of which relies upon knowledge of receptor stoichiometry. We therefore used a fluorescent subunit counting technique to resolve the stoichiometry of heteromeric KARs. Additionally, upon probing for external ion sensitivity, a trait unique to KARs, we find that GluK2/GluK5 KARs are ion-insensitive. Since external ions are known to act at the ligand-binding domain's dimer interface, these data suggest that heteromeric KARs assemble as heterodimers at this level. Taken together, our data provide evidence for the structural arrangement of heteromeric KARs as well as insight into their gating mechanisms.

Funding: FRSQ, CIHR

Title: Translational profiles and identification of a novel role for 14-3-3 proteins in learning and memory

Authors: Rachel Jeffrey¹, Michael Honsberger², Christos Gkogkas³, Christopher Kent⁴, James Wohlschlegel⁵, Alyson Fournier⁶, Karim Nader⁷, Nahum Sonenberg⁸

Affiliation: 1-4, 6-8 McGill University 5- University of California, Los Angeles

Abstract: Formation of long term memory (LTM) requires new protein translation. Synaptic plasticity, the capacity of neurons to change the strength of their connections with experience, provides a cellular mechanism for LTM and is driven by changes in translation after LTM. While select protein products have been identified for their role in LTM, many remain undiscovered. Additionally, identification of proteins specifically involved in consolidation or reconsolidation is of interest for studies in memory and psychiatric disorders. In order to elucidate new and/or unique proteins translated after LTM consolidation and reconsolidation, we performed associative fear conditioning in rats in concert with a proteomics screen. Using proteins precipitated from amygdala at time points after LTM consolidation and reconsolidation we ran samples in MudPIT mass spectrometry to compare proteomic profiles. One target that we have validated by Western blot analysis is the 14-3-3 family of signaling proteins which are central to a wide number of cellular processes. The 14-3-3 proteins regulate translation in the brain as well as numerous kinase pathways including direct interaction with PKA and PKC. Interaction with PKA in neurons is essential for axon guidance in the growth cone during development. We find that 14-3-3 proteins are upregulated 90 minutes after consolidation of LTM. Further, blocking 14-3-3 proteins using HSV-delivered inhibitory peptide prevents memory formation in rats. This provides evidence for the first time showing a role for 14-3-3 in mammalian memory formation. Future experiments will test through which pathway of 14-3-3 interactions this regulation of LTM formation is occurring.

Funding: NSERC, CIHR, HHMI International

Title: Inactivity- and TNF- α -Induced Modulation of GABA(A)R Trafficking

Authors: Horia Pribiag¹, David Stellwagen²

Affiliation: 1-2 McGill University

Abstract: Tumor necrosis factor- α (TNF- α), a pro-inflammatory cytokine, has been shown to upregulate surface expression of AMPA receptors while down-regulating surface expression of

GABA(A) receptors. Furthermore, TNF-alpha is required for one form of homeostatic synaptic plasticity - a compensatory response to chronic action potential blockade which increases excitatory synaptic efficacy while decreasing efficacy at the GABAergic synapse. We are exploring the specificity of acute TNF-alpha-induced and chronic inactivity-induced modulation of GABA(A)R trafficking in mature hippocampal neuron cultures. For several GABA(A)R subunits we find that acute application of TNF-alpha causes a rapid and persistent reduction of clustered surface receptors. Chronic inactivity achieved by incubation with tetrodotoxin achieves a similar down-regulation of surface clustered GABA(A)R subunits, associated with a reduction in the amplitude of miniature inhibitory post-synaptic currents and a reduction in clustered gephyrin immunoreactivity. These findings have implications for the regulation of mature GABAergic synapse function during inflammation and network inactivity.

Funding: "CIHR and NSERC grants to D.S.; NSERC doctoral scholarship to H.P."

Title: The direct interaction of TRPM2 with tubulin and TRPM2's role and downstream effects in macrophage and microglia cells after activation via LPS and H₂O₂.

Authors: Colin Seepersad¹, Michelle Aarts²

Affiliation: 1-2 University of Toronto

Abstract: Transient Receptor Potential Melastatin 2 (TRPM2) is a non-selective, cationic membrane channel permeable primarily to Ca²⁺. The short isoform (S-TRPM2), which contains only the N-terminus and two of six transmembrane domains, directly inhibits the activity of the long isoform (L-TRPM2). L-TRPM2 has two cytoplasmic tails that possess putative protein interaction domains. Gated by intracellular calcium and oxidative stress via an ADP ribosylase, TRPM2 has suggested roles in ischemic cell death, insulin secretion, neutrophil activation and chemotaxis. The current study aims to characterize the molecular interactions of TRPM2 and determine the role of TRPM2 in immune cell activation, namely in macrophage (RAW) and microglia (BV-2) cell lines. Using mass spectrometry and recombinant protein-binding assays we have demonstrated that the C-terminus of TRPM2 binds directly to tubulin. Stabilization of microtubules increases the surface expression of TRPM2 while depolymerization results in TRPM2 internalization. Activation of macrophage and microglia using K12 E. coli LPS causes a parallel decrease in S-TRPM2 and increase in the active L-TRPM2 isoform. A critical regulator of both microtubule dynamics and channel surface expression is PKC. Accordingly the extracellular

TRPM2 activator H₂O₂ increases calcium-dependent PKC activation in macrophages. Our preliminary evidence suggests that tubulin is a key regulator of TRPM2 localization and activity in macrophage and microglia, which may involve the downstream activation of PKC.

Funding: Work supported by: NSERC, Canada Stroke Network, CRC

Title: Optogenetic study of the role of parvalbumin and somatostatin interneurons in theta oscillations in the hippocampus

Authors: Bénédicte Amilhon¹, Carey Huh², Frédéric Manseau³, Romain Goutagny⁴, Antoine Adamantidis⁵, Sylvain Williams⁶

Affiliation: 1-6 Douglas Mental Health University Institute

Abstract: Neuronal network oscillations are a crucial component of hippocampal functions, providing a temporal metric to individual neuronal activity, and allowing the binding of cell assemblies across structures. Among the variety of rhythms that can be recorded in the hippocampus, oscillations in the theta (3-12 Hz) range are known to be essential for learning and memory. The delicate balance between inhibition, provided by GABAergic interneurons, and excitation, originating from principal cells, is a key feature of hippocampal oscillations. Among the wide variety of hippocampal interneurons, those expressing the neurochemical markers parvalbumin (Pvlb) and somatostatin (Som) are suspected to be essential in theta oscillations. Som and Pvlb interneurons indeed exhibit strikingly complementary innervation patterns and putative functions. To elucidate the functions of each of these interneuron subtypes in theta oscillations in the hippocampus, we have combined the use of an intact in vitro hippocampus preparation, spontaneously oscillating at theta frequencies, and optogenetics, that allow light-driven control of either interneurons sub-population activity. By using Pvlb-Cre and Som-Cre mice strains, and Cre-dependant viral vectors, we were able to express the yellow light-driven H⁺ pump ArchT or the blue light-driven Na⁺ channel ChETA specifically in Pvlb or Som interneurons. ChETA stimulation of Pvlb or Som interneurons allowed an assessment of their respective properties and output onto pyramidal cells. Besides, ArchT stimulation allowed silencing of Pvlb or Som interneuron populations in the oscillating hippocampus preparation. Preliminary results show that Pvlb network is essential to theta oscillations since silencing them disrupted theta rhythm in the hippocampus preparation.

Funding: CIHR, NSERC

Title: The CA3 area contains distinct slow-theta oscillators at the dorsal and ventral sections in the complete hippocampus in vitro

Authors: Ning Gu¹, Jesse Jackson², Romain Goutagny³, Germain Lowe⁴, Sylvain Williams⁵

Affiliation: 1-5 Douglas mental health university institute, McGill University

Abstract: "The hippocampus is functionally heterogeneous along the longitudinal axis; memory function occurs predominantly dorsally whereas emotional-related processing takes place ventrally. Although, significant differences in connectivity and molecular heterogeneity are present along this axis, it remains unknown if two separate networks are present dorso-ventrally. We demonstrate that two independent slow theta oscillators can be recorded simultaneously at dorsal and ventral ends of the hippocampus since these areas displayed low coherence (0.39 ± 0.03 , $n=78$) and comparable power and frequency (dorsal 2.13 ± 0.07 Hz vs. ventral 2.34 ± 0.08 Hz; $n=78$, $p < 0.05$). Both oscillators were generated through the activation of AMPA and GABA_A receptors, but NMDA receptors played a more prominent role ventrally than dorsally as assessed by its greater sensitivity to the NMDA receptor antagonist AP-5 ($50 \mu\text{M}$) or the NR2B subunit antagonist RO 25-6981 ($5 \mu\text{M}$). Interestingly, it was found that rapidly increasing $[\text{Ca}^{2+}]_o$ from 2 to 4mM for 3 minutes, a classic protocol to induce LTP in slices, induced a dramatic NMDA receptor-dependent increase in dorso-ventral coherence suggesting that coherence can be modulated as a function of plasticity. Finally, we investigated if one oscillator was leading/driving the other. In control conditions, the dorsal oscillator usually led the ventral section. However, after NMDA-dependent increase in coherence, the ventral section usually dominated the dorsal oscillator. Taken together, these data provide for the first time, in vitro physiological support for the existence of the sub-regional network heterogeneity along the dorsal-ventral CA3 axis and provide evidence of the dynamics in the interaction. "

Funding: "CHIR;FRSQ;NSERC"

Title: TRPM2 and TRPM7: Expression Patterns in the CNS

Authors: Melanie Ratnam¹, Jonathan Chan², Naghmeh Lesani³, James Eubanks⁴, Michelle Aarts⁵

Affiliation: 1-3,5 University of Toronto 4- Toronto Western Hospital

Abstract: TRPM7 and TRPM2 have previously been implicated as regulators of stroke-induced neuronal death. Other cation channels implicated in regulating stroke-induced cell death are highly enriched in cortical neurons. Thus, we determined quantitatively the expression of TRPM2

and TRPM7 in various CNS regions over perinatal development and in specific cell types. Expression patterns were examined in age-specific rat brain tissues, primary cultures and cell lines. TaqMan real-time PCR was used to measure dynamic changes in mRNA levels and western blot analyses was used to quantify protein levels. Our results indicated that TRPM7 and TRPM2 expression decreased between 1- and 3-week developmental time-points. We also found that TRPM2 was differentially expressed between cell types, present only in microglia but not cortical neurons or astrocytes. In contrast, TRPM7 was expressed at similar levels in all cell types. The decreased expression of TRPM channels between 1 and 3 weeks suggest a potential role during synaptogenesis. Our study also indicates an important role for glial TRPM2, which will inform the future development of cell-specific pharmacological interventions of TRPM channels.

Funding: CRC CSN NSERC

Title: Mechanisms underlying spreading depression in insect model systems.

Authors: Kristin Spong¹, Nicholas Hou², Esteban Rodriguez³, Meldrum Robertson⁴

Affiliation: 1-4 Queen's University

Abstract: "Spreading depression (SD) can be defined as a rapid and nearly complete depolarization of neurons caused by a massive redistribution of ions that propagates through neural tissue. It is important to understand the cellular mechanisms underlying SD due to its association with human pathologies such as stroke, migraine, and seizure. Our lab investigates such mechanisms in two insect model systems: locust metathoracic ganglion (*Locusta migratoria*) and fly brain (*Drosophila melanogaster*). Insects enter a reversible coma (SD event) in response to stress that is associated with abrupt surges in extracellular potassium concentration ($[K^+]_o$). Using K^+ sensitive microelectrodes we can monitor these SD events in both the locust and fly allowing for detailed investigations of the cellular mechanisms involved. Our insect models of SD can be modulated pharmacologically and by environmental preconditioning (heat shock). Here we show that pharmacological blockade of gap junctions with either carbenoxolone or 18β -glycyrrhetic acid reliably induces repetitive SD-like events in the locust's CNS. Furthermore, we are using fluorescence immunocytochemistry to examine subcellular localization of Na^+/K^+ -ATPase protein in tissue taken from control and heat shock preparations following coma. Results suggest that heat shock pre-treatment increases neural recovery from coma by trafficking more Na^+/K^+ -ATPase into neuronal membranes. Lastly, we investigated the role of temperature during

repetitive anoxic comas in *Drosophila melanogaster* and it appears that hypothermia has a protective effect against K⁺ homeostasis disturbance. Using pharmacological approaches in the locust and molecular genetic approaches in the fly we hope to better understand control mechanisms involved in SD."

Funding: Funded by NSERC

Title: Stress hormone corticosterone suppresses long term depression of synaptic N-methyl-d-aspartate receptor

Authors: Yiu Chung Tse¹, RC Bagot², Tak Pan Wong³

Affiliation: 1,2 Douglas Mental Health University Institute 3- Department of Psychiatry, McGill University and Douglas Mental Health University Institute

Abstract: Learning and memory can be affected by stress. Stress could affect memory by modulating hippocampal synaptic plasticity, a cellular model for learning and memory via steroidal hormone corticosterone (CORT). Most forms of hippocampal synaptic plasticity are induced by activation of N-methyl-d-aspartate receptor (NMDAR), which causes long-term changes of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPA) mediated synaptic activity. Similar to synaptic AMPAR, NMDAR can undergo activity-dependent synaptic plasticity, such as long-term potentiation (LTP-NMDAR) and depression (LTD-NMDAR). We hypothesized that CORT could modulate hippocampal function by affecting activity-dependent plasticity of synaptic NMDAR. Using a rat brain slice model (3-month old), we found that stress level CORT (100 nM for 30 min) triggered a long-lasting (up to 2 hours) increase in NMDAR activity. In addition, this increase in NMDAR function accompanied by a delay-onset (>1 hour after the end of CORT) increase in NR2A/NR2B ratio. We next investigated if LTP-NMDAR and LTD-NMDAR are altered by CORT and found that while LTP-NMDAR was largely intact after CORT treatment, LTD-NMDAR was suppressed at the time window when NR2 subunit composition had been altered. Our findings shown that a brief CORT treatment, which imitates acute stress, alters activity-dependent plasticity of synaptic NMDAR via changing its subunit composition.

Funding: CIHR, NARSAD, NSERC

Title: " Synaptically evoked Ca²⁺ release from intracellular stores is not influenced by vesicular zinc in CA3 hippocampal pyramidal neurons"

Authors: Alesya Evstratova¹, Katalin Toth²

Affiliation: 1-2 Centre de recherche Université Laval Robert-Giffard

Abstract: "Activation of hippocampal mossy fibers is known to evoke calcium release from internal stores of CA3 pyramidal cells. Recently it has been suggested that this process is regulated by vesicular zinc released from presynaptic terminals (Besser et al. J Neurosci. 2009). We used ratiometric calcium imaging in combination with patch-clamp recordings to investigate this possibility. In acute hippocampal slices repetitive mossy fiber stimulation (10/20 pulses at 50/100 Hz) produced a large, delayed postsynaptic Ca²⁺ wave that was spatially restricted to the proximal apical dendrites of CA3 pyramidal cells and was clearly distinguishable from Ca²⁺ signals associated with synaptically evoked backpropagating action potentials. This delayed calcium increase was sensitive to intracellularly applied heparin indicating reliance upon release from internal stores and was triggered by activation of both group I metabotropic glutamate and NMDA receptors. We used the membrane-impermeable zinc chelator CaEDTA to determine if synaptically released zinc has any influence on Ca²⁺ waves. In control ACSF, calcium release from intracellular stores lead to 32 ± 6.4 % $\Delta F/F$ increase, this value was not statistically different in the presence of 2.5 mM CaEDTA (35.7 ± 6 % $\Delta F/F$). Moreover, there were no statistically significant differences in calcium waves recorded in the wild type and ZnT3 knockout mice (56 ± 4.3 and 60.3 ± 3 % $\Delta F/F$, respectively). In addition, Ca²⁺ wave threshold was independent of vesicular zinc. Overall, our data do not support a role for vesicular zinc in the regulation of mossy fiber evoked Ca²⁺ release from CA3 pyramidal cell internal stores."

Funding: CIHR

Title: MyD88-adaptor protein in monocytoïd cells acts as a preventive mechanism for memory deficits in a mouse model of Alzheimer's disease

Authors: Jean-Philippe Michaud¹, Karine L. Richard², Serge Rivest³

Affiliation: 1-3 Laboratory of Endocrinology and Genomics, CHUL Research Center and Department of Molecular Medicine, Faculty of Medicine, Laval University

Abstract: "Alzheimer's disease (AD) is an age-related neurodegenerative disorder associated with brain innate immune activation mainly mediated by microglia. These cells are known to be activated in the brain of AD patients in response to Amyloid beta (A β). Accumulating evidence supports a critical role of Toll-like receptors in the clearance of A β by microglial cells. Myeloid

differentiation factor 88 (MyD88) is the adaptor molecule for most of these innate immune receptors, transducing the intracellular signal from TLRs to nucleus. Here, we report that more than 50% reduction in MyD88 expression in a mouse model of AD accelerated spatial learning deficits. Brains of APP^{swe}/PS1-MyD88^{+/-} mice were characterized by a delay in accumulation of A β plaques and increased soluble levels of A β oligomers. Furthermore, inflammatory monocyte subset and brain IL-1 β gene expression were significantly reduced in APP^{swe}/PS1 mice with impaired MyD88 signaling. In adulthood, microglia can be renewed by bone marrow-derived cells. Therefore, we investigated the role of competent MyD88 hematopoietic stem cells on the cognitive decline of APP^{swe}/PS1 mice. We used classical chimeric mouse models using irradiation and transplantation of wild type GFP cells, MyD88-deficient cells and all the control groups. Transplantation of GFP cells essentially rescued the cognitive impairment, whereas MyD88-deficient cells significantly accelerated spatial learning deficits of APP^{swe}/PS1 mice. These data indicate that MyD88 intracellular signaling pathway in myeloid cells acts as a natural innate immune mechanism to restrict disease progression of APP^{swe}/PS1 mice."

Funding: The Canadian Institutes in Health Research (CIHR) and Neuroscience Canada (Brain Repair Program) supported this research.

Title: Astrocyte-Derived SPARC Regulates the Function and Plasticity of Excitatory Synapses in the Hippocampus

Authors: Emma V. Jones¹, Yann Bernardinelli², Yiu Chung Tse³, Sabrina Chierzi⁴, Tak Pan Wong⁵, Keith K. Murai⁶

Affiliation: 1-6 McGill University

Abstract: "Astrocytes are required for the formation and maintenance of functional synapses in the CNS. Previous studies have shown that soluble factors secreted by astrocytes, such as thrombospondins, are important for synapse formation. However, when compared to astrocyte-conditioned media, synapses induced by thrombospondins alone remain postsynaptically silent. This indicates that additional astrocyte-derived factors are required to regulate glutamate receptor insertion and the formation of functional synapses. We have identified the matricellular protein Secreted Protein, Acidic and Rich in Cysteine (SPARC) as a novel glial-derived synapse modulatory factor and present data showing how astrocytes utilize SPARC to control the processes of excitatory synapse maturation. In the CNS, SPARC is expressed by astroglia and is

enriched in the mouse hippocampus during the first three postnatal weeks, a period of intense synapse formation. We also found that astrocytes regulate SPARC expression in response to changes in synaptic activity. Interestingly, synapses of neurons grown with astrocytes derived from Sparc-null mice have altered glutamate receptor levels. Furthermore, we find that loss of SPARC leads to significant alterations in miniature EPSCs and deficiencies in forms of long-term plasticity. In addition, we show that SPARC regulates the stability of AMPA receptors by acting on neuronal β 3-integrin complexes. These data indicate that SPARC can regulate synaptic function and represents a novel pathway for astrocyte-neuron communication during synapse formation and possibly following nervous system injury or disease. "

Funding: EJLB (K.K.M.) CIHR, CRC, CFI (K.K.M) MUHC (E.V.J.) Swiss National Science Foundation (Y.B.) NSERC (T.P.W)

Title: Vesicular zinc influences the fidelity of temporal coding at the hippocampal mossy fiber terminals

Authors: Nathalie Lavoie¹, Katalin Toth²

Affiliation: 1,2 Université Laval - Centre de Recherche Université Laval Robert-Giffard

Abstract: Hippocampal mossy fiber axons (MF) arise from the granule cells of the dentate gyrus and provide synaptic input to neurons in the hilus and the CA3 area of the hippocampus. MF terminals contain unusually high level of chelatable zinc in the synaptic vesicles, which is carried into the vesicles via a specific transporter (ZnT3). In this study, we investigated the physiological role of zinc in MF plastic properties. Patch-clamp recordings of CA3 pyramidal cells were performed on WT (ZnT3 +/+) and KO mice (ZnT3 -/-). The amplitude of evoked EPSCs was similar in both genotypes at various stimulus frequencies. However, decay kinetics of EPSCs was significantly different between these groups. Evoked events in WT animals could be fitted with a single exponential. In contrast, increased stimulation frequency revealed a slower component in KO animals and these events were better fitted with two exponentials. Next, we aimed to determine the physiological consequences of altered decay kinetics observed in KO animals. We used a presynaptic stimuli pattern mimicking realistic granule cell activity to investigate synaptic integration in WT and KO animals. The combination of slow and high frequency stimuli evoked similar postsynaptic responses in all cells investigated in WT animals. In contrast, postsynaptic responses from KO animals varied widely among individual cells. Our experiments show that

slight change in EPSC decay kinetics could compromise the fidelity of synaptic coding. These data suggest that fast and synchronized release of glutamate from dentate granule cells is essential for proper temporal coding at MF terminals.

Funding: CIHR

Title: NMDA receptor anchoring to PSD-95 is transiently disrupted in stimulated spines, a mechanism studied by FRET/FLIM

Authors: Kim Doré¹, Simon Labrecque², Paul De Koninck³

Affiliation: 1-3 Centre de Recherche Université Laval Robert-Giffard

Abstract: "Each neuron in the brain forms thousands of excitatory synapses onto tiny, micron-sized spines that contain an extremely high density of signaling proteins, just apposed to the pre-synaptic neurotransmitter release site. The NMDA receptor is the predominant molecular device for controlling synaptic plasticity and memory function. Mounting evidence suggests that the interaction between the NMDA receptor and PSD-95 is modified during synaptic plasticity. In fact; it has been shown that the transient leaving of PSD-95 from the spine after LTP stimuli is of crucial importance for the occurrence of synaptic plasticity (Steiner P. et al, Neuron, 2008). In this study we used GFP-tagged NMDA receptors and mCherry-tagged PSD-95 to measure directly the interaction between these two important proteins with FRET-FLIM. Dissociated cultures of rat hippocampal neurons and HEK293 cells were used to study how this interaction is regulated. Among the regulating factors, we examined the role of NMDA receptor stimulation, inhibition of the protease calpain or the kinases CaMKII and Src, and different NR2 subunit composition of the NMDARs. Our FRET-FLIM experiments show that the PSD-95/NMDAR interaction is disrupted upon NMDAR stimulation and that the inhibition of CaMKII and calpain makes the interaction more stable. Also, Src and CaMKII produce different effects on young and mature neurons. Our goal is to understand the intricate mechanism of this interaction involving numerous important players in synaptic plasticity."

Funding: NSERC, CIHR, Human Frontier Science program (HFSP)

Title: Changes in excitability of cortical pyramidal neurons following disruption of development of PV-expressing cortical interneurons in the cyclin D2 null mouse

Authors: Ahmed Gilani¹, Holly Moore²

Affiliation: 1-2 Columbia University

Abstract: "Parvalbumin-expressing (PV+) GABAergic interneurons control firing characteristics of pyramidal cells in the cerebral cortex. Altered function of these neurons has been implicated in diseases such as epilepsy and schizophrenia. Previously, Glickstein et al (2007) showed that cyclinD2 (cD2) null (-/-) mice exhibit selective and partial reduction in the number of PV+ cells in cortex including the hippocampus. The decrease in PV+ interneuron density in cD2-/- is accompanied by a decrease in GABA-A receptor-mediated inhibition and EEG abnormalities consistent with cortical disinhibition. cD2-/- mice are more sensitive to convulsant effects of a benzodiazepine receptor inverse agonist, in the absence of spontaneous seizures; and show disruption of hippocampal-dependent learning. The present study determined if the decrease in GABAergic synaptic input to hippocampal pyramidal cells is associated with changes in membrane properties and/or excitability. Whole cell recordings of CA1 pyramidal cells were conducted to measure GABAergic inhibition, resting membrane properties and action potential discharge properties. Consistent with a loss of PV+ interneurons, cD2-/- showed a reduction in the frequency of GABAergic miniature inhibitory postsynaptic currents (mIPSC) onto CA1 pyramidal cells. Current clamp recordings showed that although there was a trend towards a more depolarized resting membrane potential in CA1 neurons of cD2 nulls, these neurons paradoxically showed a significantly increased current threshold (rheobase) and reduced current evoked spike frequency. Together, these data are consistent with the hypothesis that a compensatory reduction in the excitability of CA1 pyramidal neurons occurs in cD2-/- model of selective and partial developmental deficit in PV+ interneurons. "

Funding: P01 NS048120-04 (H.M.), Fulbright Foundation (A.G.)

Title: CREB-dependent transcriptional control and quantal changes in persistent long-term potentiation in hippocampal inhibitory interneurons

Authors: Israeli Ran¹, Isabel Laplante², Jean-Claude Lacaille³

Affiliation: 1-3 Groupe de Recherche sur le Système Nerveux Central and Département de Physiologie, Université de Montréal

Abstract: A persistent form of long-term potentiation (termed cL-LTPmGluR1) is found at excitatory synapses onto hippocampal inhibitory interneurons in stratum oriens-alveus (OA-INs). cL-LTPmGluR1 is induced in slice cultures by repeated stimulation of type-1 metabotropic

glutamate receptors (mGluR1), persists for at least 24h, and is dependent on transcription and translation. However, the transcriptional control mechanisms involved in cL-LTPmGluR1, as well as the pre- and postsynaptic mechanisms underlying cL-LTPmGluR1 maintenance, remain undetermined. Thus, we examined first the role of cyclic adenosine monophosphate response element-binding protein (CREB)-dependent transcriptional control in cL-LTPmGluR1. The cL-LTPmGluR1 induction protocol stimulated CREB phosphorylation in OA-INs and this was prevented by an inhibitor of extracellular signal-regulated protein kinase (ERK) signaling. OA-IN transfection with CREB siRNA reduced CREB levels and prevented cL-LTPmGluR1. CREB overexpression in OA-INs facilitated cL-LTPmGluR1 induction. Thus, cL-LTPmGluR1 requires activation of CREB-dependent transcription via ERK. Next, we investigated cL-LTPmGluR1 expression mechanisms using quantal analysis and non-stationary fluctuation analysis (NSFA). Multi-Gaussian histogram fit of EPSCs evoked by minimal stimulation revealed an increased number of quanta released, suggesting enhancement of transmitter release, and a larger amplitude of the 1st Gaussian component, indicating increase in quantal size, during cL-LTPmGluR1. NSFA using variance-mean plots of miniature EPSCs revealed increases in single-channel conductance and receptor number, consistent with the quantal analysis results. Thus, cL-LTPmGluR1 expression involves a presynaptic increase in quanta released, as well as postsynaptic insertion of receptors and modulation of channel properties. Our findings uncover CREB-dependent transcriptional control of pre- and postsynaptic changes during persistent long-term potentiation at hippocampal interneuron synapses.

Funding: CIHR, CRC, FRSQ, and Savoy Foundation.

Title: Subcellular distribution of VAMP4 in hippocampal neurons

Authors: Philippe Lemieux¹, Modesto Peralta III², Katalin Toth³

Affiliation: 1-3 Université Laval - Centre de Recherche Université Laval Robert-Giffard

Abstract: Intracellular vesicle fusion is mediated by the interactions of Soluble N-Ethylmaleimide-sensitive factor Attachment protein Receptor (SNARE) proteins on vesicles (v-SNAREs) and on target membranes (t-SNAREs). Vesicle associated membrane protein 4 (VAMP4) belongs to the synaptobrevin family. VAMP4 is primarily present in the trans-Golgi network (TGN) and participates in transport between the TGN and endosomes. VAMP4 is also involved in the regulated exocytosis of endosomes. We have shown that endocytotic recycling plays an important

role in synaptic transmission during intense synaptic activity. Neurotransmitter release from different subsets of hippocampal interneurons has distinct temporal profile during increased activity. In this study, we aimed to determine whether VAMP4 shows cell-type specific distribution that could explain this difference. VAMP4 colocalization with parvalbumin (PV) or cholecystokinin (CCK) was investigated with immunocytochemistry and fluorescent confocal microscopy. VAMP4 staining was present at the cell bodies of all cell types in the hippocampus. In addition, VAMP4-positive synaptic terminals were observed in the strata oriens and radiatum. While VAMP4 never colocalized with PV, ~20% of CCK positive terminals were VAMP4-positive. We confirmed the subcellular distribution of VAMP4 using a transmission electron microscope (TEM). VAMP4 is strongly expressed in the Golgi apparatus of hippocampal neurons and is present in presynaptic terminals in the stratum radiatum of the CA1. Our data show that VAMP4 is present in the terminals of a subset of hippocampal interneurons. Cell-type specific expression of VAMP4 could contribute to the distinct temporal profile of the neurotransmitter release observed in different types of inhibitory cells in the hippocampus.

Funding: CIHR

Title: Serotonin 5HT1 Receptor-mediated Inhibition of Calcium Signaling in Peripheral CGRP Nociceptive Terminal Fibres

Authors: Landon Baillie¹, Sean Mulligan²

Affiliation: 1-2 University of Saskatchewan

Abstract: The neuropeptide calcitonin gene-related peptide (CGRP) has been shown to play a central role in migraine pathology, involved in both initiating and sustaining migraine attacks. The neurotransmitter serotonin (5HT) as well, has long been implicated in playing an essential role in migraine pathophysiology based largely on the success of the triptan class of therapeutics that are active at the 5HT1 class of serotonin receptors. Successful treatment of migraine headache pain with sumatriptan has been shown to correlate with normalization of CGRP levels -an effect that paralleled migraine resolution. 5HT1 receptors in the dura are restricted to neuronal fibres where they colocalize with CGRP immunoreactive fibres in high density thus providing a possible site for inhibition of the CGRP release. Intracellular calcium (Ca²⁺) plays an essential role in mediating the release of neurotransmitters from nerve terminals. We are studying Ca²⁺ signaling selectively in identified CGRP afferent fibre terminations in our recently developed en bloc dural-skull

preparation for optical microfluorometric imaging. We find that sumatriptan activation of the 5HT1 receptor causes a dose-dependent block of single action potential mediated Ca²⁺ influx in CGRP terminal fibres. This inhibition of terminal Ca²⁺ signaling appears to be mediated by the N-type Ca²⁺ channel subtype, which has been shown to play a critical role in neurotransmitter release from nociceptive fibres. High-resolution functional imaging of Ca²⁺ signaling selectively within individual CGRP fibre terminations may have important implications for understanding the pathogenesis of migraine and for developing peripherally targeted therapeutics for mitigating migraine pain.

Funding: Canadian Institutes of Health Research

Title: Cholesterol activated Ca²⁺ influx contributes to cholesterol dependent increases in synaptic strength

Authors: Alex Smith¹, Milton Charlton²

Affiliation: 1-2 University of Toronto

Abstract: Cholesterol delivery is necessary for synaptic maturation in cultured neurons and cholesterol synthesis may be required for some forms of synaptic plasticity. We have investigated the effects of acutely increasing membrane cholesterol content on synaptic transmission between granule and Purkinje neurons in dissociated culture. Treatment with saturated methyl- β -cyclodextrin:cholesterol complexes increased membrane cholesterol content whereas treatment with complexes at lower ratios of cholesterol to cyclodextrin did not significantly alter cholesterol levels. Cholesterol enrichment by this method increased the amplitude of evoked EPSCs and increased the frequency and amplitude of mEPSCs. Synaptic currents were not altered by cholesterol:cyclodextrin complexes that did not change the overall membrane cholesterol content. Cholesterol enrichment caused a sustained increase in dendritic [Ca²⁺] and removal of extracellular Ca²⁺ prevented the cholesterol induced increase in mEPSC amplitude and the increase in mEPSC frequency. Postsynaptic BAPTA dialysis or treatment with SKF96365 also blocked the increase in mEPSC amplitude. These results demonstrate that cholesterol dependent synaptic strengthening is mediated in part by activation of a cholesterol sensitive Ca²⁺ influx pathway.

Funding: Supported by CIHR operating grant MOP-82827 to M.P.C.

Title: The abused psychoactive inhalant toluene modulates synaptic transmission but not place cell activation in the rat hippocampus

Authors: Ali Gheidi¹, Jimmie Gmaz², Caleb Browne³, Brittany Matthews⁴, Mary Beth Dunn⁵, Diano Marrone⁶, Bruce McKay⁷

Affiliation: 1-3,5-7 Wilfrid Laurier University 4- University of Toronto

Abstract: Toluene, a psychoactive volatile solvent commonly found in adhesives, paint products and gasoline, is inhaled for its euphoric and intoxicating effects. Toluene inhalation additionally results in cognitive disturbances including impairments in learning and memory, suggesting that toluene may change the physiology of neurons in the hippocampus. In the present experiments we examined the effects of toluene on synaptic transmission at perforant path (PP) - dentate gyrus (DG) synapses, and on place cell activation in the hippocampus. **Synaptic Transmission:** Field potential recordings were obtained from the granule cell layer of the DG (stimulation of the ipsilateral PP) in anesthetized Long Evans rats. Exposure to toluene vapour reversibly potentiated population spike amplitude while decreasing its latency to peak, prolonged the time to recover population spike amplitudes during paired-pulse stimulation, and decreased theta rhythm power. **Place Cell Activation:** Naïve rats were exposed either to toluene vapour or control conditions for a period of 25 min, which was immediately followed by an assisted behavioural exploration procedure to activate place cells. The number of cells transcribing the immediate early gene Arc, which is coupled to the activation of place cells by behavioural exploration, was measured via fluorescence in situ hybridization and confocal microscopy. Toluene vapour did not affect the number of CA1, CA3 or DG neurons transcribing Arc. Our results demonstrate that toluene disrupts synaptic transmission at PP-DG synapses, but not place cell activation, and provides one potential explanation for the behavioural deficits noted following exposure to toluene vapours. **Funding:** Natural Sciences and Engineering Research Council of Canada (BEM, DFM) and Wilfrid Laurier University

Title: The role of different Munc18 isoforms in release of neurotransmitters

Authors: Na-Ryum Bin¹, Liping Han², Shuzo Sugita³

Affiliation: 1-3 University of Toronto

Abstract: "Neuronal exocytosis is one of the most fundamental processes in neurobiology yet

complicated such that requires orchestrations of various molecular machineries. Munc18 is regarded as one of the regulators of exocytosis. It has been known that Munc18 has several isoforms that share similarities in structures but different major isoforms in distinct tissues; Munc18-1 in nervous system, Munc18-2 in general except brain. Here, I present role of Munc18-1 and perhaps surprising, Munc18-2 in release of neurotransmitters in a neuroendocrine model cell-line called pheochromocytoma 12 (PC12). ShRNA mediated knockdown of Munc18-1 have resulted ~65% reductions of norepinephrine releases whereas Munc18-2 knockdown yielded ~20% reductions confirming Munc18-1 as a major functional isoform in nervous system. On the other hand, knockdown of Munc18-1 in Rat Basophilic Leukemia-2H3 cell (an immune cell-line that also undergoes similar SNARE mediated lysosomal fusion) has resulted almost no changes in lysosomal secretions compared to control, whereas Munc18-2 knockdown resulted ~80% reductions thus illustrating major role of Munc18-2 in general tissues. In attempt to dissect functions of Munc18 isoforms in neuronal exocytosis, stable Munc18-1/2 double knockdown PC12 cells were established that showed striking ~90% reductions in norepinephrine releases and mislocalized syntaxin-1. Re-expression of Munc18-1 has rescued secretary functions as well as trafficking of syntaxin protein. However interestingly, re-expression of Munc18-2 has resulted rescue of secretion phenotypes yet still possessing mislocalization problems of syntaxin. It seems that different Munc18 isoforms contribute to regulations of neuronal exocytosis in distinct modes of functions."

Funding:

Title: Activity-dependent translocation of CaMKII to dendritic microtubules to support synaptic plasticity

Authors: Mado Lemieux¹, Christian Tardif², Simon Labrecque³, Étienne Labrie-Dion⁴, Éric LeBel⁵, Paul De Koninck⁶

Affiliation: 1-6 Centre de Recherche Université Laval Robert-Giffard, Laval University

Abstract: Objectives: Input-specific synaptic plasticity involves the timely recruitment of multiple proteins into the postsynaptic compartment. At excitatory synapses, Ca²⁺/CaM dependent kinase II (CaMKII) plays a key role in input-specific plasticity, but the underlying mechanisms remain poorly understood. While CaMKII is thought to control the release and delivery of material to spines and dendrites, it is unclear how it is achieved with spatial and temporal specificity. To

begin to address this issue, we tested the hypothesis that CaMKII can dynamically translocate to dendritic regions specifically located near activated synapses to regulate their plasticity, and examined the underlying mechanism of this dendritic translocation. **Materials and Methods:** We performed time-lapse imaging of GFP-tagged CaMKII (WT or mutated), Ca²⁺ (GCaMP2), AMPARs (SEP-GluA1) and spines (mCherry) in cultured hippocampal neurons, combined with immunocytochemistry and Fluorescence Recovery After Photobleaching (FRAP). **Results:** We show that synaptic stimulation in cultured hippocampal neurons leads to the recruitment of CaMKII to dendritic subdomains adjacent to synapses. This dendritic enrichment of CaMKII i) involves an interaction with microtubular elements, ii) requires localized elevation in dendritic free Ca²⁺ and CaMKII activation, iii) leads to localized substrate phosphorylation and iv) is accompanied by spine remodeling and synaptic insertion of AMPARs. **Conclusion:** This novel observation of an activity-dependent translocation of CaMKII to dendritic sites near activated synapses provides a mechanism to supply material to synapses undergoing input-specific plasticity.

Funding: CIHR, NSERC, FRSQ and FQRNT.

Title: Immunohistochemical localization of c-Fos in the adult rat brain following toluene inhalation

Authors: Kristina Perit¹, Caleb Browne², Jimmie Gmaz³, Mary Beth Dunn⁴, Tanya Raaphorst⁵, Paul Mallet⁶, Bruce McKay⁷

Affiliation: 1-7 Wilfrid Laurier University

Abstract: Toluene is a psychoactive chemical inhaled for its euphoric effects. Toluene inhalation is additionally associated with a variety of short- and long-term changes including impairments in cognition, memory deficits, and motor dysfunction. Such diverse behavioural outcomes suggest a myriad of brain structures may be implicated in the effects of toluene, yet to date there has been no systematic examination of the brain structures activated by toluene vapours. In the present study we examined the functional activation of neurons throughout the brain by assessing immunoreactivity for the immediate early gene c-Fos. Adult rats were exposed to toluene vapour (5000 ppm - an abuse-relevant concentration) or control conditions for 0, 5, 10, or 30 minutes. 1.5 hours later rats were anesthetized and then perfused transcardially. Cryoprotected brains were sectioned, slices were immunostained with a c-Fos antibody, and cells showing c-fos

immunoreactivity were quantified via brightfield microscopy. Numerous brain regions implicated in reward and addiction were activated by toluene, including the ventral tegmental area, the nucleus accumbens, subnuclei of the amygdala, and specific regions within the hypothalamus. Immunoreactivity was further observed in areas of the brain associated with motor function, including select regions of the cerebellum as well as the motor cortices. Finally, c-Fos immunoreactivity was noted in the medial and lateral entorhinal cortices, but not in the hippocampus itself. Our results reveal the extent of neural activation associated with exposure to abuse-relevant concentrations of toluene vapour, with patterns of neural immunoreactivity consistent with behavioural outcomes of toluene exposure.

Funding: Natural Sciences and Engineering Research Council of Canada (BEM, DFM) and Wilfrid Laurier University

Title: Inflammation and A-beta induce similar changes in dendritic spine morphology and lipidomics in the mature hippocampus

Authors: Philip Keng-Yu Chang¹, Armen Khatchadourian², Sebastien Boridy³, Dusica Maysinger⁴, Anne McKinney⁵

Affiliation: 1-5 McGill University

Abstract: "Alzheimer's disease (AD), the most prevalent form of dementia for people over 65, causes a progressive cognitive decline that cannot be reversed with current treatments. It is widely accepted that the brain microcircuitry defects in AD are exacerbated by the soluble oligomer β -amyloid ($A\beta$) protein accumulation. The exact mechanisms of $A\beta$ in AD progression are still unclear. Therefore, by studying the initial effects of $A\beta$ prior to formation of plaques and tangles we can gain insight to AD pathophysiology. Our study capitalizes on an emerging finding that lipid abnormalities are important in AD. We modeled the initial insult of $A\beta$ in mature organotypic hippocampal slice cultures with $A\beta$ for 24 hours. We report an abnormal accumulation of lipid components in CA1 pyramidal neurons and microglia in the form of tiny "inflammatory organelles" called lipid droplets (LD) following $A\beta$ treatment. Strikingly similar alteration in LD distribution can be observed when inflammatory response is elicited with lipopolysaccharide (LPS). Furthermore, we found decrease in dendritic spine densities after both $A\beta$ and LPS treatments. Accompanying this morphological change, we saw an increase in AMPA-mediated mEPSC frequency. Interestingly, when we supplemented omega-3 fatty acid

docosahexanoic acid (DHA) to restore abnormal LD distribution all morphological and electrophysiological phenotypes were indistinguishable from that of control. It remains to be determined whether deficits in synaptic plasticity can be rescued by re-establishment of LD status. Our focus on the prevention and repair of the compromised brain circuitry will provide an innovative approach for AD therapies."

Funding: CIHR, NSERC, CFI

Title: EPHA4 Plays a role in the regulation of the neuronal-glia structural plasticity induced by salt loading in the supraoptic nucleus

Authors: Daniella Isacu¹, Diane Gingras², Sylvie Laforest³, Guy Drolet⁴, Wafaa Jammow⁵, Michel Lauzon⁶, Luc Desgroseillers⁷, Sabrina Chierzi⁸, Keith Murai⁹, Elena Pasquale¹⁰, Guy Doucet¹¹

Affiliation: 1,2,5-7,11 Montreal University, GRSNC 3,4 Laval University, CHUQ 8,9 McGill University, MUHC 10- Sanford-Burnham Medical Research Institute

Abstract: The supraoptic nucleus (SON) shows striking neuro-glia structural changes following salt-loading in adult rodents, with retraction of astrocytic leaflets covering of oxytocin (OT) soma and dendrites which then receive new synapses. To test the role of the tyrosine kinase receptor, EphA4, in these cellular movements, we compared the astrocytic and synaptic coverage of OT-immunostained dendrites, by electron microscopy, in the SON of C57BL/6 (wt, n=4) and EphA4^{-/-} (KO, n=4) mice submitted to a 7-day, 2% NaCl regimen, with wt (n=4) and KO (n=3) controls. As expected, salt-loaded wt mice showed a significant reduction in their astrocytic coverage of OT dendrites, accompanied by an increase in synaptic contacts, compared to untreated wt controls (p<0,0001). In contrast, there was no change in astrocytic coverage or synaptic contacts in salt-loaded KO mice, compared to wt or KO untreated controls, indicating the requirement for EphA4 expression for such plasticity. In situ hybridization (ISH) showed moderate expression of EphA4 in the SON, but not in astrocytes of the glia limitans, suggesting that dendritically-expressed EphA4 acts by reverse signalling, activating an astrocytic ephrin. Measurements of autoradiographic grains following ISH showed a significant decrease in density accompanied by an increase in volume of the SON in salt-loaded rats (n=6) compared to untreated controls (n=4), indicating no net change in the expression of EphA4 following salt-loading. These results indicate a role for EphA4 in the neuro-glia structural plasticity, which would not involve modifications

in its expression.

Funding: Supported by NSERC and an infrastructure grant from FRSQ (GRSNC).

Title: Neto2 is a novel regulator of the potassium chloride co-transporter KCC2 and GABA-mediated synaptic inhibition

Authors: Evgueni Ivakine¹, Brooke Acton², Vivek Mahadevan³, Melanie Woodin⁴, Roderick McInnes⁵

Affiliation: 1,3,5 Program in Stem Cell and Developmental Biology, Hospital for Sick Children Research Institute 2,4- Department of Cell & Systems Biology, University of Toronto

Abstract: "Recently, we and others reported that Neto1 and Neto2 proteins are important regulators of excitatory neurotransmission (Ng et al. PLoS Biol. 2009; 7(2):e41; Zhang et al. Neuron. 2009; 12;61(3):385-96). The objective of the current study was to define the role of Neto2 in GABA-mediated synaptic inhibition. Using an unbiased GST pulldown approach we identified KCC2 as a putative Neto2 interactor. KCC2 is a K⁺-Cl⁻ cotransporter required for synaptic inhibition in the mature CNS, where it establishes a Cl⁻ ion gradient. We validated the Neto2:KCC2 interaction by co-immunoprecipitating them from both hippocampal protein lysates and HEK-293 cells. Using a series of Neto2 deletion constructs we determined that the Neto2:KCC2 interaction was mediated by the Neto2 ectodomain. Importantly, in vivo Neto2 predominantly associated with the active oligomeric form of KCC2. To define the functional consequences of this interaction, we measured the strength of synaptic inhibition, as determined by the Cl⁻ equilibrium potential (E_{Cl} = membrane potential where net flow of chloride ions is zero). We found that in Neto2^{-/-} neurons E_{Cl} were significantly depolarized vs. wild type neurons (-53.74 ± 2.43 mV vs. -75.22 ± 2.05 mV; p < 0.001), suggesting a decrease in KCC2 activity. Importantly, shRNA-mediated knock-down of Neto2 in mature wild type cultured neurons also causes a depolarization of E_{Cl} (-54.43 ± 2.12 mV; p = 0.016), indicating that maturation of Neto2^{-/-} neurons is not affected. We conclude that Neto2 is a regulator of neuronal Cl⁻ homeostasis through its interaction with KCC2, thereby profoundly influencing GABA-mediated synaptic inhibition."

Funding: CIHR, NSERC

Title: Loss of Ca²⁺ permeable AMPA receptor function in tonic neurons of the substantia gelatinosa after sciatic chronic constriction injury

Authors: Yishen Chen¹, Peter Smith²

Affiliation: 1,2 University of Alberta

Abstract: "Peripheral nerve injury can promote chronic neuropathic pain. This is initiated by the appearance and persistence of ectopic spontaneous activity in primary afferent neurons and a secondary, enduring increase in excitability of sensory circuits in the spinal dorsal horn. This leads to the phenomenon of 'central sensitization'. We hypothesized that chronic constriction injury (CCI) of sciatic nerve would alter the properties of synaptic AMPA receptors in substantia gelatinosa (SG) neurons in the dorsal horn. To test this, rats (18d) were subjected to 14-24d CCI and whole-cell recordings made from inhibitory (tonic firing) and excitatory (delay firing) SG neurons. A selective blocker of Ca²⁺ permeable AMPA receptors, IEM1460 (50 μ M) reduced evoked EPSC's, spontaneous EPSC's (sEPSC's) and mini EPSC's (mEPSC's) amplitude by 40% in excitatory neurons from both sham operated and CCI animals. IEM1460 also produced 50% inhibition of these events in inhibitory neurons from sham operated animals but lost its effectiveness after CCI. Preliminary estimates of single channel conductance (γ) using non-stationary fluctuation analysis revealed γ = 29pS for synaptic AMPA receptors on tonic, inhibitory neurons in sham-operated animals and 14.5pS after CCI. We suggest that CCI downregulates or promotes internalization of Ca²⁺ permeable AMPA receptors on tonic, inhibitory neurons but not those on excitatory, delay neurons. This contrasts with the findings of Park et al. (J. Neurosci., 29:3206, 2009) who found that chronic inflammatory pain increased the effectiveness of blockers of Ca²⁺ permeable AMPA receptors. Thus, distinct molecular mechanisms of synaptic modifications may underlie different forms of chronic pain. "

Funding: Supported by CIHR

Title: Dynein Light Chain (LC8) is an interacting partner of Myelin Associated Glycoprotein (MAG) in vitro and in vivo

Authors: Dalinda Liazoghli¹, Steve Salomon², Ajit Singh Dhaunchak³, Omar De Faria⁴, Robert Dunn⁵, David R. Colman⁶

Affiliation: 1-6 Montreal Neurological Institute and hospital

Abstract: Myelin Associated Glycoprotein (MAG) mediated axon-glia interactions at the periaxonal membranes, in both CNS and PNS, have been shown to regulate myelin formation and axonal integrity. Molecules restricting MAG to the periaxonal membranes are not known. In order

to identify protein regulating MAG distribution within the myelin sub-compartment, we performed a yeast-two-hybrid screen using the intracellular domain of MAG as bait. Interestingly, 5 out of 6 identified clones were nearly full length copies of the dynein light chain LC8. LC8 and MAG interaction was further verified by co-immunoprecipitation and Glutathione-S-Transferase pull down assay. We also show that LC8 is a novel myelin associated protein in humans and rodents. Interestingly, in developing mice brain LC8 expression peak coincides with myelination peak (around postnatal week 2-3). We also found significant amount of co-localization between LC8 and MAG in myelinating co-cultures. Currently, we are investigating whether LC8 expression is required for MAG distribution within myelin.

Funding:

Title: New in vitro models to decipher the role of the innate immune response in Huntington's disease

Authors: Giulia Cisbani¹, Francesca Cicchetti²

Affiliation: 1,2 CHUL-Universite Laval

Abstract: Huntington's disease (HD) is a devastating neurodegenerative disease caused by a genetic mutation in the huntingtin gene. The mutant Huntingtin (mHtt) is pathogenic and induces the formation of aggregates that interfere with the normal cell physiology, possibly creating abnormal cell-cell interactions. Despite the fact that the CNS is severely targeted in HD, abnormalities resulting from the ubiquitously expressed mHtt are also seen within several organ systems. Compelling evidence that a strong immune response is involved both in the brain and in the blood has led us to hypothesize on the pathological role of mHtt in microglia and monocytes.

Objectives: Development of new cell models of microglia and monocytes expressing the normal and pathogenic huntingtin gene under the control of an inducible promoter. **Methods:** 1. Cloning of EGFP-tagged pathogenic and not pathogenic huntingtin genes under the control of a lactose-inducible promoter. Thereafter, the resulting plasmids are stably introduced in microglia and macrophages/monocytes cell lines expressing the repressor protein. 2. Evaluation of the immune response and cell viability upon the inducible expression of huntingtin. 3. Co-culture and conditioned media experiments to study cell-cell interaction. **Results:** The cells have been successfully transfected and are undergoing characterization for the expression of both the repressor and the gene of interest. Co-culture studies with either neurons or microglia expressing

mHtt are performed to analyze their pathogenic interplay in vitro. Conclusion: The study of the mHtt in immune cells will allow to decipher the molecular mechanisms involved in mHtt toxicity and will contribute to discover potential therapeutic targets.

Funding: Huntington Society Canada

Title: A realistic neuron model indicates that loss of inhibition caused by impaired Cl⁻ extrusion cannot be fully restored by increasing GABAA mediated activity.

Authors: Nicolas Doyon¹, Steve Prescott², Annie Castonguay³, Antoine Godin⁴

Affiliation: 1,3 Université Laval 2- Pittsburgh University 4- McGill University

Abstract: We constructed a realistic multi compartment electro-diffusion model of a neuron bombarded by both inhibitory and excitatory distributed synaptic input. Apart replicating the electric properties of a cell, the model tracks spatio-temporal changes in concentration of several interacting ionic species. We used the model to investigate the interplay between Cl⁻ extrusion, GABAA mediated activity and their impact on cell excitability. Simulations indicated that loss of inhibition due to impaired Cl⁻ extrusion caused by KCC2 downregulation cannot be efficiently restored by increasing GABAA mediated activity. Several of the model predictions were validated by performing measurement of intracellular Cl⁻ concentration using fluorescence lifetime imaging (FLIM) under various conditions of synaptic activity and Cl extrusion capacity. These measurements indicated a standing somato-dendritic Cl⁻ gradient which renders dendritic inhibition even more inefficient. Furthermore, these measurements indicated that under scenario of impaired Cl⁻ extrusion, excitatory activity can play a role in exacerbating Cl⁻ accumulation. Moreover, a new method of image analysis (SPIDA) was used to estimate the membrane density of active KCC2 along the somato-dendritic axis. This data is both highly valuable by itself and critical for validation of the model. Simulations also indicated that under physiological input, the presence of KCC2 does not exacerbate extracellular K⁺ accumulation. Finally under depleted KCC2 activity, Cl⁻ accumulation occurring in scenario where GABAA mediated activity fails to prevent spiking can lead to catastrophic failure of inhibition.

Funding:

Title: Effects of Ras knockout on Infarct Volume after Photothrombotic Cerebral Ischemic

Authors: Shakib Rahman¹, Ian Winship², Nancy Dower³, Kimberly Wong⁴, James Stone⁵, Ashfaq

Shuaib⁶, Kathryn Todd⁷

Affiliation: 1-7 University of Alberta

Abstract: Introduction: Cerebral ischemia, a reduction in blood flow to the brain, is known to activate many signalling pathways. The Rat Sarcoma (Ras) family of GTPases are important signalling pathways that have been shown to have varying effects on cellular growth, differentiation and survival. For example, reports have shown that Ras has anti-apoptotic effects via PI3-K/Akt signalling, yet Ras can also activate pro-apoptotic factors through Nore-1 and Mst1/2 signalling. The aim of our study was to investigate the effects of Ras knockout on infarct volume after photothrombotic ischemic stroke. Methods: A photothrombotic stroke was induced in the forelimb sensorimotor cortex of wild type and Ras knockout mice. After a one week recovery period, mice were euthanized and the brains collected. Sequential cryosections were obtained and assayed to quantify the volume of infarction, astrocyte proliferation (glial fibrillary acidic protein [GFAP] immunoreactivity) and dying cells (fluorochrome [FJ] staining). Results: Field counts for GFAP and FJ-positive cells were not significantly different between RasKO and WT controls (n=5). Interestingly, RasKO mice trended towards larger stroke volumes (more than double) compared to WT mice. Discussion: Ras activation and its signalling cascade have been associated with both anti-apoptotic and pro-apoptotic processes. In the present study we found a strong trend towards larger stroke volumes in RasKO mice suggesting that, under ischemic conditions, altering the Ras signalling pathway will tip the balance toward pro-apoptotic signalling. Further studies to evaluate up-regulation of specific Ras isoforms are warranted. **Funding:** CIHR, AHFMR, AIHS, Heart and Stroke Foundation, Davey Endowment Fund, Faculty of Medicine and Dentistry (University of Alberta).

Title: GABAA Transmission Regulates Dendritic Spine Density in the Developing Hippocampus

Authors: Christopher Salmon¹, Emma Jones², Keith Murai³

Affiliation: 1-3 McGill

Abstract: The neurotransmitter γ -aminobutyric acid (GABA) plays an important role in CNS development and function. Remarkably, synaptic transmission through GABAA receptors is excitatory in immature neurons during early postnatal development. During this time, excitatory glutamatergic synapses are actively forming and maturing into dendritic spines. Here we investigated the effects of GABAergic transmission on the formation and stability of dendritic

spines on CA1 neurons in mouse organotypic hippocampal slices. We monitored the detailed morphology of dendrites, including spines, by expressing membrane-targeted enhanced green fluorescent protein in CA1 neurons of mouse organotypic slices followed by confocal microscopy and image analysis. We found that at 3 days in vitro (DIV), inhibiting GABAA receptors with bicuculline increased spine density by 33%. Driving GABAA transmission with muscimol, in contrast, decreased spine density. However, the effects of manipulating GABAA transmission on spine density were transient, and by 5 DIV inhibition of GABAA receptor transmission significantly reduced spine density. We then followed up on the differential effects of GABAA receptor inhibition on spines by monitoring the developmental time course of potassium-chloride cotransporter-2 (KCC2), which helps establish the mature Cl⁻ gradient in neurons. KCC2 levels increased between 3 and 7 DIV, indicating that a switch in the action of GABA (excitatory to inhibitory) may account for the observed switch in the effect of bicuculline on spine density. Together, our findings indicate that early GABAA transmission regulates excitatory synapse formation in the developing hippocampus.

Funding:

Title: Differential oligomerization state of the G protein-coupled receptor Neurokinin-1 between cell culture and native tissue

Authors: Louis-Étienne Lorenzo¹, Antoine Godin², Jody Swift³, Benjamin Rappaz⁴, Annie Castonguay⁵, Santiago Costantino⁶, Alfredo Ribeiro-da-Silva⁷, Paul Wiseman⁸, Yves De Koninck⁹

Affiliation: 1,2,5,9 Université Laval 3,4,7,8- McGill University 6- Université de Montréal

Abstract: "Expression systems such as cell culturing are universally used in biological sciences and neuroscience to study receptor oligomerization states and to better understand protein interactions. Here, we demonstrate that the oligomerization state of the neurokinin-1 receptor (NK-1r) is cell-line dependant. Based on spatial intensity distribution analysis (SpIDA, Godin et al., PNAS 2011), we confirmed that the NK-1rs were almost exclusively monomeric at the cell membrane of CHO-k1 and HEK cells. The receptors were shown to be active, as substance P (SP) induced internalization and formation of endosomes. We obtained similar results for both cell-types, with receptors tagged either with fluorescent proteins or with antibody labeling. Fluorescence lifetime imaging microscopy (FLIM) confirmed results obtained with SpIDA, as no significant changes of measured lifetimes were measured in the presence of NK-1r tagged with

Förster resonance energy transfer (FRET) partners (mCerulean and mYFP) equally transfected in cells. Conversely, in spinal cord native tissue, SpIDA revealed that NK-1r formed homodimers at cell membranes and is primarily monomeric in the cytoplasm of spinal dorsal horn neurons. Stimulated receptor internalization by SP release, revealed a decrease in the number of membrane homodimers ($38\% \pm 2$ to $21\% \pm 1$ μm^{-2}). Electron microscopic ultrastructural analysis of NK-1r gold-immunolabellings confirmed the distributions of NK-1r monomers-homodimers in the subcellular compartments. This major difference in protein organization between native tissue and cell culture is crucial as role and function of a GPCR highly depend on its surrounding proteins. Conclusions drawn from expression systems might be indubitably biased by the cell-line choice."

Funding:

Title: Voltage sensitive dye imaging reveals that repeating cortical motifs in spontaneous activity reflect underlying functional sensory circuits

Authors: Majid Mohajerani¹, David McVea², Timothy Murphy³

Affiliation: 1-3 University of British Columbia

Abstract: Spontaneous neuronal activity is not noise, but reflects the orchestrated action of underlying brain circuitry. Although its importance is recognized, exactly what determines the spatial and temporal dynamics of spontaneous activity in mammalian brain is unknown. Here, using fast voltage sensitive dye (VSD) imaging, we compared the spatiotemporal structure of ongoing spontaneous and stimulus-evoked activity in the primary, secondary and association (retrosplenial, cingulate) cortices of anaesthetized adult mice. We find that visual, somatosensory, and auditory sensory-evoked responses activate a common area of motor, cingulate and retrosplenial cortices. These areas were also the most active spontaneously, in the absence of any sensory inputs. In addition, we assessed whether repeated sensory stimulation would alter the spatiotemporal dynamics of subsequent cortical activity. Sensory circuits that were repeatedly activated remained active during the subsequent sequences of spontaneous activity, even though stimulation had been discontinued. To test whether this effect was simply a reflection of generalized changes in excitability, or reflected plasticity within localized sensory circuits, we stimulated visual and somatosensory sensory systems at different frequencies. Such stimulation of the cortex led to activation patterns that were repeated during spontaneous activity. These effects

persisted for 20 min without additional sensory stimulation. We interpret these results as strong evidence that spontaneous cortical activity reflects underlying structural circuitry in the cortex and that it is influenced by the spatial and temporal patterns of previous brain activity. These findings add further support to important functional roles for spontaneous brain activity.

Funding: CIHR Operating Grant MOP-48695 to THM, CIHR postdoctoral fellowship to MHM, and Vanier Canada Graduate Scholarship to DM.

Title: Functional interaction between alphaCaMKII and GluN2B controls ERK-dependent structural plasticity.

Authors: Farida El gaamouch¹, Olivier Moustie², Mado Lemieux³, Paul De Koninck⁴, Alain Buisson⁵, Olivier Nicole⁶

Affiliation: 1,3,4 Centre de recherche Université Laval Robert-Giffard 2- CNRS UMR Ci-NAPS 6232 5- Centre de Recherche Inserm U 836 - UJF - CEA - CHU 6- CNRS UMR 5293 - Institut des Maladies Neurodégénératives

Abstract: One key neurobiological mechanism underlying memory formation and storage resides in activity-driven modifications of synaptic strength and structural remodeling of synapses. Over the past decade, the ERK/MAPK pathway has emerged as a central player in signaling mechanisms involved in the activity-driven synaptic changes. One of the signaling events that activates ERK cascade is a rise in free intracellular Ca²⁺ concentration through the NMDA receptors. Despite the key role of prolonged ERK activation in synaptic plasticity, the role of NMDAR subunit composition in ERK signaling cascade is still under debate and the mechanisms by which a short elevated Ca²⁺ concentration induces a long lasting ERK activation remains unknown. We demonstrate that only GluN2B-containing NMDAR activation leads to ERK phosphorylation. In addition, we show that alphaCaMKII, but not betaCaMKII or CaMKI, is involved in synaptic NMDAR-induced sustained ERK activation. To determine if the binding of alphaCaMKII to GluN2B is required for synaptic NMDAR-induced ERK activation, we used an alphaCaMKII mutant unable to interact with GluN2B, our results indicate that 1) a direct interaction between GluN2B and alphaCaMKII is essential to induce a prolonged ERK phosphorylation, 2) blocking of GluN2B-alphaCaMKII interaction as well as applying ERK inhibitors or a selective GluN2B antagonist alters the increase of dendritic spine size induced by synaptic activity. Thus the interaction between GluN2B and alphaCaMKII may serve to support long-lasting changes in synaptic plasticity, via ERK

signaling. Altogether these functions suggest that the GluN2B/alphaCaMKII/ERK triad may be considered as a major contributor for the formation of new memories.

Funding:

Title: Rab8 Modulates Group I Metabotropic Glutamate Receptor Signalling and Intracellular Trafficking

Authors: Jessica Esseltine¹, Fabiola Ribeiro², Lianne Dale³, Stephen SG Ferguson⁴

Affiliation: 1- University of Western Ontario 2-4 Robarts Research Institution

Abstract: Metabotropic Glutamate Receptors (mGluR) mediate the action of glutamate, a major excitatory neurotransmitter in the central nervous system. Group 1 mGluRs (mGluR1 and mGluR5) are primarily coupled to Gq leading to the activation of phospholipase C and the formation of diacylglycerol and inositol 1, 4, 5-trisphosphate leading to release of intracellular calcium stores and PKC activation. Desensitization, endocytosis and recycling are major mechanisms of receptor regulation and intracellular trafficking of GPCRs is linked to the Rab family of small G proteins. Rabs mediate various processes in vesicular transport, targeting, docking and fusion. Rab8 specifically is involved in the regulation of secretory/recycling vesicles, modulation of the actin cytoskeleton and cell polarity and has been shown to regulate the synaptic delivery of AMPA Receptors during long term potentiation and differentially associate with and modulate the plasma membrane delivery of the alpha 2B and beta 2 adrenergic receptors. Here we show that Rab8 associates with the carboxyl terminal tail of mGluR1a in an agonist-dependent manner and blocks the endocytosis of this receptor. Interestingly Rab8 expression leads to decreased receptor-mediated IP formation in a PKC dependent manner and also alters mGluR1 phosphorylation. Taken together the data presented here indicates an important role for Rab8 in the modulation of mGluR1 signalling and intracellular trafficking.

Funding: Ontario Graduate Scholarship Ontario Graduate Scholarship in Science and Technology

Title: ORPHAN NUCLEAR RECEPTORS NURR1 AND NUR77 IN THE MODULATION OF DOPAMINE NEUROTRANSMISSION: POSSIBLE INTERACTIONS AND DISTINCT ROLES.

Authors: Antoine Hone-Blanchet¹, Brigitte Paquet², Joanie Baillargeon³, Daniel Levesque⁴

Affiliation: 1-3 CRCHUL; Université Laval 4- Faculté de Pharmacie; Université de Montréal

Abstract: "OBJECTIVES: Orphan nuclear receptors of the NR4A subfamily are closely related to

the functioning of dopaminergic systems. Nurr1 is essential at developing and maintaining the survival of dopamine neurons whereas Nur77 is known as an important modulating agent of the dopamine neurotransmission. Knowing that locomotor activity is strongly influenced by the dopaminergic systems, we have investigated the possibility of an interaction between the two transcription factors in the modulation of the dopaminergic neurotransmission. **METHODS:** We have measured the behavioral output of different wild type and knockout mice (Nur77 $-/-$; Nurr1 $+/-$; Double knockout (Nur77 $-/-$ Nurr1 $+/-$)) in different basal and drug-challenged motor procedures. We have also measured the mRNA levels of different striatal peptides and nuclear receptors using in situ hybridization, in the same basal and drug-challenged conditions. **RESULTS:** Nur77 $-/-$ and Double knockout mice display a significantly increased short-term basal locomotor activity and a significantly reduced cataleptic output following haloperidol injection. Double knockout mice display a significantly increased locomotor activity following amphetamine treatment in comparison to all other wild type and knockout mouse types. Enkephalins mRNA expression is significantly higher in Double knockout mice following amphetamine drug treatment, but significantly unchanged following haloperidol treatment in comparison to all other mouse types. **CONCLUSION:** NR4A subfamily receptors are important modulators of the dopamine mediated behavioral outputs. We suggest that the possibility of an interaction between Nurr1 and Nur77 exists, based on the biochemical and behavioral differences displayed by the Double knockout mice. "

Funding: This work was supported by the Canadian Institute of Health Research (CIHR).

Title: Endogenous phasic GABAA receptor-mediated electrical suppression prevents cell death in anoxia-tolerant turtle cortex.

Authors: David Hogg¹, Leslie Buck²

Affiliation: 1-2 University of Toronto

Abstract: "In mammalian brain, anoxia induces hyper-excitability and cell death; however, the western painted turtle *Chrysemys picta bellii* is anoxia tolerant, neuronal activity is depressed by anoxia and cell death does not occur. In anoxic turtle brain, [γ -aminobutyric acid] (GABA) is rapidly elevated 80-fold suggesting that the mechanism(s) responsible for anoxia-tolerance involve GABAergic synaptic transmission. The objective of this study was to investigate the neuroprotective role of GABA receptor currents during anoxia in turtle pyramidal neurons. Using

whole-cell and perforated patch clamp techniques we identified phasic GABAA receptor-mediated currents that double in amplitude with anoxia (41.2 ± 1.6 to 82.1 ± 2.1 pA) resulting in a 60-70% decrease in spontaneous action potential frequency. Anoxia or GABA perfusion depolarized membrane potential (-88.8 ± 1.7 mV) to the GABA reversal potential (-80.8 ± 2.3 mV) and prevented further depolarization. Application of gabazine, a GABAA receptor antagonist, inhibited phasic GABAA receptor currents and resulted in excitotoxic cell death. We conclude that anoxia increases phasic GABAA receptor peak currents resulting in a "shunting current" that prevents further depolarization and hyper-excitability. "

Funding: Research supported by an OGSST grant to DWH and and NSERC grant to LTB.

Title: Retinoic Acid exposure reduces intracellular Ca²⁺ in cultured molluscan neurons

Authors: Taylor Dawson¹, Nicholas Vesprini², Ye Yuan³, Doug Bruce⁴, Gaynor Spencer⁵

Affiliation: 1-5 Brock University

Abstract: "The vitamin A metabolite, retinoic acid (RA), is known to play a role in the development and regeneration of the nervous system in vertebrates, influencing patterning, neuritogenesis and axonal pathfinding. Traditionally, RA has been thought to act in conjunction with its nuclear receptors, exerting its effects by altering gene expression; however recent evidence suggests a non-genomic role for RA in regenerating neurons. Our lab has shown that exposure to RA can influence neurite outgrowth and growth cone turning in cultured Lymnaea neurons, and that this behavior is dependent on Ca²⁺ influx. Recently, we have also shown that acute RA exposure elicits significant changes in the firing properties and action potential waveform in cultured Lymnaea neurons. Utilizing the ratiometric calcium indicator dye Indo-1 AM we now show that RA exposure results in a significant decrease in intracellular Ca²⁺ that correlates with the time course of the RA-induced electrophysiological changes. The RA-mediated effects on electrophysiological properties and intracellular Ca²⁺ also appear to be dose- and isomer-specific. We hypothesize that voltage-gated Ca²⁺ channels (VGCCs) are involved in the RA-induced effects, but there is no current evidence that RA can modulate the properties of VGCCs in mature neurons. As such we extend these studies, utilizing whole-cell patch clamp techniques, to characterize the effects of RA on the biophysical properties of VGCCs. This work demonstrates that RA can influence intracellular Ca²⁺ levels, which may underlie some of the physiological roles

played by RA during nervous system development and regeneration."

Funding: "Acknow: NSERC Discovery grant to GES; NSERC PGS-D to NV, TFD. "

Title: Screening of PDZ proteins involved in scaffolding the 5-HT₂ /CRFR1 receptors complex

Authors: Ammar Mahmood¹, Pradeep Ramana², Faisal Beg³

Affiliation: 1-3 Simon Fraser University

Abstract: Our group has shown that activation of CRFR1 receptors causes an increase in 5-HT₂ signalling due to an increase of these receptors at the cell surface, a mechanism named heterologous sensitization. This synchronized serotonin receptor recruitment to the cell surface is dependent on CRFR1 activation and consequent internalization followed by Rab 4-dependent rapid recycling. Our studies also showed that the association with PDZ proteins is crucial for this mechanism since PDZ binding motif removal from both receptors abolishes the observed effect. More importantly, this molecular mechanism has been shown to be physiologically relevant in vivo since stimulation with CRF peptide followed by challenge with a serotonin agonist showed increased serotonin-mediated anxiety behavior. We are now concentrating on identifying the PDZ protein(s) that is (are) crucial for the scaffolding of these receptor complexes. We started with 16 PDZ proteins that are important to the regulation of GPCR trafficking and signalling and among them, we found a few potential candidates. Now we will knock down these proteins to assess their role in the CRF-mediated heterologous sensitization of 5-HT_{2A/C} receptors. This mechanism presents a way to integrate different signalling pathways that are important for normal and pathophysiological states, opening a new line of research on the molecular pharmacology field and translational science.

Funding: CIHR

Title: Screening of PDZ proteins involved in scaffolding the 5-HT₂ /CRFR1 receptors complex

Authors: Ana Cristina Magalhaes¹, Stephen Ferguson², Henry Dunn³

Affiliation: 1-3 Robarts Research Institute, University of Western Ontario

Abstract: Our group has shown that activation of CRFR1 receptors causes an increase in 5-HT₂ signalling due to an increase of these receptors at the cell surface, a mechanism named heterologous sensitization. This synchronized serotonin receptor recruitment to the cell surface is dependent on CRFR1 activation and consequent internalization followed by Rab 4-dependent

rapid recycling. Our studies also showed that the association with PDZ proteins is crucial for this mechanism since PDZ binding motif removal from both receptors abolishes the observed effect. More importantly, this molecular mechanism has been shown to be physiologically relevant in vivo since stimulation with CRF peptide followed by challenge with a serotonin agonist showed increased serotonin-mediated anxiety behavior. We are now concentrating on identifying the PDZ protein(s) that is (are) crucial for the scaffolding of these receptor complexes. We started with 16 PDZ proteins that are important to the regulation of GPCR trafficking and signalling and among them, we found a few potential candidates. Now we will knock down these proteins to assess their role in the CRF-mediated heterologous sensitization of 5-HT_{2A/C} receptors. This mechanism presents a way to integrate different signalling pathways that are important for normal and pathophysiological states, opening a new line of research on the molecular pharmacology field and translational science.

Funding: CIHR

Poster Category: C - Disorders of the Nervous System

Title: Protective effect of resveratrol, a wine polyphenol, on cellular degeneration of dopaminergic neurons.

Authors: Maria-Grazia Martinoli¹, Julie Bournival², Justine Renaud³, Geneviève Bureau⁴

Affiliation: 1-4 Cellular Neurobiology, Dept. of Biochemistry UQTR

Abstract: Pathological signs of oxidative stress and inflammation have been observed in the brain of patients with Parkinson's disease (PD), a movement disorder characterized by progressive loss of nigrostriatal dopaminergic neurons. Interestingly, the neuroprotective potential of several naturally-occurring dietary polyphenols appears to be rooted in their anti-oxidant properties. In this context, we have studied the neuroprotective role of the stilbene resveratrol (RESV), abundantly present in red wine, in a cellular model of PD, neuronal dopaminergic PC12 cells. Our data showed that RESV reduced the cytotoxicity and apoptotic death of neurons induced by MPP⁺, a well-known parkinsonian toxin. Then, DNA fragmentation, Bax/Bcl-2 ratio, nuclear translocation of the apoptosis-inducing factor (AIF) as well as poly(ADP-ribose) polymerase (PARP) cleavage were significantly reduced by RESV administration, sustaining its anti-apoptotic character. We also studied its modulation of mitochondrial function. Our findings clearly demonstrated that the increase of cytochrome c levels in cytosolic fractions of neurons treated with MPP⁺ was attenuated by pre-treatment with RESV. We then integrated our two cell-culture systems (glia-neurons) to elucidate the effect of RESV on dopaminergic neuronal death induced by glial cell inflammation. Our data demonstrated that RESV reduced dopaminergic neuronal death evoked by glial cell inflammation. In conclusion, we demonstrated that RESV can reverse parameters of cell distress as well as cell inflammation in dopaminergic neurons. Our present results strongly suggest that natural anti-oxidative compounds, such as RESV, could be used as complementary and/or preventive therapy of neurodegenerative diseases caused by inflammation and/or oxidative stress.

Funding: NSERC Discovery Grant to MGM

Title: Synergistic effects of stroke and Alzheimer's pathologies on neuronal survival of adult born neurons in the hippocampus

Authors: Lulu Gao¹, David Cechetto², Martin Wojtowicz³

Affiliation: 1, 3 University of Toronto 2- University of Western Ontario

Abstract: "Alzheimer's disease (AD) is characterized by a progressive decline in cognition in the aging population. It is advanced by risk factors for vascular cognitive impairment, such as stroke. Our team has generated animal models to investigate the underlying pathological interactions between stroke and AD. Four groups of young adult male Sprague-Dawley rats were used in the study: sham controls (n=3), AD model (n=4), stroke model (n=4) and the combined (AD & stroke) model (n=3). The stroke model was produced by a single injection of endothelin-1 into the right striatum. The AD model was created by a bilateral injections of A β 25-35 into the lateral ventricles. It has been previously shown that the combined model presented with a synergistic increase in inflammation in the hippocampal CA1 region and striatum, and in infarct size around the ischemic region. This study further examined adult neurogenesis in the dentate gyrus by using immunohistochemical markers of new neurons: BrdU, Doublecortin, NeuN and Ki67. Stroke or AD treatment alone, significantly impaired dendritic development (38% reduction) in the middle molecular layer 3 weeks after the treatment. The combined treatment further reduced neuronal survival by over 90% via a synergistic mechanism. No significant long-term changes in cell proliferation were observed. This is the first evidence that a stroke in the striatum can impinge on the neuronal maturation of adult born neurons in the dentate gyrus. Combined treatment can further impact neuronal survival, leading to more dramatic consequences that could contribute to the early onset and progression of Alzheimer's dementia. "

Funding: This is a collaborative work by the CIHR Vascular and Cognitive Impairment Team. L. Gao is supported by BBDC studentship.

Title: A bimanual object hit task to quantify sensorimotor dysfunction of subjects with stroke

Authors: Kathrin Tyryshkin¹, Angela Coderre², Sean Dukelow³, Janice Glasgow⁴, Stephen Scott⁵

Affiliation: 1,2,4,5 Queen's University 3- University of Calgary

Abstract: Clinical assessment provides a foundation for all aspects of patient care assisting diagnosis, prognosis and overall patient care. Stroke can impact a broad range of sensory, motor and cognitive functions, but existing assessment tools tend to be largely subjective in nature and use relatively course rating systems. The present study highlights a bimanual task that quantifies the behaviour of subjects that must plan and control goal-directed movements of both limbs simultaneously. The task is performed using the bimanual KINARM robot that permits planar

movements of the upper limb in concert with a 2D virtual reality system that displays hand position and targets in the horizontal workspace. In this task a subject is instructed to use their right and left hands (represented as green paddles) to hit red balls that move towards the subject. At the beginning of the task, only one ball is displayed and moves relatively slowly, but the number and speed of the balls progressively increases. In addition to completing this ball drop task, each subject underwent a standardized clinical stroke assessment. The collected data were analyzed and different parameters were extracted that characterize task performance, hand temporal and spatial characteristics. The parameters, taken individually, identified between 23% - 74% of right-side affected stroke subjects and 20% - 95% of the left-affected stroke subjects as different from controls. Some of these parameters correlated with Functional Independence Measure (FIM) scores.

Funding: CIHR, NSERC

Title: Identification of myeloid immune cells as a source of neurotrophins in sciatic nerve lesion model.

Authors: Alexandre Paré¹, Sylvain Nadeau², Nicholas Vallières³, Steve Lacroix⁴

Affiliation: 1-4 CHUL Research Center

Abstract: Using various transgenic mice and mouse models of peripheral nerve and spinal cord injury, we have recently shown that immune cells are essential for axonal regeneration. More specifically, we found that immune cells of the myeloid subset (CD11b+) play a key role in axonal regeneration through their capacity to 1) clear myelin debris and its associated inhibitory molecules, 2) produce neurotrophins, and 3) support the formation of new blood vessels that help irrigate the lesion site and provide growth support for regenerating axons. We now report that neurotrophins are expressed during the first week after sciatic nerve lesion, with a peak of expression at 4 days. By taking advantage of the implantation of diffusion chambers containing sciatic nerve segments, we demonstrate that neurotrophin-producing cells are not resident cells but rather infiltrating cells. Studies performed in NOD/CB17-Prkdcscid, IL-1R1/TNFR1-knockout (ko) et CCR2-ko mice allowed us to exclude lymphocytes, neutrophils and pro-inflammatory M1 monocytes as cellular sources of neurotrophins. Finally, depletion of CD11c+ cells by the administration of diphtheria toxin in CD11c-DTR/EGFP mice led to a 10% decrease in the number of neurotrophin-expressing cells, suggesting that dendritic cells or anti-inflammatory M2

monocytes are responsible for neurotrophin synthesis after peripheral nerve injury.

Funding: This study was supported by NSERC

Title: Visual Ability, Learning and Memory in Young and Old APP^{swe}/PS1^{dE9} Alzheimer's Disease Model Mice

Authors: Kurt Stover¹, Richard Brown²

Affiliation: 1-2 Dalhousie University

Abstract: Mouse models of Alzheimer's disease are often tested for learning and memory deficits using visuo-spatial tasks such as the Morris water maze. Performance on these tests is dependent on visual ability, which may be confounded in the APP^{swe}/PS1^{dE9} mouse model of Alzheimer's disease as they have amyloid beta plaques in their retinas and evidence of retinal degeneration. We studied the relationship between visual ability, learning and memory in these mice. Both young (5-8 months old) and old (20-27 months) transgenic mice performed significantly worse on the visual acuity task in the visual water box than their wildtype litter mates and old mice performed significantly worse on all measures of vision than young mice. There was no difference in performance in the Morris water maze between young transgenic and wildtype mice, but old transgenic mice had deficits in visuo-spatial learning and memory on the Morris water maze. There were no significant differences between transgenic and wildtype mice on the non-visually dependant conditioned odour preference or the taste aversion tasks. Visual ability was significantly negatively correlated with age and learning and memory in the Morris water maze but not in the non-visually dependant tasks. Performance in the Morris water maze was significantly negatively correlated with age, but performance on the non-visually dependant tasks was not correlated with age. These results indicate that the APP^{swe}/PS1^{dE9} mice have an age related deficit in visual ability, and indicate that visually dependant measures alone should not be used to assess age related learning and memory deficits.

Funding: NSERC & Alzheimer's Association

Title: Developing a therapeutic model for TARDBP mutations in ALS using zebrafish

Authors: Alexandra Lissouba¹, Edor Kabashi², Nathalie Champagne³, Edna Brustein⁴

Affiliation: 1-4 Département de pathologie et biologie cellulaire, Université de Montréal

Abstract: Amyotrophic lateral sclerosis (ALS) is a rapidly progressing neurodegenerative disease

affecting the neuromuscular system and leading to a lethal paralysis in 2-5 years. 38 mutations of the gene TARDBP, coding for the RNA binding protein TDP-43, have been identified in ALS patients. To assess the biopathology and therapeutic treatment of these mutations, we use the zebrafish as a genetic model. We have generated two stable transgenic lines expressing either the wild-type human TARDBP gene or its ALS-related mutation G348C. We used eGFP linked to a cardiac promoter as a reporter gene. Our genes of interest were Myc-tagged and placed under the control of the heat-shock inducible Hsp70 promoter, to control the time of the expression and its level. Heat-shocking embryos at 18 hours post-fertilization at 38.5°C for 30min was sufficient to induce ubiquitous expression of TDP-43 in both lines, as assayed by RT-PCR, anti-Myc immunoblotting and immunolabelling. The induced mutant embryos showed significantly shorter and hyperbranched motoneuron axons compared to the control embryos, as shown by anti-SV2 immunolabelling. Moreover, we have assayed the motor behavior of the embryos using the touch-evoked escape response. The mutant embryos responded to the stimuli, but more than 60% were unable to swim away, displaying weak and uncoordinated muscular contractions. In a preliminary screen of compounds used to treat neurological disorders, we have identified several which partially rescue the mutant phenotype. These results indicate that zebrafish expressing human TARDBP mutations is a promising model for understanding ALS biopathology and advancing therapeutic drug discovery.

Funding: Génome Québec, CIHR, ALS society of Canada, Frick Foundation for ALS Research, US Department of Defense.

Title: Disinhibition unmasks spontaneous and synchronous activity across spinal lamina I: an in vivo functional imaging study

Authors: Sophie Laffray¹, Stéphane Pagès², Paul De Koninck³, Daniel Côté⁴, Yves De Koninck⁵

Affiliation: 1-5 Centre de Recherche Université Laval Robert-Giffard

Abstract: Neuropathic pain is a widespread, highly debilitating condition commonly resulting from injury to peripheral nerves. Spinal lamina I is one of the main nociceptive ascending pathways and, in contrast to lamina V, has neurons that present little or no spontaneous activity. Yet spontaneous neuropathic pain requires ongoing or episodic activity in nociceptive pathways. Here, we investigated the possibility that suppression of inhibition mediated by GABA and glycine receptors in the dorsal horn transforms lamina I neuron networks from silent to

displaying spontaneous activity. Optical recording of network activity by monitoring Ca²⁺ dynamics in live animals has however remained a major challenge due to breathing and cardiac movements. To overcome this limitation, we used a fast, non-contact adaptive movement compensation approach, allowing real-time functional imaging from intrinsically moving tissue. The strategy involves enslaving the position of the microscope objective to that of the tissue surface in real-time. In adult rats, superficial dorsal horn neurons were loaded with a calcium dye. Under control conditions, almost no neuron from lamina I neither displayed spontaneous nor evoked activity. After blocking GABA_A and glycine receptor-mediated transmission, spontaneous activity occurred within most of the neurons in the field of view and high levels of synchronicity occurred between cells even in absence of peripheral stimulation. This activity can logically be interpreted as noxious at the supraspinal level. Our in vivo network activity mapping data thereby suggests that disinhibition of spinal lamina I represents a likely substrate of spontaneous pain characteristic of neuropathic pain.

Funding: Funded by CIHR, NSERC, FRSQ, CIPI

Title: Pathological features of ALS/FTLD in transgenic mice produced with genomic fragments encoding wild-type or mutant forms of human TDP-43

Authors: Vivek Swarup¹, Christine Bareil², Daniel Phaneuf³, Janice Robertson⁴, Jasna Kriz⁵, Jean-Pierre Julien⁶

Affiliation: 1-3,5,6 Centre de Recherche du CHUQ 4- Centre for Research in Neurodegenerative Diseases University of Toronto

Abstract: Objectives : Neuronal cytoplasmic and intranuclear aggregates of RNA-binding protein TDP-43 are a hallmark feature of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Frontotemporal lobar degeneration (FTLD). Mutations in TDP-43 are known to cause both the familial and the sporadic form of ALS in about 3% of patients. The goal of this study is to overexpress two such TDP-43 mutations - A315T and G348C and the wild-type human TDP-43 protein in transgenic mice, thereby modeling the human disease. Generation of Transgenic Mice: TARDBP (NM_007375) was amplified by PCR from a human BAC clone (clone RPCI-11, clone number: 829B14) along with the endogenous promoter (~5kB). Site-directed mutagenesis using the primer-based approach was used for the generation of mutants (TDP-43A315T and TDP-43G348C). Results: TDP-43 mice developed abnormal peripherin mRNA

splicing and peripherin aggregates reminiscent of those found in human ALS. One such neurotoxic peripherin splice variant, Per61, was highly upregulated in TDP-43G348C mice. The TDP-43 mutant mice also exhibit alterations in neurofilament organization with ensuing age-related reduction in axon calibre. The TDP-43G348C and TDP-43A315T mice and to a lesser extent the TDP-43Wt mice exhibited during aging impaired learning and memory capabilities as well as motor dysfunction. Analysis of the brain and spinal cord sections from TDP-43 transgenic mice showed ubiquitin positive TDP-43 inclusions and microgliosis/astrogliosis. These novel TDP-43 transgenic mice mimic several aspects of the pathological and biochemical features of human ALS/FTLD and they should provide valuable animal model for testing therapeutic approaches. **Funding:** This work was supported by the Canadian Institutes of Health Research (CIHR).

Title: Characterization of prostaglandin E2 receptors (EP 1-4) in CNS during experimental autoimmune encephalomyelitis

Authors: Sukhdev Kamboj¹, Jennifer Berard²

Affiliation: 1-2 Research Institute of the McGill University Health Center

Abstract: Prostaglandin E2 (PGE2) is known to play an important role in the regulation of pro and anti-inflammatory mechanisms in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of Multiple Sclerosis. PGE2 mediate these effects by binding to four different G-protein coupled receptors namely EP1-4. Though these receptors are well characterized in the periphery, their distribution in the CNS during EAE is largely unknown. In the present study, we characterize the expression of PGE2 receptors in relapsing-remitting EAE. RR-EAE was induced in C57/BL6 mice by subcutaneous injection of MOG30-35 in complete Freund adjuvant along with pertussis toxin. Spinal cords were obtained at onset, peak and remission stages of EAE for RT-PCR, Western blotting, FACS and double immunofluorescence labelling. EP (1, 2 & 4) receptors showed alterations in mRNA and proteins levels at onset and peak phase of EAE, and restored to normal levels by the remission phase. No changes were seen in EP3 receptor mRNA expression. FACS analysis revealed that the PGE2 receptors were predominantly expressed in CD11b+/Gr1+ monocytes/neutrophils and CD11b+/Gr1- macrophages/microglia. A smaller population of cells that expressed the EP receptors also co-expressed T and B lymphocyte markers. Double immunofluorescence labelling revealed robust expression of EP receptors in infiltrating immune cells and GFAP+ astrocytes adjacent to EAE

lesions. These data show that most of expression of PGE2 receptors is observed in infiltrating immune cells and astrocytes and suggest that these receptor-mediated pathways in these cells may modulate the inflammatory response during EAE.

Funding: "Multiple Sclerosis Society of Canada; CIHR Neuroinflammation Training Program "

Title: Influence of myelin phagocytosis on macrophage polarization

Authors: Antje Kroner¹, Samuel David²

Affiliation: 1-2 Centre for Research in Neuroscience, Research Institute of the McGill University Health Centre

Abstract: Macrophages are important members of the innate immune system function in immune surveillance, phagocytosis of dead cells and pathogens, and maintain tissue homeostasis. They are activated following injury to the central nervous system and in neurodegenerative and autoimmune diseases. However, macrophages and microglia appear to have both detrimental and beneficial effects after CNS injury. The tissue environment is known to influence macrophage polarization towards different phenotypes, frequently described as M1 and M2. M1 polarization includes the production of nitric oxide and cytotoxic pro-inflammatory cytokines, while M2 macrophages are viewed as anti-inflammatory with a strong effect in wound healing and tissue repair. Previous studies have shown that myelin phagocytosis reduces secretion of pro-inflammatory cytokines. In this study, we used flow cytometry to investigate the influence of myelin phagocytosis on macrophage polarization towards M1 or M2 phenotypes in vitro. Our results indicate that myelin phagocytosis by bone marrow derived macrophages stimulated with IFN-gamma + LPS or LPS alone induces the expression of the M2 markers CD204 (scavenger receptor), arginase-1 and CD206 (mannose receptor). Under the same conditions, the M1 markers CD16/32 and TNF-alpha were expressed at lower levels. Interestingly, myelin phagocytosis also increased the secretion of nitrite, a marker for nitrite oxide production, a possible indicator of oxidative burst during phagocytosis. Taken together, these results indicate that myelin phagocytosis induces a shift towards an M2 phenotype.

Funding: CIHR operating grant to SD CIHR postdoctoral fellowship to AK

Title: Functional characterization in zebrafish of the WNK1/HSN2 isoform causing a human peripheral neuropathy

Authors: Valerie Bercier¹, Edna Brustein², Meijiang Liao³, Patrick Dion⁴, Guy A. Rouleau⁵, Pierre Drapeau⁶

Affiliation: 1-3,6 University of Montreal 4,5 Centre for Excellence in Neuroscience of the University of Montreal (CENUM)

Abstract: Human sensory and autonomic neuropathy type 2 (HSN2) is a rare human hereditary disease characterized by an early onset severe sensory loss due to autosomal recessive mutations confined to exon HSN2, an alternatively spliced exon conferring nervous system specificity to the WNK1 (with-no-lysine protein kinase 1) serine-threonine kinase. Little is known about the role of WNK1 in the nervous system. We hypothesized that the truncating mutations present in the HSN2 exon lead to a loss-of-function of the WNK1 kinase impairing development of the peripheral sensory system. In order to investigate the mechanisms by which the lack of the WNK1/HSN2 isoform acts to cause HSN type 2, we examined its expression pattern in zebrafish and observed strong expression in neuromasts of the peripheral sensory lateral line system. We then knocked down the HSN2 exon in zebrafish embryos using antisense morpholino oligonucleotides. Our three approaches to knockdown the WNK1/HSN2 isoform led to embryos with a defective lateral line as well as a depopulation of spinal cord sensory neurons. The potassium chloride co-transporter KCC2 is a target of WNK1 and it also has a role in promoting neurogenesis. Semi-quantitative RT-PCR showed an increased expression of KCC2 in WNK1/HSN2 knockdown embryos and overexpression of human KCC2 RNA in embryos led to an impaired mechanosensory lateral line system, as with the WNK1/HSN2 knockdown. We propose that WNK1/HSN2 regulates the level of expression and activation of KCC2 in sensory neural progenitor cells, where it is expressed in a timely manner to promote differentiation into mature neurons.

Funding: Funded by the CIHR (G.A.R) and the GRSNC of the FRSQ (P.D)

Title: Defective neuromuscular transmission in zebrafish expressing human TARDBP (TDP-43) with a mutation related to ALS and FTLN.

Authors: Gary Armstrong¹, Pierre Drapeau²

Affiliation: 1-2 Université de Montréal

Abstract: Mutations in the TARDBP gene encoding TDP-43 have been found in ALS and FTLN patients. Although several animal models for these TDP-43 mutations have been described, none have characterized the pathophysiological deficits that underlie the resulting phenotype. To

advance our understanding of the pathogenicity of these mutations we used a zebrafish model previously described by our lab in which we transiently expressed mutant human TARDBPG348C as well as wildtype TARDBPWT mRNA in zebrafish larvae. Following over expression of mutant but not WT TDP-43 we observed specific deficits at the neuromuscular junction (NMJ). Immunohistochemistry revealed hyperbranched motor neuron endings with AChR clusters at NMJs in fish expressing TARDBPG348C but not TARDBPWT or WT fish. We next examined spontaneous miniature endplate currents (mEPCs) at the NMJ. Two striking features were observed: the amplitude of mEPCs was significantly larger and occurred at a lower frequency in fish expressing mutant (but not WT) TARDBPG348C. We next evoked swimming-related activity and observed normal rhythmic EPCs at NMJs from fish expressing TARDBPWT. In contrast, fish expressing TARDBPG348C displayed a slower frequency of rhythmic EPCs with shorter bouts of swimming-related activity. In paired recordings action potentials were elicited in motor neurons while recording EPCs in the muscle. We observed an impairment in the fidelity of sustained synaptic transmission. These data represent the first electrophysiological description of TARBP-related mutations and indicate that over branched motor neurons form NMJs each with exaggerated spontaneous release and overall weaker sustained release at the NMJ.

Funding: NSERC/CRSNG

Title: CNS remyelination requires iron delivered from astrocytes

Authors: Katrin Schulz¹, Nancy Andrews², Magdalena Goetz³, Samuel David⁴

Affiliation: 1, 4 The Research Institute of the McGill University Health Centre 2- Duke University School of Medicine 3- HelmholtzZentrum Munich, German Research Center for Environmental Health

Abstract: Iron plays a role in myelination because it is a cofactor for enzymes involved in lipid and cholesterol synthesis, and in energy metabolism in oligodendrocytes. We investigated whether oligodendrocytes acquire iron for myelination from astrocytes. Since astrocytic processes form endfeet around blood vessels, they are ideally located to take up iron from the circulation and to distribute it to other CNS cells. We hypothesize that astrocytes deliver iron to oligodendrocytes for remyelination to occur. We generated mice that lack the expression of the iron exporter ferroportin (Fpn) specifically in astrocytes, and induced localized demyelination by intraspinal lysophosphatidylcholine (LPC) injections into the dorsal column white matter. Ten days after LPC

injections, remyelination was analyzed by electron microscopy. Quantification of the g-ratio (axon diameter/fiber diameter) revealed reduced remyelination in the astrocyte-specific Fpn knockout mice as compared to wildtype controls. Double immunofluorescence analysis showed that proliferation of oligodendrocyte precursor cells is reduced in the knockout animals, which likely contributes to impaired remyelination. We further investigated whether lack of iron affects the ability of microglia to express cytokines involved in remyelination, such as TNF α and IL-1 β . Iron-deficient microglia in culture express reduced levels of these cytokines. This in turn could affect the ability of astrocytes to produce growth factors required for myelination, as astrocytes expressed high levels of bFGF in response to IL-1 β , and IGF-1 in response to TNF α stimulation. These data suggest that iron efflux from astrocytes is required for remyelination by either direct effects on oligodendrocytes or indirectly by affecting microglial activation.

Funding: This work was funded by a CIHR grant. KS is funded by a studentship from the MS Society of Canada.

Title: The involvement of lipocalin-2 in experimental autoimmune encephalomyelitis and multiple sclerosis.

Authors: Jennifer Berard¹, Nathalie Arbour², Alexandre Prat³, V. Wee Yong⁴, Samuel David⁵

Affiliation: 1,5 McGill University Health Center 2,3 Université de Montréal 4- University of Calgary

Abstract: Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS). Several of the pathological features of MS are shared by the animal model experimental autoimmune encephalomyelitis (EAE). EAE pathogenesis involves numerous cell types, cytokines/chemokines, and adhesion molecules. It is likely, however, that many immune mediators remain undiscovered. To identify novel immune mediators, we performed an Affymetrix gene array on the spinal cords of animals at the onset of disease. One of the most highly upregulated was lipocalin-2 (Lcn2). Lcn2 is an iron-binding protein involved in iron transport, apoptosis, and glial activation. We therefore assessed the expression and role of Lcn2 in the pathogenesis of a MOG-induced model of EAE. Western blot analysis of spinal cord revealed that Lcn2 protein expression was increased at all stages of EAE, being maximal at the peak of disease. Lcn2 was expressed primarily in CD11b⁺/Gr-1^{low}/CD45⁺ monocytes, as well as in reactive astrocytes. Additional Western blotting revealed that the Lcn2 receptor, 24p3R, was present in the naïve spinal cord, but shifted to a lower molecular weight form during disease. Additional FACS

analysis revealed that 24p3R was expressed by monocytes, as well as by CD11b+ macrophages/microglia. In addition, EAE severity was increased in Lcn2^{-/-} mice relative to wildtype controls. Importantly, Lcn2 expression in MS patients also exhibited changes when compared to healthy controls. These data reveal that Lcn2 plays a protective role in EAE and suggest that Lcn2 may also be involved in the pathogenesis of MS.

Funding: CIHR Operating Grant, CIHR Neuroinflammation Training Program Fellowship

Title: Chronic food restriction increases cue-induced heroin seeking in rats.

Authors: Tracey D'Cunha¹, Firas Sedki², Josie Macri³, Cristina Casola⁴, Uri Shalev⁵

Affiliation: 1-5 Concordia University

Abstract: Previous research with an animal model of relapse has shown that acute food deprivation will increase drug-seeking behaviours. Recent evidence in humans, however, suggests that chronic food restriction, not acute food deprivation, is related to increases in drug taking, emphasizing a need for an animal model to elucidate the neural mechanisms mediating the effects of chronic food restriction on drug-seeking behaviour. Here we report that a mild chronic food restriction induced an increase in cue-induced heroin seeking behaviour. Male Long-Evans rats were trained to self-administer heroin for 10 consecutive days in operant boxes. Following heroin training rats were housed in the animal colony for 14 days with either free access to food or a mild restricted diet (~80% of sated rats' body weight). In experiment 1, rats were tested under extinction conditions in the operant boxes following 14 days of abstinence. In experiment 2, food restricted rats were given 24 hours of free access to food on day 15 of the abstinence period, and drug seeking was assessed on the next day under extinction conditions. Food restricted rats demonstrated a robust increase in cue-induced heroin seeking compared to the sated rats. However, when rats with a history of food restriction were satiated during testing, there was no difference in drug-seeking behaviour compared to continuously sated rats. Future studies will explore the brain mechanisms underlying this chronic food restriction-induced increase in incentive salience of drug cues.

Funding: provided to Dr. Uri Shalev from NSERC, FRSQ and CRC.

Title: The role of the MYD88-dependent pathway in MPTP-induced brain dopaminergic degeneration

Authors: Janelle Drouin-Ouellet¹, Melanie Bousquet², Claire Gibrat³, Martine Saint-Pierre⁴, Jasna Kriz⁵, Frédéric Calon⁶, Denis Soulet⁷, Francesca Cicchetti⁸

Affiliation: 1-8 Laval University

Abstract: "Myeloid differentiation primary response gene (88) (MyD88) is the most common adaptor protein implicated in toll-like receptor (TLR) signaling pathways leading to inflammation, a phenomenon suspected to play a critical role in the pathophysiology of Parkinson's disease but for which the key players have not clearly been identified. To specifically address this question, we subjected MyD88^{-/-} mice to a 7-dose regime of MPTP administered over a 5-day period and observed a deleterious role of MyD88 in the MPTP-induced myenteric nervous system dopaminergic degeneration via infiltrating macrophages. In the brain, HPLC analyses demonstrated that MyD88^{-/-} mice are as vulnerable to dopamine and DOPAC striatal depletion as wild type mice ($p < 0.001$). This was accompanied by similar dopamine transporter loss ($p < 0.001$), as assessed by [125I]-RTI autoradiography, a decrease in tyrosine hydroxylase levels in the striatum (Western blot, $p < 0.001$), as well as dopaminergic cell loss in the substantia nigra pars compacta (SNpc; $p < 0.05$). Microglial density analysis, performed by stereological counts of Iba-1+ cells, revealed a modest microglial response in the SNpc, accompanied by an astrocytic reaction in the striatum, which was of similar magnitude both in wild type and MyD88^{-/-} mice (Western blot, $p < 0.001$). Unlike our observations in the ENS, these results suggest that the MyD88-dependent pathway is not involved in the MPTP-induced dopaminergic degeneration leading to parkinsonism in the CNS, at least not when exposed to a mild inflammation. This could be due, in part, to different infiltrating processes occurring in the brain as a consequence of the blood-brain barrier."

Funding: "Canadian Institute of Health Research (CIHR); Canadian Foundation for Innovation (CFI)"

Title: Enhanced motor cortex excitability and improved motor performance during high frequency stimulation of the subthalamic nucleus in a rat model of Parkinson's disease

Authors: Andrew Brown¹, G Campbell Teskey², Michael Antle³, Bin Hu⁴

Affiliation: 1-4 University of Calgary

Abstract: Loss of frontal neocortical activation is one of the main neurophysiological abnormalities of Parkinson's disease (PD) and can be observed in rodent models of nigrostriatal degeneration. High-frequency deep brain stimulation (DBS) of the subthalamic nucleus improves

motor deficits in PD. However, it is unknown whether this general therapeutic effect is associated with a restoration of frontal output function. To address this question, chronic stimulating electrodes were implanted bilaterally into the subthalamic nuclei of adult rats that received either bilateral intrastriatal 6-hydroxydopamine (6-OHDA) or vehicle infusion to induce nigrostriatal degeneration. Forelimb use and locomotor activity was assessed based on the cylinder and open field tests in intact, post-lesion+sham DBS, and post-lesion+DBS conditions. Intracortical microstimulation was then used to probe frontal output function of forelimb motor areas. DBS was found to improve motor deficits arising from 6-OHDA lesions, increase forelimb map area, and decrease movement thresholds relative to baseline. These effects were significantly greater in 6-OHDA lesion rats compared to vehicle controls. Results indicate that enhanced motor cortex activation takes place during Subthalamic DBS following dopamine depletion in a rodent model of PD.

Funding: NSERC, CIHR, AI-HS

Title: The Role of Prefrontal Cortex Dopamine and Glutamate Transmission in Acute Food Deprivation-Induced Reinstatement of Heroin Seeking

Authors: Stephanie Tobin¹, Firas Sedki², Uri Shalev³

Affiliation: 1-3 Concordia University

Abstract: "Addiction is a chronic disease characterized by periods of abstinence and relapse. Amongst human drug abusers functional magnetic resonance imaging studies have consistently shown an association between prefrontal cortex (PFC) activation and drug craving. Moreover, the reinstatement model, an animal model of relapse, has been used to demonstrate the involvement of PFC dopamine (DA) in drug seeking. For instance, DA infusion into the prelimbic cortex has been shown to reinstate cocaine seeking; whereas, blockade of DA receptors in the medial PFC has been shown to attenuate both priming and stress-induced reinstatement. Furthermore, PFC glutamatergic transmission has been shown to regulate nucleus accumbens and ventral tegmental area DA release, suggesting an important role for glutamate receptors in reinstatement. Glutamate receptors have also been strongly implicated in the maladaptive learning associated with chronic drug seeking. Here, we study the potential involvement of DA D1 receptors and glutamate receptors in acute 24h food deprivation (FD)-induced reinstatement of heroin seeking behavior, an alternative model of stress-induced reinstatement. We found that acute FD-induced

reinstatement is suppressed following infusion of a high (2.0 ug/side), but not low (0.2 ug/side) dose of the DA D1-receptor antagonist, SCH 23390, into mPFC. We also report a reduction in PFC GluA2 receptor subunit expression in rats which have been trained to self-administer heroin and subsequently exposed to acute FD. Taken together, the current experiments indicate a role for both PFC dopamine and glutamate transmission in acute FD-induced reinstatement."

Funding: Uri Shalev CFI, CRC and NSERC Stephanie Tobin CIHR

Title: NMDAR receptor subtypes underlying modulation of prepulse inhibition in rats

Author: Susanne Schmid

Affiliation: University of Western Ontario

Abstract: Prepulse inhibition (PPI) of the acoustic startle response is a paradigm commonly used as an operational measure of sensorimotor gating, a pre-attentive process which is disrupted in schizophrenic patients. NMDA antagonist-induced PPI disruption has become a model for the study of the cognitive symptoms of schizophrenia and sensorimotor gating deficits. However, the mechanism by which NMDA antagonists exert their effects has yet to be elucidated. Evidence suggests that medial prefrontal cortex (mPFC) lesions occlude the PPI disrupting effects of systemically administered NMDA antagonists. Furthermore, NMDA receptors are expressed by the startle mediating neurons of the caudal pontine reticular nucleus (PnC). This study aims to examine the possible roles of the mPFC and the PnC in NMDA antagonist-induced prepulse inhibition deficits in rats. Sprague-Dawley rats received local injections of MK-801, ifenprodil, or vehicle (0.9% saline) either to the mPFC or the PnC. Both MK-801 and ifenprodil caused potent disruption of PPI when administered to the dorsal region of the mPFC (prelimbic cortex), however administration of MK-801 and ifenprodil to the ventral mPFC (lower prelimbic cortex or infralimbic cortex) had no significant effect on PPI. When administered to the PnC, MK-801 and ifenprodil had no effect on PPI. Current and future studies aim to dissociate the seemingly variable effects of NMDAR antagonists depending on dorsoventral extent in the mPFC with respect to their effect on PPI, and to evaluate the mechanisms by which NMDAR affect modulation of PPI in the mPFC in order to potentially better understand the pathophysiology of schizophrenia.

Funding: This study was funded by OMHF and NSERC

Title: Neurorescue properties of cystamine following MPTP-induced parkinsonism in mice

Authors: Claire Gibrat¹, Mélanie Bousquet², Karl Gué³, Martine Saint-Pierre⁴, Frederic Calon⁵, Claude Rouillard⁶, Francesca Cicchetti⁷

Affiliation: 1-7 CRCHUL

Abstract: We have recently shown that cystamine has beneficial effects in parkinsonian animals when administered prior to the toxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The present study was designed to determine if cystamine, in addition to its neuroprotective action, could overturn an initiated neurodegenerative process. Mice were subcutaneously exposed to MPTP and administered 10 mg/kg of cystamine i.p. daily either 1) 2 days before the start of MPTP injections or 2) 24 hours after the last MPTP dose, and which continued for 14 days post injury.

Administration of cystamine to MPTP-treated mice commencing 24 hours after the impairment of the nigrostriatal system, induced a significant recovery of the number of nigral DAergic neurons. However, cystamine treatment did not have significant beneficial effects on striatal levels of DA, DOPAC and HVA 14 days after MPTP intoxication, suggesting a potential discrepancy of cystamine's properties within the nigro-striatal pathway. The pre-MPTP cystamine treatment corroborated our previous work, ascribing neuroprotective properties to this molecule. The beneficial action of cystamine was more specifically associated with an upregulation of the anti-apoptotic factor Bcl2 in the ventral mesencephalon and with increased levels of the protein BDNF in the striatum ($p < 0.05$). These factors may halt of the apoptotic process that occur in this parkinsonian model and may thus account for the beneficial effect of cystamine post-injury. Although the demonstration of cystamine's neuroprotective action is of great value, neurorescue properties of this molecule will be of immense clinical value for early stage pharmacologic intervention.

Funding: This study was supported by the Fondation Canadienne pour l'innovation, the Canadian Institutes of Health Research

Title: High-Fat Diet, Exercise, and Altered Cerebrovascular Function in a Mouse Model of Alzheimer's Disease

Authors: Jenna Boulanger¹, Marielle Young-Bernier², Steffany Bennett³, Claude Messier⁴

Affiliation: 1-4 University of Ottawa

Abstract: "The CRND8 mouse model of Alzheimer's Disease (AD) is particularly relevant as it

contains a double-mutated form of the APP gene. As such, CRND8 mice show one of the most rapid progressions towards amyloidogenesis. Diet and exercise are well-known modulators of cerebral health and function. It is unclear whether the angiogenic effect of exercise is positive in AD, as angiogenic vessels are not as effective as longstanding vessels. A diet rich in lipids also has an angiogenic effect, as hypoperfusion and inflammation are also known to stimulate the formation of new blood vessels. A pilot study examined the relationship between diet, exercise, and angiogenesis more closely in CRND8 mice. While no significant differences in cognitive function and plaque burden were found between groups, animals exposed to a high-fat diet showed increased von Willebrand factor expression, though no blood vessels were exclusively stained with CD105 and not also with CD34. Immunohistochemistry also revealed beta-amyloid (A β) build-up along the walls of the blood vessels of the transgenic animals. This condition is known as cerebral amyloid angiopathy (CAA). Evidence of CAA is demonstrated in wildtype mice used as familial controls and in age-matched C57BL/6 mice, genetic ancestors to the CRND8 mouse. No A β build-up was found along the vessels of CD-1 mice, genetically unrelated to the CRND8 mouse. It was thus hypothesized that cerebrovascular function of the entire CRND8 mouse line may be compromised. This compromised function seems to be exacerbated by a high-fat diet but rescued by exercise."

Funding: NSERC Graduate Scholarship and CIHR Training Program in Neurodegenerative Lipidomics Graduate Supplement Scholarship to J.B.

Title: Role of the Receptor for Advanced Glycation End-Products (RAGE) in the Onset of Diabetic Autonomic Neuropathy

Authors: Andrew Chandna¹, Veronica Campanucci²

Affiliation: 1-2 University of Saskatchewan

Abstract: "People with diabetes often suffer from complications of the autonomic nervous system resulting in conditions such as cardiac arrhythmias, myocardial infarction, orthostatic hypotension, gastrointestinal abnormalities, and urinary tract problems. To understand the mechanisms underlying these autonomic complications in diabetes, a recent study found that diabetic mice showed a depression in autonomic synaptic transmission that leads to the onset of autonomic neuropathy. These effects were mediated by hyperglycemia-induced oxidative conditions that lead to the inactivation of postsynaptic nicotinic acetylcholine receptors (nAChRs)

(Campanucci et al., 2010). Hyperglycemia was found to induce the accumulation of reactive oxygen species (ROS) in autonomic neurons (Campanucci et al., 2010); however, the site where these species are generated remains unknown. In this study, we hypothesize the missing link is the receptor for advanced glycation end-products (RAGE), which is up-regulated during hyperglycemia triggering the accumulation of ROS. ROS accumulation in turn leads to nAChRs inactivation and the onset of autonomic neuropathy. The main purpose of this project was to determine the role of RAGE in the inactivation of nAChRs in sympathetic neurons in diabetes. To do this we used: 1) the natural RAGE ligands S100 and HMGB-1 proteins and 2) a blocking function antibody against RAGE. Our findings show that both S100 and HMGB-1 induce the inactivation of nAChRs in sympathetic neurons. Interestingly, we also found that hyperglycemia-induced inactivation of nAChRs is significantly prevented by the RAGE function blocking antibody. This study suggests for the first time a pivotal role for RAGE in diabetic autonomic neuropathy. "**Funding:** NSERC grant to Dr. Veronica Campanucci and the Biomedical Undergraduate Summer Research Scholarship to Andrew Chandna

Title: Tau is hyperphosphorylated in type 2 diabetes mouse model

Authors: Noura El Khoury¹, François Marcouiller², Françoise Morin³, Emmanuel Planel⁴

Affiliation: 1, 2, 2 Université Laval 3- Centre de Recherche du CHUL

Abstract: Background: Hyperphosphorylated tau is the major component of paired helical filaments in neurofibrillary tangles found in Alzheimer's disease (AD) brains, and tau hyperphosphorylation is thought to be a critical event in the pathogenesis of the disease since it correlates with the degree of cognitive impairment in AD. Only a small proportion of AD is due to genetic variants, the large majority of cases (~95%) is late onset and sporadic in origin. The cause of sporadic AD is likely to be multifactorial, with external factors interacting with biological or genetic susceptibilities to accelerate the manifestation of the disease. Diabetes mellitus (DM) might be such factor, as there is extensive data from epidemiological studies suggesting that DM is associated with an increased relative risk for Alzheimer's disease. Moreover, there is increasing evidence supporting a link between insulin signaling dysfunction and AD. Methods: We investigated tau phosphorylation and its mechanisms in db/db mice, a well-established mouse model of type 2 diabetes mellitus. Results: We observed tau hyperphosphorylation in the brain of db/db mice, and we show that this hyperphosphorylation is probably due to a deregulation of

specific kinases/phosphatases activities. Conclusions: This study reports that diabetes induces AD-like tau hyperphosphorylation in the mouse brain, with biochemical and regional patterns resembling those in early AD brains. This research will help understanding the link between diabetes and AD, for the development of future treatments or life style strategies destined to check the advance of the disease.

Funding: IRSC, FRSQ, NSERC

Title: Effects of chronic food restriction on Fos immunoreactivity in the Nucleus Accumbens and Amygdala in heroin seeking rats

Authors: Firas Sedki¹, Tracey D'Cunha², Shady Awadallah³, uri shalev⁴

Affiliation: 1-4 Concordia University

Abstract: Chronic food restriction (FR) is a commonly used manipulation shown to enhance drug seeking behavior in an animal model of drug relapse. Previous research in our laboratory revealed an increase in cue-induced heroin seeking behavior in rats with a history of chronic FR in comparison to sated controls. Identification of brain sites implicated in this effect, is integral for a better understanding of the neuronal mechanisms involved in relapse. Here, male Long-Evans rats were trained to self-administer heroin for a total of 10 days, in operant chambers. Following self-administration training, rats were moved to the animal colony and subjected to either 14 days of free access to food (sated group) or a mild chronic FR (FR group), leading to a reduction of 80% of the sated groups body weight. On day 14, rats were returned to the operant chambers and given a 1 hr test under extinction conditions. Rats were sacrificed post-test and the expression of the immediate early gene, c-Fos, an indicator of neuronal activity, was measured using immunohistochemical techniques. Behaviorally, rats in the FR group showed a statistically significant increase in cue-induced heroin seeking behavior in comparison to the sated group. Interestingly, a statistically significant decrease in Fos immunoreactivity in the NAc shell, was revealed for the FR group compared to the sated group. No differences in Fos expression were found in the NAc core, the basolateral amygdala or the central extended amygdala.

Funding: NSERC, FRSQ, CRC

Title: The effect of hemorrhagic stroke on the expression of the extracellular matrix molecule SC1/hevin

Authors: Starlee Lively¹, Lyanne Schlichter²

Affiliation: 1-2 Toronto Western Research Institute 2- Toronto Western Research Institute

Abstract: Intracerebral hemorrhage (ICH), caused by blood vessel rupture in the brain, represents 15-20% of all strokes, but results in a higher mortality rate than ischemic stroke with poor prognosis for survivors. In order to effectively develop treatments for this devastating form of stroke, a greater understanding of the changes that occur following the insult is essential. SC1/hevin is an anti-adhesive matricellular molecule involved in plastic processes in the rodent brain. Following neural injury, SC1 is prominent in reactive astrocytes. In the present study, we examined the effect of hemorrhagic stroke on the expression of SC1 in the adult rat striatum. ICH was induced by injecting 0.2 units of collagenase dissolved in 0.5 ul physiological saline into the adult rat striatum. Animals were sacrificed at 1, 3 and 7 days after the onset of ICH. Using immunohistochemistry, the distribution of SC1 protein was examined. SC1 localized to damaged axon bundles bordering the lesion. These damaged axon bundles were positive for both degraded myelin and APP, a marker of axonal injury. In the penumbra region, SC1 was also situated in axon bundles that were positive for APP, but not degraded myelin, indicating that axon damage and the disruption of axonal transport preceded the degradation of myelin. In saline-injected control bundles, SC1 localized to APP-positive axon bundles situated along the needle entry site. These findings suggest that in addition to the involvement of SC1 in astrogliosis, SC1 may also play a role in the remodeling of axons following neural injury.

Funding: Work was supported by HSF operating grants to LCS and a CIHR Fellowship to SL.

Title: TRPM7 Activity Stimulates Cofilin Rod Formation after Oxygen Glucose Deprivation via Activation of nNOS and Free Radical Stress.

Authors: Russell Bent¹, Michelle Aarts²

Affiliation: 1,2 University of Toronto Scarborough

Abstract: "Transient Receptor Potential 'Melastatin' 7 (TRPM7) is a ubiquitously expressed, non-selective divalent cation channel that also contains an enzymatic tail. TRPM7 has been implicated in numerous diverse cellular functions including actomyosin cytoskeletal remodelling, calcium and magnesium homeostasis, and anoxic neuronal death. Inhibition of TRPM7 activity and siRNA knockdown has proven to be neuroprotective after oxygen-glucose deprivation (OGD); however, the mechanisms behind TRPM7's physiological roles remain undetermined. The present study

aims to investigate the role of TRPM7 in modulating cofilin-1 (an actin-binding protein) after OGD. Cofilin-1 activation after energy depletion is thought to play a role in cytoskeletal disruption and neurite degeneration, and we hypothesized that TRPM7 could play a role in this process. Using in vitro rat primary cortical cultures and western blotting analysis, we show that siRNA knockdown and pharmacological inhibition of TRPM7 attenuates cofilin activation after OGD. Immunostaining of cofilin rod formation after OGD also showed that TRPM7 activity contributes to the formation of long cofilin-actin inclusions ('cofilin rods'), which are significantly shorter in resting cells. Furthermore, via use of the free-radical scavenger MnTBAP and the neuronal nitric oxide synthase inhibitor L-NAME, we show that TRPM7-mediated activation of cofilin is at least partially reliant upon free radical signalling and nitric oxide synthase activity. This study provides direct evidence for a novel link between ischemic-induced TRPM7 activity and cofilin-1 activation, which suggests an additional mechanism through which TRPM7 activity may be harmful to neuronal cells after OGD."

Funding: Work was supported by

Funding: from the Canadian Stroke Network and the Natural Sciences and Engineering Research Council of Canada.

Title: Chronic inflammation induces changes in the NGF metabolic pathway in the glabrous skin of the rat paw

Authors: Maria Osikowicz¹, Geraldine Longo², Claudio Cuello³, Alfredo Ribeiro-da-Silva⁴

Affiliation: 1-4 McGill University

Abstract: We have previously shown a sprouting of sympathetic fibers into the upper dermis of the skin over the inflamed joints following a subcutaneous injection of complete Freund's adjuvant (CFA) in the hind paw. This sprouting correlates with an increase in pain sensitivity. We hypothesize that the sprouting and pain related behavior are due to the increase in nerve growth factor (NGF) levels. Recent evidence indicates that NGF is a major mediator of chronic pain. It was shown that NGF is secreted from the cells as proNGF, together with a full enzymatic cascade necessary to its conversion into the mature form. Although this is now well established in the CNS (Bruno and Cuello, PNAS, 2006), it has been much less studied in the periphery. In particular, there has never been a study showing the levels of proNGF breakdown-related molecules within the skin over the inflamed joints. In the present study using a mono-arthritis model, we have

observed that intra-articular CFA injection induced sensitization to both mechanical and thermal stimulation. Using immunocytochemistry, we observed an invasion of sympathetic fibres into the upper dermis of CFA-treated animals. In addition, the protein levels of NGF itself, as well as of plasminogen, tissue plasminogen activator and matrix metalloproteinase-9, which are known to play a major role in mature NGF production and degradation, were altered following chronic inflammation. Therefore, modulation of NGF levels would provide an attractive opportunity to develop a novel class of therapeutic agents for the management of inflammatory pain associated with arthritis.

Funding: Funded by CIHR, the Louise and Alan Edwards Foundation and MITACS Accelerate Program

Title: Antagonism of purinergic P2X7 receptor blocks tumor progression in a C6 glioma tumor model

Authors: James McLarnon¹, Nattinee Jantaratnotai², Patrick McGeer³

Affiliation: 1-3 University British Columbia

Abstract: Pharmacological inhibition of ionotropic P2X7R in glioma and microglial cells has been examined for modulatory effects on tumor growth in an animal tumor model. Intrastratial injection of C6 glioma caused marked upregulation of P2X7R immunoreactivity (ir) relative to control vehicle injection. Double staining analysis showed P2X7R ir was co-localized with glioma (LacZ marker) and microglia (OX-42 marker) but not endogenous astrocytes. Administration of the P2X7R antagonist brilliant blue G (BBG) was effective in significantly reducing volume and spatial progression of tumors. BBG treatment had no effects on microgliosis, astrogliosis or vasculature in C6-injected animals. In vitro studies supported animal model data with BBG effective in blocking expression of P2X7R and glioma migratory responses induced by agonist stimulation of cultured C6 cells. The relevance of animal model results to human tumors was demonstrated by a considerably increased level of P2X7R expression in human brain tumor, relative to control, tissue. The overall findings suggest that pharmacological inhibition of ionotropic purinergic P2X7R may have efficacy in slowing the progression and development of brain tumors.

Funding: Pacific Alzheimer Research Foundation

Title: MiRNA regulation of hAPP 3'UTR is affected by Alzheimer specific target site polymorphisms

Authors: Charlotte Delay¹, Claudia Goupil², Sébastien S. Hébert³

Affiliation: 1-3 Laval University

Abstract: Background: It is becoming increasingly acknowledged that polymorphisms in microRNA (miRNA) target sites (PolymiRTS) may influence neurological disorders, including Parkinson's disease and frontotemporal dementia. A number of PolymiRTS in the 3' untranslated region (3'UTR) of the APP gene have recently been identified, some of which are found exclusively in patients suffering from Alzheimer's disease (AD). Aims: Most complex diseases are caused by a combination of genetic risk variants. Given recent findings, we hypothesize that PolymiRTS could contribute significantly to risk for AD by affecting miRNA binding and increasing the expression of genes like APP involved in the amyloid cascade. Methods: Using various bioinformatics algorithms, we established a detailed list of potential miRNA binding sites in the 3'UTRs of APP. The corresponding miRNAs were tested through luciferase reporter assays as well as by Western blotting for their potential to alter APP expression. In order to further establish whether polymorphisms in the 3'UTR of APP affect the function of the identified miRNAs, mutagenesis of the 3'UTR of these genes and subsequent luciferase reporter assays were performed. Results: We have identified novel miRNAs that regulate APP expression. Our results suggest that certain polymorphisms influence miRNA binding and therefore expression. Conclusions: These data could help to focus future association studies aimed at identifying novel risk factors for AD. The identification of novel miRNAs involved in the physiological regulation of APP may provide novel targets for potential future diagnostics and therapy purposes.

Funding: Canadian Alzheimer Society

Title: Seizure tracking in a genetic model of absence epilepsy using simultaneous fMRI / EEG recording.

Authors: Stuart Cain¹, Andrew Yung², Christopher Lapish³, Jenny Chien-Hsin Tso⁴, Terence O'Brien⁵, Piotr Kozlowski⁶, Terrance Snutch⁷

Affiliation: 1-4, 6, 7 University of British Columbia 5- University of Melbourne

Abstract: The Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model displays a well characterized absence epilepsy phenotype. Seizures involve highly synchronized, oscillatory

activity in neurons of the thalamocortical system proposed to originate as rhythmic oscillations in the somatosensory cortex. In this study we aimed to identify the location of seizure initiation and attempted to track seizure progress using simultaneous functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) recording during spontaneous seizures in GAERS. Animals were maintained under fentanyl-haloperidol neurolept analgesia and immobilized with tubocurarine. Echo-Planar imaging (spin-echo and gradient-echo) was used to acquire Blood Oxygen Level-Dependent signal from the level of the forebrain to occipital cortex in vivo. We most frequently observed increased BOLD signal in the primary somatosensory cortex, insular cortices and hippocampus when comparing control vs seizure data. Interestingly, we often saw a decrease in BOLD signal in thalamic regions, potentially as a result of a differing hemodynamic response to other regions of interest. Furthermore, by acquiring other datasets in 500ms repeats we observed BOLD signal changes at high temporal resolution, potentially validating the theory that fMRI might be able to track epileptic seizures. This method of acquiring fMRI at high temporal resolution may provide insights into the initiation and progression of many seizure types, allowing for both a better understanding of the regions involved and for the sites of action of existing and novel anti-epileptic drugs to be elucidated.

Funding: Supported by the Canadian Institutes of Health Research

Title: Understanding the Neuronal Function of MeCP2 Isoforms

Authors: Robby Zachariah¹, Carl Olson²

Affiliation: 1-2 Regenerative Medicine Program, Department of Biochemistry and Medical Genetics, Faculty of Medicine, University of Manitoba, Canada

Abstract: "Rett Syndrome (RTT) is a progressive neurological disease caused by mutations in the Methyl CpG Binding Protein 2 (MECP2) gene. Two MeCP2 protein isoforms exist; E1 and E2, which are both widely expressed. The distinct expression pattern of the two isoforms in the developing brain as well as the distribution of known mutations in *Mecp2* strongly suggests that the two isoforms have non-redundant functions within neurons. To date, RTT has no effective treatment. However, reactivation of the *Mecp2* gene after the onset of disease in mouse models rescues major RTT phenotypes. This raises hope towards RTT gene therapy prospects. Understanding the neuronal function of the MeCP2 isoforms would be critical in developing an efficient gene therapy strategy for RTT. The present study attempts to elucidate the neuronal function of the two MeCP2

isoforms through gene therapy delivery of MECP2 isoforms into Mecp2 deficient neurons. The role of individual MeCP2 isoforms in neuronal differentiation and maturation will be studied via MECP2 isoform-specific lentiviral delivery. Different functional aspects of MeCP2 will be analyzed through this system, including the interaction of individual MeCP2 isoforms with known MeCP2 targets as well as the identification of novel isoform-specific targets. It is believed that the identification of non-redundant functions of the MeCP2 isoforms will further our knowledge on the pathology of Rett Syndrome, thus aiding ongoing efforts to devise an efficient therapeutic strategy for this disease."

Funding: Scottish Rite Charitable Foundation of Canada, CFI, NSERC, MHRC, MICH, University of Manitoba (URGP and Paul H Thorlakson)

Title: Regional and Intranuclear Neuronal Expression of MeCP2 in Mammalian CNS: Insights Into Rett Syndrome.

Authors: Carl Olson¹, Robby Zachariah², Mojgan Rastegar³

Affiliation: 1- Regenerative Medicine Program, Department of Biochemistry and Medical Genetics, Faculty of Medicine, University of Manitoba 3- Regenerative Medicine Program, Department of Biochemistry and Medical Genetics, Faculty of Medicine, University of Manitoba; Department of Immunology, Faculty of Medicine, University of Manitoba"

Abstract: Methyl-CpG-binding protein 2 (MeCP2) is ubiquitously expressed in mammals, and functions as a transcriptional regulator through its unique ability to bind genes at methyl-CpG dinucleotides. Despite a ubiquitous expression, mutations in the gene encoding MeCP2 negatively impacts central nervous system (CNS) function. Loss of MeCP2 is considered to be the primary cause of Rett Syndrome (RTT), a delayed onset neurodevelopmental disorder and an Autistic Spectrum Disorder. Overexpression of MeCP2 also negatively impacts neurological function indicating tight regulation of MeCP2 expression is necessary for proper neural development and activity. Despite global expression of MeCP2 in the CNS, the clinical manifestations of autism and RTT display distinct phenotypes that may be attributed to effects on subregions of the brain. Further, MeCP2 expression is coincident with the onset of synaptogenesis and neuronal maturation in the developing brain, and MeCP2 loss of function significantly affects these occurrences. However, a thorough study describing the regional and intranuclear expression of MeCP2 protein throughout the mammalian CNS has not been done to date. Using large scale

microscopy immunofluorescent scanning, we show immunofluorescent detection of MeCP2 in wild-type and MeCP2 heterozygous mouse brain tissue. We describe the regional distribution, intranuclear immunofluorescent intensity variations and labelling of MeCP2 in neuronal subtypes in mouse brain. By studying the expression of MeCP2 protein we aim to elucidate the regional and neuronal relevance of MeCP2 protein to regional and neuronal subtype function. The results of our studies will significantly increase our understanding of the role of MeCP2 in Rett Syndrome.

Funding: Scottish Rite Charitable Foundation of Canada, CFI, NSERC, MHRC, MICH, University of Manitoba (URGP and Paul H Thorlakson)

Title: Selective disruption of dopamine D2-receptors/arrestin signaling by mood stabilizers

Authors: Del'Guidice Thomas

Affiliation: Université Laval-Centre de Recherche Université Laval Robert-Giffard

Abstract: Mood stabilizers are a structurally heterogeneous class of drugs used for the management of bipolar disorder, depression and schizophrenia. Here we show that the mood stabilizers lamotrigine, lithium and valproate exert behavioral effects in mice by disrupting beta arrestin 2-mediated signaling pathways engaging Akt/GSK3 downstream of dopamine seven transmembrane domain receptors (7TM). Disruption of different combinations of arrestin-dependent signaling pathways by mood stabilizers was also associated with distinctive profiles of pharmacological effects in mice. This identifies beta arrestin 2 as a common signaling hub involved in the action of mood stabilizers and suggests that cross-talk between diverse arrestin-mediated signaling pathways may be critical for mood regulation and potentially other functions of 7TMs.

Funding: Akt GSK3 dopamine mood stabilizers Beta-Arrestine2

Title: Role of endothelial brain-derived neurotrophic factor on subventricular zone cells recruitment to the ischemic striatum

Authors: Sofia Grade¹, Yuan C. Weng², Jasna Kriz³, J. O. Malva⁴, Armen Saghatelian⁵

Affiliation: 1- "a. Centre de Recherche Université Laval Robert-Giffard; b. Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal" 2,3- Centre de Recherche du CHUL (CHUQ), T3-67, Université Laval 4- Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal 5- Centre de Recherche Université Laval Robert-Giffard

Abstract: Upon an ischemic stroke, subventricular zone (SVZ) neural stem cells are activated, and a subpopulation of newly generated neuronal progenitors is mobilized to the area of brain damage. These de-routed neuronal progenitors use blood vessels as a physical scaffold for their migration in a manner resembling the constitutive migration of neuronal precursors from the SVZ into the olfactory bulb (OB) via the rostral migratory stream (RMS). In the adult RMS, BDNF released by blood vessels fosters neuroblasts migration through low-affinity BDNF receptor, p75NTR. The mechanisms guiding the injury-induced migration remain, however, elusive. Herein, we demonstrate that striatal blood vessels synthesize BDNF after ischemia and that neuroblasts migrating along blood vessels express p75NTR. Moreover, reactive astrocytes widespread throughout the damaged area ensheat blood vessels and express TrkB, a high affinity BDNF receptor. Our data reveal that astrocytes in the ischemic striatum do not express mRNA for BDNF, but are immunopositive for this trophic factor. Importantly, the same expression pattern was observed in the adult RMS where TrkB-expressing astrocytes trap extracellular BDNF allowing thus entrance of migrating cell into the stationary phase. Using time-lapse imaging in acute brain slices, we characterized the dynamics of neuroblasts migration in the ischemic striatum. Neuroblasts migrating in the ischemic striatum have a higher exploratory behavior and therefore longer stationary periods as compared to cells migrating in the RMS. Our data suggest that cellular and molecular mechanisms involved in the injury-induced migration recapitulate the BDNF-mediated mechanism observed during constitutive migration in the RMS.

Funding: Supported by Canadian Institutes of Health Research (CIHR) and Fundação para a Ciência e a Tecnologia (FCT).

Title: "Stimulation of central IL-1R1 and IL-1 β derived from bone marrow cells are required for induction of experimental allergic encephalomyelitis"

Authors: Sébastien Lévesque¹, Sylvain Nadeau², Steve Lacroix³

Affiliation: 1-3 Université Laval

Abstract: "Several proinflammatory cytokines including IL-1 and TNF are key differentiation factors for autoaggressive Th1 and Th17 lymphocytes and inflammation-mediated damage to myelin. However, MS patients that received anti-cytokine therapies (e.g. anti-TNF) aimed at preventing this damage unexpectedly experienced a worsening of disease, and evidence gathered over the past decade indicates a role for IL-1 and TNF in CNS remyelination. The objective of the

present study is to investigate and clarify the possible dichotomous effects of proinflammatory cytokines, in particular IL-1, during MS. To achieve this goal, we undertook an investigation of experimental allergic encephalomyelitis (EAE) in chimeric mice deficient in various genes of the IL-1 system. In brief, bone marrow cells were injected into γ -irradiated recipient mice (e.g. IL-1R1-KO into WT, IL-1 α -KO into WT, IL-1 β -KO into WT, WT into WT, and vice-versa) and animals injected with MOG35-55 and Pertussis toxin to induce EAE. Mice lacking the IL-1 receptor type 1 (IL-1R1-KO) failed to develop EAE. Interestingly, IL-1R1-KO chimeric mice transplanted with wild-type (WT) bone marrow cells (i.e. WT into IL-1R1-KO) developed less EAE symptoms (and later) than control mice. Similar studies with IL-1 α - and IL-1 β -KO mice revealed that IL-1 α is dispensable for disease development (independently of its origin), whereas IL-1 β -KO into WT bone marrow chimeras are resistant to EAE. These results suggest that peripheral IL-1 β and central IL-1R1 signaling are required for the induction and progression of EAE. IL-1 β is not detectable in serum by ELISA during EAE. We are currently identifying the peripheral cell(s) responsible(s) for IL-1 β production."

Funding: This investigation was supported by a grant and a postdoctoral fellowship from the Multiple Sclerosis Society of Canada.

Title: Deciphering the role of the IL-1 system in spinal cord injury

Authors: Dominic Bastien¹, Isabelle Pineau², Alexandre Paré³, Steve Lacroix⁴

Affiliation: 1-4 Laval University

Abstract: "CNS injury stimulates the expression of several proinflammatory cytokines and chemokines, some of which including CXCL1, CXCL2 and CCL2 act to recruit Gr-1+ leukocytes at lesion sites. While earlier studies have reported that neutrophils and monocytes/macrophages contribute to secondary tissue loss after spinal cord injury (SCI), recent work has shown that depletion of Gr-1+ leukocytes compromised tissue healing and worsened functional recovery. Here, we show that astrocytes contribute to early inflammation at sites of SCI by producing CXCL1, CXCL2 and CCL2 at 3-12 hours, and that this expression is significantly decreased in mice deficient in MyD88 and IL-1R1. We also demonstrate that neutrophil and M1 monocyte recruitment depends on MyD88/IL-1R1 signaling at 12 hours and 4 and 28 days after SCI. Flow cytometry analysis of cells recovered from the spinal cord of MyD88- and IL-1R1-knockout mice confirmed the presence of significantly fewer M1 monocytes and neutrophils at 12 hrs and 4 days post-SCI.

Importantly, IL-1 α and IL-1 β were found to contribute equally to the recruitment of these immune cells. Finally, we found that mice lacking IL-1 α , IL-1 β or IL-1R1 exhibited significantly better locomotor recovery after SCI, as determined using the BMS, BMS subscore and grip walk behavioural tests. Together, these results indicate that the entry of neutrophils and M1 monocytes is regulated through activation of the Myd88/IL-1R1 pathway in the injured mouse spinal cord, and that this innate immune response is likely to contribute to early pathogenesis after SCI. "

Funding: Canadian Institutes of Health Research (CIHR)

Title: Development of a Fusion Protein that Crosses the Blood-Brain Barrier for Detection of Beta-Amyloid by Optical Imaging

Authors: Benoit Leclerc¹, Chantal Gaudet², Michel Menard³, Danica Stanimirovic⁴, Balu Chakravarthy⁵, Abedelnasser Abulrob⁶

Affiliation: 1-6 National Research Council of Canada

Abstract: "Brain accumulation of beta-amyloid, a key pathological feature of Alzheimer's disease, could be used to evaluate disease progression by PET molecular imaging. The objective of the study was to evaluate a fusion protein consisting of a high-affinity (1 nM) beta-amyloid-binding peptide (ABP; 5.6kD) and the single-domain antibody FC5 (13.2kD) selected from phage-display libraries for its ability to transmigrate across the blood-brain barrier (BBB), using in vitro BBB model and in vivo optical imaging techniques. In contrast to a control single-domain antibody EG2, FC5 and FC5ABP fusion protein bands were identified by Western Blot to cross the in vitro BBB model consisting of SV40 transfected immortalized adult rat brain endothelial cells (SV-ARBEC) cultured in the presence of astrocyte-conditioned media. FC5, FC5ABP and EG2 were labelled with the near-infrared fluorescent probe IR800 and conjugates were IV injected at a dose of 1mg/kg into wild-type mice to determine their pharmacokinetic properties. Measured plasma half-lives of FC5 and FC5ABP were 28 minutes and 64 minutes, respectively. Both IV injected 5mg/kg FC5-IR800 and FC5ABP-IR800 were detected in the brain at higher levels than EG2-IR800 as early as 30 minutes, and showed further increase at 60 minutes post-injection when assessed by ex vivo brain imaging following perfusion. The results suggest that the FC5ABP fusion protein crosses the BBB in vitro and in vivo and could potentially be used to detect beta-amyloid accumulation in the brain by using optical imaging technique in appropriate transgenic mice models of Alzheimer's

disease."

Funding: Alzheimer's Association (www.alz.org)

Title: "Comparative interactome analyses of PS1- and PS2-specific $\gamma\gamma$ -secretase complexes reveals distinct molecular environments"

Authors: Amy Hye Won Jeon¹, Christopher Böhm², Fusheng Chen³, Stephen Lu⁴, Peter St. George-Hyslop⁵, Gerold Schmitt-Ulms⁶

Affiliation: 1-3,5,6 University of Toronto 4- University of Cambridge

Abstract: " γ -Secretase is an intramembrane cleaving protease (i-CLIP) that plays a pivotal role in the production of neurotoxic amyloid β -peptide ($A\beta$), the principal component of plaques observed in the brains of individuals afflicted with Alzheimer's disease (AD). γ -Secretase consists of a heterotetrameric core complex of presenilin (PS), nicastrin, anterior pharynx-defective 1 (APH-1), and presenilin enhancer 2 (pen-2) proteins. The human genome contains two presenilin genes which code for 67% identical presenilin 1 (PS1) and presenilin 2 (PS2) proteins. These presenilins harbor the catalytic aspartates required for regulated intramembrane proteolysis and contribute to the assembly of distinct subpopulations of γ -secretases with functional variability. To understand the underlying causes for this dichotomy, in-depth quantitative comparisons of the molecular neighbourhood of γ -secretase complexes containing PS1- or PS2-subunits were undertaken. The study made use of stable integrant clones in HEK cells which code for PS paralogs equipped with N-terminal tandem-affinity purification (TAP) tags, a three-step purification scheme and isobaric labeling of co-purifying peptides for quantitative mass spectrometry based interactome comparisons. The analysis corroborates the previously known composition of γ -secretases and revealed a subset of novel candidate interactors. Consistent with previous data, members of the catenin/cadherin family of proteins were almost exclusively found associated with PS1. Interestingly, another subset of proteins was pre-dominantly found to co-purify with PS2-containing γ -secretase complexes. Current efforts are directed toward the validation of these interactors, which may elucidate the biological significance of their association with γ -secretase and may provide insights into the etiology of AD."

Funding: CIHR, Scottish Rite Foundation of Canada, University of Toronto Fellowships

Title: The effect of ephrin-B3 on the survival of transplanted neural stem/progenitor cells (NSPC) in an adult rat spinal cord injury (SCI) model

Authors: Xin Yan Susan Fan¹, Andrea Mothe², Charles Tator³

Affiliation: 1-3 University of Toronto; Toronto Western Research Institute

Abstract: Purpose: Ephrin-B3 was shown to reduce endogenous NSPC death by inhibiting EphA4 receptors in the mouse brain. This project aims to determine the effect of ephrin-B3 on NSPC survival after SCI. Methods: (1) In cultures of rat spinal cord NSPC and in rat spinal cord sections, we detected the expression of EphA4 receptors. (2) In cultures of NSPC, we tested cytotoxicity of recombinant ephrin-B3-Fc and viability of NSPC after 3 days of ligand infusion. Fc fragments and PBS were used as controls. (3) In 26g clip compression SCI rats, we examined the survival of transplanted NSPC. Ephrin-B3-Fc was delivered intrathecally starting at the time of transplantation for 3 or 7 days. Fc fragments and PBS were used as controls. Results: (1) EphA4 receptors were expressed in cultured NSPC and in healthy and injured rat spinal cord, although expression is lower in the healthy cord. (2) In vitro, ephrin-B3-Fc did not affect NSPC viability except at the highest concentration tested, where viability was significantly reduced. Infusion of Fc also reduced NSPC viability. (3) 3 days after transplantation, all groups contained many live transplanted NSPC. Conversely, 14 days after transplantation, there were no surviving transplanted cells in the ephrin-B3-Fc or Fc groups. At 3 and 14 days, transplanted NSPC and degraded remnants were localized to the sites of injection, and some were in macrophages. Conclusions: Cultured NSPC express EphA4 receptors and can respond to ephrin-B3. However, ephrin-B3 and Fc are toxic to NSPC in vitro and after transplantation in SCI rats.

Funding: Canadian Institute of Health Research, James Crothers Family Fellowships, Unilever/Lipton Neuroscience Fellowship, University of Toronto Institute of Medical Science Award

Title: Neuroprotective effect of activation of G protein-coupled estrogen receptor 1 (GPER1) on MPTP toxicity

Authors: Mélanie Bourque¹, Marc Morissette², Thérèse Di Paolo³

Affiliation: 1-3 Molecular Endocrinology and Genomic Research Center, Centre de recherche du CHUQ (CHUL), and Faculty of Pharmacy, Laval University

Abstract: "The neuroprotective effect of 17 β -estradiol on the nigrostriatal dopaminergic system

against MPTP toxicity has been well documented previously whereas the mechanism implicated remains to be investigated. The G protein-coupled estrogen receptor 1 (GPER1) has been proposed to function as a membrane estrogen receptor (ER) and is highly expressed in the striatum. Using the specific GPER1 agonist G1, with no detectable activity on either classical ERs α or β , we investigated the implication of this receptor in the effect of 17β -estradiol on MPTP toxicity. Intact male mice were treated with 17β -estradiol ($1 \mu\text{g}$, B.I.D.) or with G1 (1 or $5 \mu\text{g}$, B.I.D.) during 10 days and received 4 injections of MPTP (4.75 mg/kg) on day 5. Administration of MPTP decreased striatal dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations and increased HVA/dopamine ratio. Both 17β -estradiol and G1 treatment decreased MPTP toxicity on dopamine and DOPAC concentrations and completely prevent the increase of HVA/dopamine. Autoradiography of striatal dopamine transporter (DAT) and vesicular monoamines transporter (VMAT2) show a reduction in MPTP effect in mice treated with both 17β -estradiol or G1 ($5 \mu\text{g}$). Decreased of DAT specific binding in substantia nigra of MPTP mice was observed whereas 17β -estradiol or G1 ($5 \mu\text{g}$) treated MPTP mice were at control values. VMAT2 specific binding in substantia nigra remains unchanged in MPTP mice as compared to control. These results suggest that G1 has a similar effect as 17β -estradiol and that activation of GPER1 can contribute to the neuroprotective effect of 17β -estradiol on dopaminergic systems against MPTP toxicity."

Funding: Canadian Institutes of Health Research (CIHR) to TDP and Fonds de la Recherche en Santé du Québec (FRSQ) to MB

Title: Increase of 5-HT_{2A} receptor binding in striatum and cerebral cortex of dyskinetic MPTP monkeys

Authors: Golnasim RIAHI¹, Marc Morissette², Martin Parent³, Thérèse Di Paolo⁴

Affiliation: 1,4 Faculty of Pharmacy Université Laval 2- Centre de Recherche du CHUL 3- Centre de Recherche Université Laval Robert-Giffard

Abstract: Levodopa-induced dyskinesias (LIDs) are abnormal involuntary movements induced by the chronic use of levodopa (L-Dopa) limiting the quality of life of Parkinson's disease (PD) patients. In this study, we evaluated changes of the serotonin 5-HT_{2A} receptors in control monkeys, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys, and in L-

Dopa-treated MPTP monkeys, without or with adjunct treatments to inhibit the expression of LID: CI-1041, a selective NR1A/2B subunit antagonist of glutamate N-methyl-D-aspartic acid (NMDA) receptor or Cabergoline, a long acting dopamine D2 receptor agonist. All treatments were administered for one month and euthanasia was done 24 hours after the last dose of L-Dopa. The striatal concentrations of serotonin were decreased in all MPTP monkeys investigated, as measured by high-performance liquid chromatography. [3H]Ketanserin specific binding to 5-HT2A receptors was measured by autoradiography. L-Dopa treatment, that induced dyskinesias, increased 5-HT2A receptors specific binding in the caudate nucleus and the anterior cingulate gyrus (AcgG) compared to control monkeys. Moreover, [3H]Ketanserin specific binding was increased in the dorsomedial caudate nucleus in L-Dopa-treated MPTP monkeys compared to saline-treated MPTP monkeys. Nondyskinetic monkeys treated with CI-1041 or Cabergoline showed low 5-HT2A specific binding in the posterior dorsomedial caudate nucleus and the anterior AcgG compared to dyskinetic monkeys. No significant difference in 5-HT2A receptor binding was observed in any brain regions examined in saline-treated MPTP monkeys compared to control monkeys. These results confirm the involvement of serotonergic pathways and the glutamate/serotonin interactions in LID. They also support targeting 5-HT2A receptors as a potential treatment for LID.

Funding: "CIHR to TDP; FER (Fonds d'enseignement et de recherche) of faculty of Pharmacy, Laval University to GR"

Title: Persistent Changes in Central Stress Response Mechanisms in a Developmental Rat Model of Epilepsy

Authors: Daphne Gill¹, Melissa Perry², Emily McGuire³, Anabel Pérez-Gómez⁴, R. Andrew Tasker⁵,

Affiliation: 1,2,4,5 Atlantic Veterinary College, UPEI 3- Dalhousie University

Abstract: Appropriate stress responses rely on a finely-tuned neuronal balance that must continually adapt to a frequently changing external environment. Alterations in this balance can result in susceptibility to a variety of stress-related disorders, as well as exacerbate already existing conditions, including epilepsy. We have previously reported that rat pups injected with a very low dose (20ug/kg) of domoic acid (DOM) during the second postnatal week of life display low-grade seizure behaviours when challenged with stressful tasks, and also exhibit a variety of structural and functional changes similar to those seen in temporal lobe epilepsy. The current

study was designed to investigate markers of altered stress-response in this model. Following neonatal treatment, adult rats (male and female) were tested in the elevated plus maze, as well as two water maze tasks, both of which involved a platform reversal challenge. Results indicated significantly heightened anxiety, increased perseveration, and alterations in search strategy for all DOM-treated rats, as well as male-specific deficits in cognitive flexibility. In addition, 80% of treated males and 20% of treated females exhibited seizure behaviour. Western blot analysis revealed male-only increases in adrenergic receptor (A2a and A2c) and mineralocorticoid receptor (MR) expression, but no change in glucocorticoid receptor (GR), corticotropin-releasing factor (CRF) receptors I/II, or dopamine D2 receptor expression. A significant decrease in GR:MR ratio was also noted. We conclude that early exposure to DOM alters central mechanisms underlying stress response, and that this model may be valuable for investigating the connection between stress and neurological disorders.

Funding: Supported by the Atlantic Innovation Fund

Title: Identification of a novel JNK-interacting protein as a potential therapeutic target for ischemic stroke

Authors: Max Cynader¹, Guang Yang²

Affiliation: 1,2 Brain Research Centre, UBC

Abstract: Clinical applications of neuroprotection strategies in ischemic stroke have been disappointing due to problems of efficacy and side effects. We have identified a novel interacting protein (NIP) of c-jun N-terminal kinase (JNK) as a key player in acute ischemic brain injury. NIP interacts with JNK and activates downstream neuronal cell death signals in response to pathological stressors that include excitotoxicity. We have developed novel peptides targeting a previous unknown JNK-interacting motif on NIP that block the enhancement of the NIP-JNK interaction induced by excitotoxicity and selectively abolish the activation of JNK isoforms 2 and 3, while leaving JNK1 unaffected. Application of these peptides successfully blocks JNK activation and neuronal cell death pathways, protects cultured neurons from excitotoxicity, and dramatically reduces brain damage and behavioural deficits in a rat model of focal ischemic stroke. The combination of strong neuroprotective efficacy and the potential advantages of isoform- and scenario-selective inhibition of JNK in reducing side effects suggest that targeting this novel interaction of NIP and JNK may be of therapeutic value for ischemic stroke and other neurological

diseases.

Funding: CIHR

Title: "Amyloid beta and NMDA-activation share common pathways that affect tau phosphorylation; implications for Alzheimer's disease"

Authors: Siddhartha Mondragon-Rodriguez¹, Catherine Bourgeois², Jannic Boehm³

Affiliation: 1-3 Universite de Montreal

Abstract: "Extracellular amyloid- β ($A\beta$) plaques and intracellular neurofibrillary tangles, mainly comprised of phosphorylated tau protein, are the main hallmark of Alzheimer's disease (AD). Mechanistically, it has been suggested that $A\beta$ causes alterations in synaptic transmission as well as tau phosphorylation; however the involved intracellular cascade remains largely elusive. To determine whether synaptic activity is related to tau phosphorylation we used rat organotypic hippocampal slice cultures and primary neurons from rat. By using confocal imaging and synaptosomal preparations we observed that phosphorylated tau protein was present not only in the axon but also in the dendrite and specifically into the spines. Additionally, we found that NMDA receptor activation induces up-regulation of tau phosphorylation at sites Ser199/202, Thr231/Ser235 and Ser396/404 but not at sites Thr212/Ser214. Since it was reported that $A\beta$ could affect synaptic NMDA responses, we analyzed the effect of $A\beta$ on tau phosphorylation. In this regard, we found that $A\beta$ increases tau phosphorylation at sites Thr231/Ser235 and Ser396/404 but not at sites Ser199/202 and Thr212/Ser214 after five days incubation in hippocampal slice cultures. Our results suggest a common pathway between $A\beta$ and NMDA receptor activation. Furthermore our data showed that tau phosphorylation caused by NMDA receptor activation is occluded by prior incubation with $A\beta$."

Funding: CIHR, GRSNC

Title: Synchrotron X-ray fluorescence microprobe can detect and quantify iron in SPIO labeled stem cells

Authors: Angela Auriat¹, Weili Zheng², Helen Nichol³, Michael Kelly⁴, Raphael Guzman⁵

Affiliation: 1,5- Stanford University 2- Wayne State Univ 3,4 University of Saskatchewan

Abstract: We used synchrotron X-ray fluorescence (XRF) imaging to detect and quantify iron in SPIO labeled stem cells. Rats were subjected to a 1hour middle cerebral artery occlusion stroke.

On day 3 following stroke SPIO labeled mouse neural progenitor cells (NPCs) expressing the bioluminescence imaging (BLI) reporter gene synthetic Renilla luciferase and green fluorescent protein (GFP) were transplanted. Control stroked animals received saline injection. BLI was performed 24 hours after cell injection and once on the day of euthanasia. The animals were euthanized 1, 10, 30, or 42 days following transplantation. Ex vivo MRI images of the brain were acquired. Brains were then sectioned and XRF mapping of iron was completed. Iron concentration in stem cells was also assessed in vitro. Iron was quantified by comparing the signal strength of the iron fluorescence with an iron calibration standard designed for XRF. MRI demonstrated significant homing of NPC to the ischemic hemisphere but not to the contralateral hemisphere. Iron XRF maps showed distinct areas of iron signal distributed in the ischemic hemisphere correlating with MRI findings. High-resolution scans depicted single cells with an average iron content of $2.4\text{pg}\pm 2.0\text{SD}$, similar to the concentration identified with the in vitro analysis $2.5\pm 3.1\text{SD}$. Immunohistochemistry and Prussian Blue staining confirmed the presence of SPIO labeled stem cells in the brain areas of interest. We found excellent correlation between MRI, XRF and histology. XRF is a powerful tool for high resolution ex vivo morphologic imaging and quantitative iron analysis of SPIO labeled stem cells.

Funding: The Synchrotron Medical Imaging team is funded by CIHR and the Heart and Stroke Foundation of Canada.

Title: Estrogen receptors and gonadal steroids in vulnerability and protection of dopamine neurons in a mouse model of Parkinson's disease

Authors: Sara Al-Sweidi¹, Marc Morissette², Melanie Bourque³, Therese Di Paolo⁴

Affiliation: 1-4 Faculty of Pharmacy, Laval University and Endocrinology and Genomic Axis of the CHUQ-CHUL Research Center

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder of the central nervous system, characterized by the loss of dopaminergic neurons of the substantia nigra (SN) which project into the striatum. More men than women suffer from PD suggesting that estrogens are implicated in preventing or delaying the disease. 17beta-estradiol, the essential female estrogen, induces neuroprotective effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. Both DA transporters DAT and VMAT2 are located on nigrostriatal DA terminals and are used as markers of neuronal loss caused by MPTP.

We hypothesize that estrogen receptors (ERalpha and ERbeta) are involved in neuroprotection, so we used ERalpha and ERbeta intact or knockout (KO) C57Bl/6 male mice receiving acute MPTP treatments of different doses (7, 9, 11 or 15 mg/kg). The striatum and SN were used in autoradiographic binding and in situ hybridization for DAT, VMAT2 and tyrosine hydroxylase (TH). MPTP caused a dose-dependant loss of both transporters and TH, but ERKOalpha were more vulnerable to MPTP. We also observed correlations between DAT and VMAT2 specific binding. 17beta-estradiol treatment increased estradiol plasma levels in all genotypes, while ERKOalpha mice blood plasma had higher testosterone, dihydrotestosterone (DHT) and 3beta-diol compared to WT and ERKObeta mice. Hence, our results unveil a role for ERalpha in modulating the degree of vulnerability of dopaminergic neurons to MPTP toxicity. Endogenous estrogens acting on ERs are shown to play an important protective role against MPTP toxicity and ERalpha is dominant in mediating these protective effects.

Funding: TDP was supported by a grant from the CIHR. SAS held a ULaval FER studentship. MB holds a FRSQ studentship.

Title: Modulation of netrin-1 in the hematopoietic system affects invasion of systemic immune cells at the injury epicenter and lower locomotor recovery after spinal cord contusion.

Authors: Audrey Petit¹, Carol L. Wilson², Jingjing Tang³, Jurate Lasiene⁴, Elaine W. Raines⁵, Timothy E. Kennedy⁶

Affiliation: 1- University of British Columbia 2-5 University of Washington 6- McGill University

Abstract: The initial trauma after a spinal cord injury (SCI) is caused by shearing and laceration of the neural tissue and is associated with blood brain barrier disruption and an early invasion of cells from the systemic immune system as well as microglia activation. Macrophages are the major players in the inflammatory response and are associated with a positive outcome after injury, involving removal of myelin and tissue debris, and promotion of neuroprotection, axonal regeneration and remyelination. Macrophages can also be detrimental to repair, by promoting secondary injury, and their depletion reduces tissue damage and improves functional recovery after SCI. Invading macrophages express a repulsive cue, netrin-1 (Ntn-1) and we demonstrated that it plays a major repulsive role in the migration pattern of adult spinal cord progenitors (aSCP). We postulate that the development of gliotic/necrotic zones is dictated by the immune-derived Ntn-1 that drives aSCP to migrate away from the site of injury. We hypothesize that by

blocking Ntn-1, aSCP will proliferate and remain in the lesion zone remodeling the injury core. We created a chimeric mouse with hematopoietic stem cells lacking Ntn-1 expression. We evaluated the aSCP presence and immune cell invasion at the injury site, correlated with evaluation of sensory/motor function recovery. Mice with immune cells lacking Ntn-1 have shown lower locomotor recovery score, larger lesion zone and increased immune cell invasion at the injury site. Suggesting Ntn-1 express by invading immune cells plays a key role in the evolution of the injury compartments, decreasing the recovery of function.

Funding: (NIH RO1 NS46724/ Craig H. Neilsen Foundation/ FRSQ)

Title: The pattern of human tau phosphorylation is the result of priming and feedback events in primary hippocampal neurons

Authors: Johanne Bertrand¹, Vanessa Plouffe², Patrick Sénéchal³, Nicole Leclerc⁴

Affiliation: 1-3 Université de Montréal

Abstract: Tau, an axonal microtubule-associated protein, becomes hyperphosphorylated in several neurodegenerative diseases including Alzheimer disease (AD). In AD brain, tau is phosphorylated at pathological multiple-site epitopes recognized by antibodies AT8 (S199/S202/T205), AT100 (T212/S214/T217), AT180 (T231/S235) and PHF-1 (S396/S404) and at sites such as S262 and S422. Although it is believed that hyperphosphorylation of tau occurs in a precise cascade of phosphorylation events, this cascade remains to be demonstrated in mammalian neuronal cells. Here, human tau mutants in which disease-related sites were mutated in alanine to inhibit their phosphorylation were overexpressed in primary hippocampal neurons to examine their impact on the phosphorylation of other pathological sites. Mutation in alanine of S262 decreased the phosphorylation of AT8 and PHF-1 epitopes and that of T217. When sites included in the AT8 epitope were mutated in alanine, phosphorylation of T217 and PHF-1 epitope was significantly reduced indicating that the decrease of AT8 phosphorylation was a key event in the impaired phosphorylation of T217 and PHF-1 by S262A. Most interestingly, mutating T217 in alanine increased the phosphorylation of the AT8 epitope, indicating the presence of a feedback loop between AT8 and T217. Phosphorylation of the AT180 epitope was increased when S262 and the sites forming the AT8 epitope were mutated in alanine. All together, our data show that the sites forming the AT8 epitope could play a central role in regulating the phosphorylation of tau and that priming and feedback events take place to regulate the overall level of tau

phosphorylation in rat hippocampal neurons.

Funding: Alzheimer Society of Canada, CIHR, NSERC, FRSQ infrastructure grant to the GRSNC, GRSNC

Title: Knock down of the ataxia protein saccin causes mitochondrial defects and alterations in neuronal morphology

Authors: Martine Girard¹, Roxanne Larivière², David A. Parfitt³, Esmeralda G.M. Vermeulen⁴, Guennadi Kozlov⁵, George Prenosil⁶, Marie-Josée Dicaire⁷, R. Anne McKinney⁸, Kalle Gehring⁹, J. Paul Chapple¹⁰, Bernard Brais¹¹

Affiliation: 1- Montreal Neurological Institute/McGill University 2,11 Laboratoire de Neurogénétique/Centre de Recherche du CHUM 3,4,10- Centre for Endocrinology William Harvey Research Institute/Queen Mary University of London 5,9 Department of Biochemistry/McGill University 6,8 Department of Pharmacology and Therapeutics/McGill University 7 Laboratoire de Neurogénétique/Centre de Recherche du CHUM

Abstract: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset neurodegenerative disorder. Originally identified in the Charlevoix and Saguenay regions of Quebec, where as many as 1 in 22 individuals are carriers, the disease is now recognized worldwide and appears much more common than originally presumed. ARSACS results from mutations in the SACS gene that encodes saccin, a massive 4579 amino acid protein. Saccin is composed of multiple domains with chaperone activity indicating that it may operate in protein quality control, but little is known regarding its cell biological properties or functional roles. We now demonstrate that saccin localizes to mitochondria and that targeted disruption of the protein (the major mutations in Québec are loss-of-function) leads to altered mitochondrial morphology and makes mitochondria more sensitive to oxidative stress. In neurons, loss of saccin function leads to an accumulation of mitochondria in proximal dendrites. We also observe alterations in dendritic morphology, notably neurons have quantifiably fewer dendrites of greater diameter. Similar alterations are seen in cerebellar Purkinje neurons of saccin knockout mice, which also display loss of Purkinje cells. We propose that saccin functions in mitochondrial protein quality control mechanisms and that disruption of this process leads to altered neuronal morphology with subsequent neuronal loss.

Funding: Fondation de l'Ataxie Charlevoix-Saguenay

Title: Lithium ions uncover the role of the dimer interface in kainate receptor activation

Authors: Michael Accardi¹, David MacLean², Elizabeth Andrews³, Derek Bowie⁴

Affiliation: 1-4 McGill University

Abstract: Kainate-selective (KAR) ionotropic glutamate receptors (iGluRs) fulfill numerous neuromodulatory roles in the vertebrate CNS. Although they have been subject to both structural and functional analysis in recent years, it remains to be established how KAR stimulation leads to responses with both transient and sustained components. Recently, we have shown that the transient activation of KARs is governed by a pre-gating step that determines agonist efficacy. As yet, it still remains to be established what factors contribute to the steady-state activation of KARs. Accordingly, we report a novel effect of lithium ions (Li⁺) on pyrrolidine-containing agonists, such as kainate (KA) and domoate (Dom), which provides information on the role of dimer stability in KAR steady-state activation. Compared to responses in external Na⁺, Li⁺ slowed the decay kinetics of KA responses about 2-3 fold whilst promoting steady-state activation by 10-fold. Single-channel recordings reveal that these effects can be largely attributed to the appearance of a higher conductance state which is only found in external Li⁺. Ongoing experiments suggest that Li⁺ achieves these effects by stabilizing the dimer interface of KARs.

Funding: McGill University (Presenter), Canadian Institutes of Health Research (Supervisor)

Title: Viral Transfection of the M5 Gene in Wildtype Mice Enhances Morphine Induced Locomotion in VTA Sites, but Blocked Morphine-induced Locomotion in RMT Sites.

Authors: David Wasserman¹, John Yeomans², Asim Rashid³, Sheena Josselyn⁴, Haoran Wang⁵

Affiliation: 1,2 University of Toronto 3,4 The Hospital for Sick Children Research Institute 5- University of Toronto

Abstract: Mesopontine cholinergic neurons activate tegmental dopamine (DA) neurons via nicotinic and M5 muscarinic receptors. In rats, ventral tegmental area (VTA) muscarinic receptors are needed for brain-stimulation reward or food reward sensitivity, and for morphine-induced DA output. M5 knockout mice emit fewer ultrasonic vocalizations (USVs) during mating, and show less locomotion or DA output in response to morphine than wild-type mice. Using a Herpes simplex virus, the M5 receptor gene was transfected into the VTA or rostromedial tegmentum (RMT) of wild-type mice along with a green fluorescent protein (GFP) marker gene. M5 transfection into VTA DA and non-DA neurons increased morphine-induced locomotion at both 10

and 30mg/kg doses. Immunocytochemistry showed increased M5 receptors in HSV-M5-GFP infected neurons. M5 transfection into RMT inhibited morphine-induced locomotion at 10mg/kg and especially at 30mg/kg. Transfection of the excitatory M5 receptor in RMT is hypothesized to excite GABA neurons that inhibit VTA DA neurons. Immunocytochemical staining of GAD67 co-localized with the transfected HSV-M5-GFP. These results support previous evidence that the RMT is a critical inhibitory system for DA-reward functions, and provides new evidence that RMT is especially important for controlling opiate functions.

Funding: OMHF and CIHR grants to JY

Title: Exaggerated corticosterone and brain monoamine activity in repeatedly defeated mice housed in an enriched environment

Authors: Robyn McQuaid¹, Marie-Claude Audet², Hymie Anisman³

Affiliation: 1-3 Carleton University

Abstract: An enriched environment may protect animals from the harmful effects of stressors. However, environmental enrichment in group-housed male mice can also induce aggression and sensitize biological processes so that exaggerated responses are elicited by subsequent exposure to a mild stressor. In the current investigation we examined whether housing mice in an enriched environment would influence the neuroendocrine and neurochemical effects of a more potent stressor (i.e. social defeat) in a strain of mice known to be highly anxious and moderately aggressive (BALB/cByJ). Forty male BALB/c mice were housed in groups of 3 in either an enriched (EE) or a standard (SE) environment for 30 days. After the 21st day mice were exposed to a 15-min social defeat session for 7 consecutive days or were left undisturbed (10/group). Three min following the 7th stressor session, brain tissue and blood were collected. Mice that had experienced social defeat weighed less than the non-stressed mice, and this weight loss was more pronounced in EE mice than in their SE counterparts. Both SE and EE mice displayed increased corticosterone levels after the 7th social defeat session compared to non-stressed mice, but in EE mice the corticosterone response was enhanced. Utilization of hippocampal norepinephrine (NE) and serotonin (5-HT) was only increased in EE mice after experiencing the repeated social stressor, whereas levels of the parent amines were unaffected. It is suggested that competition for resources in an enriched environment may have adverse consequences as reflected by exaggerated behavioral and biological changes.

Funding: This research was supported by the Canadian Institutes of Health Research(CIHR)and the Natural Sciences and Engineering Council of Canada(NSERC).

Title: Intrastriatal calpain inhibition improves TH presynaptic expression and reverses L-dopa-induced dyskinesia.

Authors: Laure Chagniel¹, Christine Robitaille², Michel Cyr³

Affiliation: 1-3 Université du Québec à Trois-Rivières 2- Université du Québec à Trois-Rivières 3- Université du Québec à Trois-Rivières

Abstract: L-dopa-induced dyskinesia (LID) is a well-recognized side effect of chronic L-dopa therapy in Parkinson disease. The mechanism of LID is not clearly understood but several lines of evidence suggest an overactive glutamate and dopamine receptors transmission that affects calcium flux in the striatum structure. We investigated the role of calcium-dependent proteins calpains in a rat model of LID. Hemiparkinsonian rats were generated by unilateral complete dopamine-denervating lesions through injection of 6-hydroxydopamine. Lesioned-rats received an intermittent L-dopa therapy, to induce dyskinesia, paired with a continuous intrastriatal infusion of the calpains inhibitor MDL28170. Evaluation of dyskinesia and the antiparkinsonian effects of L-dopa were performed. Western blot and immunofluorescence techniques were assessed to investigate neurochemical changes. Intrastriatal infusions of the calpains inhibitor significantly reduced the severity of dyskinesia without affecting the antiparkinsonian effect of L-dopa. L-dopa therapy in combination with MDL28170 increased levels of tyrosine hydroxylase (TH) in the striatum but did not affect the dopaminergic cell numbers in the substantia nigra. We observed that TH positive neurons the lesioned rats treated with the combination of L-DOPA and MDL28170 displayed higher numbers of discernible varicosities with a larger nucleus. Calpain inhibition reversed the striatal molecular changes associated with L-dopa therapy such as the phosphorylation of ERK1/2 and dynamin. These data provide evidence that calpain is implicated in pre and post maladaptive plastic changes induced by L-dopa. These data suggests that the status of presynaptic, such as striatal TH levels, is a critical determinant of the emergence of LID.

Funding: This work was supported by the Parkinson Society of Canada and the Canada Research Chair in Molecular Neuropharmacology.

Title: Discovery of new drugs which can affect the viability and transformation of human glioma stem cells

Authors: Norbert F. Ajeawung¹, Robert Faure², Donald Poirier³, Harish Joshi⁴, Deepak Kamnasaran⁵

Affiliation: 1-3, 5 Université Laval 4- Emory University

Abstract: Background: Gliomas are the most common primary brain tumours in adults and children. Despite current treatments by surgery, radiation and temozolomide (TMZ), the median survival for patients diagnosed with malignant gliomas is still below two years. Within the tumor mass is a population of therapeutically resistant cells known as glioma stem cells. Current therapies therefore exhibit extreme limitations in preventing glioma stem cells from inducing tumor re-growth, and hence death of the patients. OBJECTIVE: The inability of current therapies in eradicating the tumour mass and the increase in toxicity associated with TMZ treatment has prompted us to identify and characterize new potent drugs in our effort to improve the survival of patients with this deadly disease. METHOD/RESULTS: Through a chemical genetic screen of 400 compounds, we have identified an inhibitor of steroid biogenesis, a potent bisperoxovanadium compound and a derivative of an opium alkaloid, having profound inhibitory effect on the growth of human glioma stem cells, and with little or no toxicity on non-transformed cells. Glioma stem cells treated with our drugs succumb to significant cell cycle arrest and apoptosis, which subsequently causes profound decreases in viability, proliferation, migration and even anchorage independent growth. Further testing reveals that these drugs also enhance the radiosensitivity of glioma stem cells in-vitro. CONCLUSION: We have discovered three novel drugs that can significantly inhibit the viability of human glioma stem cells. Further pre-clinical in-vivo studies would determine if these compounds can replace or complement existing therapies within a clinical setting in due course.

Funding: Fondation des étoiles, Canadian Foundation for Innovation (Leaders Opportunity Funds) and Fonds de la recherche en santé du Québec,

Title: Changes in exploratory behaviours and cognitive deficits in a chronic-relapsing model of experimental autoimmune encephalomyelitis (EAE)

Authors: Camille Olechowski¹, Travis Musgrave², Bradley Kerr³

Affiliation: 1-3 University of Alberta

Abstract: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that leads to severe neurological disabilities. In addition to the impairments in sensory and motor function, MS is also associated with a high incidence of depression and neuropathic pain. Recent reviews have reported a 30-60% co-occurrence rate between depression and pain. Previously, our lab demonstrated that neuropathic pain behaviours are present before and at disease onset in an animal model of MS, experimental autoimmune encephalomyelitis (EAE) in female C57BL/6 mice. We have now monitored changes in open field/exploratory behaviours as well as cognitive ability to determine if altered pain sensitivity is associated with behavioural signs indicative of anxiety, fatigue and/or depression in this model. Open field behaviours were categorized as either positive (locomotion, rearing, grooming, activity level) or negative (duration spent in sedentary postures). We find an increase in negative behaviours and a decrease in positive behaviours in EAE mice prior to and at disease onset. Using the Novel Object Recognition (NOR) test, we find that EAE mice also develop significant impairments in cognitive abilities very early in the disease course. Behavioural changes in the open field and NOR tests arise with a similar time course to the onset of neuropathic pain behaviours in EAE mice, suggesting a related underlying mechanism. Work is currently underway to look at the mechanisms that lead to these symptoms.

Funding:

Title: Changes in Proteolytic Processing of proBDNF Lead to Increased Brain-Derived Neurotrophic Factor Levels in Fusiform Gyrus of Subjects with Autism

Authors: Margaret Fahnstock¹, Kristine L. P. Garcia², Guanhua Yu³, Diego J. Garzon⁴, Victor S. Chiu⁵, Bernadeta Michalski⁶, Jeremy Goldberg⁷, Peter Szatmari⁸, Enrico Tongiorgi⁹

Affiliation: 1-8 McMaster University 9- University of Trieste

Abstract: Recent genetic studies suggest defects in synaptic development and plasticity may lead to autism. Brain-derived neurotrophic factor (BDNF) plays a critical role in synaptogenesis and synaptic plasticity. BDNF is synthesized as a precursor, proBDNF, which can be processed into either a truncated form or into mature BDNF, which is neurotrophic. ProBDNF and mature BDNF have opposing activities and roles in the brain, but truncated BDNF has unknown biological activity. Previous studies reported increased BDNF-immunoreactive protein in autism, although neither the mechanism of this increase nor the responsible BDNF protein isoform was

investigated. In this study, BDNF mRNA was examined by real-time RT-PCR in post mortem fusiform gyrus tissue from 9 autism and 14 control subjects. BDNF protein was examined in 9 autism and 9 controls using Western blotting and ELISA. BDNF mRNA levels were unchanged in the autism group compared to controls. However, BDNF-like immunoreactive protein, measured by ELISA, was increased in autism samples compared to controls. Western blotting revealed increased proBDNF, reduced truncated BDNF and a trend towards increased mature BDNF levels in autism compared to controls. These data demonstrate that increased levels of BDNF-immunoreactive protein in autism are not transcriptionally driven. Instead, increased proBDNF and reduced truncated BDNF implicate defective processing of proBDNF to its truncated form. This leads to distortion of the balance between the three isoforms of BDNF which could lead to changes in connectivity and synaptic plasticity and hence behaviour. Defective proteolytic maturation is a possible new mechanism for altered synaptic plasticity leading to autism.

Funding: Supported by an Ontario Mental Health Foundation grant (MF) and a studentship from the National Alliance for Autism Research (KLPG).

Title: Huntington disease phenotypes influenced by cleavage of huntingtin at aa586: further characterization of HD YAC128 mice expressing caspase-6-resistant mutant huntingtin.

Authors: Rona Graham¹, Mahmoud Pouladi², Yu Deng³, Yuanyun Xie⁴, Nagat Bissada⁵, Michael Hayden⁶

Affiliation: 1-6 University of BC

Abstract: "The amelioration of behavioral and neuropathological deficits in mice expressing caspase-6-resistant (C6R) mutant huntingtin (mhtt) highlights proteolysis of htt at the 586aa caspase-6 site as a key mechanism in the pathology of Huntington disease (HD). To determine whether inhibiting cleavage of mhtt at aa586 prevents other HD-like pathology we have performed additional characterization of C6R mice. Since the initial discovery of htt inclusions, there has been continued controversy over the role of htt aggregates in the pathogenesis of the disease. In contrast to the YAC128 mice, YAC128-shortstop mice exhibit widespread htt inclusions yet do not manifest an HD-related phenotype. Comparison of YAC128 and C6R striatum at 18m demonstrate a significant increase in nuclear inclusions in C6R striatum compared to YAC128 (YAC128= 39±2, C6R= 68±7; percentage of cells/inclusions, p<0.001). Several lines of evidence suggest aberrant activation of GSK3 in neurodegenerative diseases. Use of GSK3β inhibitors

protect against mhtt induced toxicity and is associated with an increase in inclusions. We assessed GSK3 activity in WT, YAC128 and C6R striatum and observed a significant increase in GSK3 activation in YAC128 striatum ($p < 0.01$) but not C6R striatum ($p = 0.513$) compared to WT. Based on findings of testicular atrophy in HD patients and in YAC128 mice, we assessed for testicular degeneration in the C6R mice. At 12m testicular weight in C6R mice is similar to WT ($p = 0.780$). These data provide additional evidence that cleavage of mhtt at aa586 plays a critical role in HD and supports efforts towards casp6 inhibition as a therapeutic treatment for HD. "

Funding:

Title: smARF induces Parkin/Pink1-dependent mitophagy.

Authors: Karl Grenier¹, Maria Kontogiannea², Edward Fon³

Affiliation: 1-3 Montreal Neurological Institute (McGill)

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting 1-2% of the population over 65 years old. Its main neuropathological hallmark is the degeneration of dopaminergic neurons in the substantia nigra pars compacta. Many causative genes have been identified in the last decade and have tremendously helped to focus the research on potential pathogenic pathways. Of those, Parkin, an E3-ligase, and Pink1, a mitochondrial kinase, have recently been linked in a common genetic pathway affecting mitochondrial homeostasis, providing the first genetic link between PD genes. More particularly, parkin was shown to be recruited at the mitochondria to trigger its autophagy following mitochondrial depolarization and Pink1 accumulation at the mitochondria. However, this was mainly shown using high concentration of a chemical uncoupler, cyanide m-chlorophenylhydrazone (CCCP). We demonstrate here that parkin and pink1 are also involved in the smARF autophagic pathway, where the uncoupler is proteinaceous (smARF). This suggest that the parkin/pink1 mitophagic pathway might be important for programmed mitochondria clearing as opposed to only responding to mitochondrial damage. This might be of key importance for cells such as dopaminergic neurons, whose survival rely heavily on mitochondrial ATP production and calcium buffering capacity.

Funding: Canadian Institute of Health Research (CIHR), Montreal Neurological Institute (MNI)

Title: The effects of paraquat on central monoamine activity, hippocampal BDNF expression and neurobehavioral function in female mice

Authors: Darcy Litteljohn¹, Eric Nelson², Shawn Hayley³

Affiliation: 1-3 Carleton University

Abstract: Accumulating evidence implicates pesticides such as paraquat in the development of Parkinson's disease (PD). Indeed, paraquat exposure is associated with an increased risk of PD and, when administered to rodents, the pesticide recapitulates many of the neuropathological and behavioural features of the disease. However, it is unclear whether any sexual dimorphism exists in the in vivo murine response to paraquat intoxication. Accordingly, we sought to determine the impact of the pesticide on a range of neuronal (brain regional monoamine activity, hippocampal brain-derived neurotrophic factor (BDNF) content) and behavioural outcomes (home-cage locomotor activity, open field exploration) in female C57BL/6J mice. The present investigation revealed that the female mice were largely resistant to the paraquat-induced nigrostriatal dopamine changes and locomotor deficits often seen in males. Yet, similar to our previous findings in males, paraquat increased norepinephrine utilization within the hippocampus and prefrontal cortex in the females, and altered exploration of a novel open field arena (increased exploration among females vs. reduced in males). Further, the paraquat-treated females appeared to display elevated hippocampal BDNF levels, whereas we previously detected a reduction in the trophic factor among males. These data suggest the possible presence of sexual dimorphism in the neurobehavioral response to paraquat: while limbic monoamine changes are similar in both sexes, female mice may be less susceptible than males to the nigrostriatal dopaminergic and motor effects of paraquat. Moreover, the heightened hippocampal trophic support observed in paraquat-treated females could conceivably help mitigate the development of psychological pathology often associated with environmental toxins.

Funding: D.L. and S.H. were supported by funds from the Canadian Institutes of Health Research (CIHR).

Title: Identification of a novel JNK-interacting protein as a potential therapeutic target for ischemic stroke

Authors: Max Cynader¹, Guang Yang²

Affiliation: 1,2 Brain Research Centre, UBC

Abstract: Clinical applications of neuroprotection strategies in ischemic stroke have been disappointing due to problems of efficacy and side effects. We have identified a novel interacting protein (NIP) of c-jun N-terminal kinase (JNK) as a key player in acute ischemic brain injury. NIP interacts with JNK and activates downstream neuronal cell death signals in response to pathological stressors that include excitotoxicity. We have developed novel peptides targeting a previous unknown JNK-interacting motif on NIP that block the enhancement of the NIP-JNK interaction induced by excitotoxicity and selectively abolish the activation of JNK isoforms 2 and 3, while leaving JNK1 unaffected. Application of these peptides successfully blocks JNK activation and neuronal cell death pathways, protects cultured neurons from excitotoxicity, and dramatically reduces brain damage and behavioural deficits in a rat model of focal ischemic stroke. The combination of strong neuroprotective efficacy and the potential advantages of isoform- and scenario-selective inhibition of JNK in reducing side effects suggest that targeting this novel interaction of NIP and JNK may be of therapeutic value for ischemic stroke and other neurological diseases.

Funding: CIHR

Title: The influence of ketamine exposure on glial cell differentiation and migration

Authors: Ushananthini Shanmugalingam¹, Matt Coyle², Xudong Cao³, Eve C. Tsai⁴

Affiliation: 1,2,4 Ottawa Hospital Research Institute 3- University of Ottawa

Abstract: Post spinal cord injury, there is an increase in astrocyte proliferation and migration towards the injury site. This contributes to the inhibitory scar which prevents regenerating axons from passing through the transection site. While ketamine has been shown to significantly reduce the migration of certain cell types, the effect of ketamine on glial migration is not well known. The aim of this study was to determine the dose response of spinal cord derived endogenous neural progenitor cell's (eNPC's) glial differentiation and migration following ketamine exposure in vitro. Adult female Sprague Dawley rat eNPCs were isolated from the subependymal zone of the spinal cord. Following a seven day proliferation period, cells were collected and exposed to ketamine concentrations ranging from 0-100ug/mL. An additional three days was allotted for the cells to differentiate, after which the cells were fixed and immunocytochemistry was performed to visualize the various glial cells. There was no significant differences in eNPCs glial differentiation following ketamine exposure (ANOVA, $p < 0.05$). Following 100ug/mL ketamine exposure there

was a significant increase in radial glia migration and a significant decrease in oligodendrocyte and astrocyte migration (Student's t-test, $p < 0.05$). Therefore, administration of high doses of ketamine may be useful in decreasing astrocyte and oligodendrocyte migration at the injury site following spinal cord injury.

Funding: Canadian Institutes of Health Research

Title: The critical role of the MYD88-dependent pathway in non-CNS MPTP-mediated toxicity.

Authors: Méli \u00e9 ssa C\u00f4t\u00e9¹, Janelle Drouin-Ouellet², Francesca Cicchetti³, Denis Soulet⁴

Affiliation: 1-4 Research Center of CHUL (CHUQ), Axis of Neurosciences

Abstract: A growing body of evidence supports a role of inflammation in the loss of central nervous system neurons both to acute and chronic insults, while its contribution to the loss of neurons in the enteric nervous system remains largely uninvestigated. We have addressed this issue by exploring the role of inflammation in dopaminergic (DAergic) myenteric neuronal degeneration secondary to MPTP lesioning in mice deficient in MyD88, a protein implicated in the cascade of events leading to the innate immune response. Our results show that MPTP-treated MyD88 knock out (MyD88^{-/-}) mice were protected against the toxin-induced TH-immunoreactive neuronal degeneration at the level of the myenteric plexus of the distal ileum, which causes a 50% loss of such neurons in MPTP-treated WT mice. Interestingly, the density of macrophages was the same in the MyD88^{-/-} mice subjected to MPTP, as opposed to the increase in density observed in wild-type (WT) mice treated with the toxin, which was due to an infiltration of monocyte from the blood to the myenteric tissue. Furthermore, in MPTP-treated MyD88^{-/-} mice, resident macrophages exhibited a predominant pro-repair phenotype, which could have contributed to the protection of DAergic neurons in the myenteric plexus. Taken together, our results suggest a critical role for the MyD88-dependent pathway in the gastrointestinal DAergic degeneration induced by MPTP.

Funding: Banting Research Foundation, Laval University and the CHUQ Research Center

Title: Cortical laminar profile of spontaneous seizures in anesthetized cats

Authors: Laszlo Grand¹, Igor Timofeev²

Affiliation: 1-2 Centre de recherche Universit\u00e9 Laval Robert-Giffard

Abstract: Cortically generated seizures include spike-wave (SW), polyspike-wave (PSW)

complexes, runs of fast EEG spikes and ripples (80-200Hz). Many studies have investigated the horizontal synchrony of seizures, but none have examined the laminar profile. Using multichannel silicon probes, we investigated depth distribution of local field potentials during different components of neocortical seizures in cats anesthetized with ketamine-xylazine. Current source density (CSD) maps showed very similar and strong source-sink pairs in the deep and less powerful pairs in superficial layers during the slow oscillation (peak frequency 0.9 Hz) and SW/PSW complexes (peak frequency 1.6 Hz). This characteristic CSD pattern was completely lost during fast runs (10-25 Hz), which can be described with gradually changing multiple sink-source pairs that occurred with different patterns in superficial and deep layers. The maximal ripple power was recorded during SW/PSW complexes and less during the slow oscillation. During fast runs the ripple activity fluctuated from very high power to absence and each individual ripple sequence was of highest power at a particular depth. We conclude that generation of the slow oscillation and SW/PSW complexes share some intracortical mechanisms with leading role of deep layer neurons. EEG fast runs and ripples have multiple and unstable cortical sources of generation.

Funding: Supported by NIH, CHIR, NSERC and FRSQ.

Title: A neurodevelopmental profile of the 3xTg-AD mouse model of Alzheimer's Disease

Authors: Caitlin Blaney¹, Richard Brown²

Affiliation: 1-2 Dalhousie University

Abstract: Alzheimer's disease (AD) is currently the most prevalent dementia worldwide. Currently, the most valuable tool in assessing the biochemistry and epigenetic factors of this disorder is the transgenic mouse model. The triple transgenic mouse model, 3xTg-AD, is considered one of the most complete models as it recapitulates both the alpha-beta plaques and neurofibrillary tangles associated with human AD. However, the full effect of the transgenes on both animal phenotype and adult behavior is undetermined. In this study the early neurodevelopment of the 3xTg-AD model, relative to its wild-type counterpart, was examined to assess the potential interactions between the transgenes APPSwe and tauP301L and neurodevelopment as well as maternal care of the 3xTg mother. The 3xTg-AD pups demonstrated earlier eye opening, pinnae detachment and earlier development of the hind limb grasp and rooting reflexes than wildtype. 3xTg-AD mothers also demonstrated lower levels of maternal care

than wildtype mothers. Although wildtype mothers were found to provide more maternal care than 3xTg-AD mothers, wildtype pups demonstrated advancement development in the rooting reflex, hindlimb grasp reflex and loss of the cross extensor reflex when raised by 3xTg-AD mothers. Results indicate an unexpected interaction between the transgenes and development as well as between 3xTg maternal care and wildtype pup development.

Funding: NSERC

Title: Evaluating spatial working memory in the APPSwe/PS1dE9 mouse model of Alzheimer's disease

Authors: Ahmed Hussin¹, Richard Brown²

Affiliation: 1-2 Dalhousie University

Abstract: Alzheimer's disease (AD) is characterized by memory impairments and significant neural degeneration. It is the most common form of dementia, accounting for at least 60% of the cases of dementia in patients older than 65. Using transgenic mouse models of AD enables researchers to investigate the mechanisms by which the observed neuropathology (amyloid plaques and neurofibrillary tangles) cause cognitive deficits. In this study, we examined spatial working memory in a double transgenic (APPSwe/PS1dE9) mouse model of AD where plaque deposition is aggressive and occurs in the hippocampus and cortex as early as 6 months of age. Using a repeated-reversal task in the Morris water maze, APPSwe/PS1dE9 mice were unimpaired in spatial working memory at 8 months of age, and habituation of their exploratory behaviour was not significantly different than wildtypes in the open field. At 10 months of age, their prepulse inhibition was unimpaired compared to wildtypes. At 11 months of age, APP/PS1 mice were unimpaired in spatial working memory assessed by the Y-maze. At 12 months of age, they showed spatial working memory impairment compared to wildtypes in the Morris water maze.

Funding: NSERC & Alzheimer's Association

Title: Peripheral Theta Burst Stimulation in cerebral palsy: a 3-case pilot study on spasticity, motor function and cortical motor excitability

Authors: Véronique Flamand¹, Marie-Christine Guimond², Line Nadeau³, Cyril Schneider⁴

Affiliation: 1,4 CHUL Research Center (CHUQ) / Université Laval 2- Institut de réadaptation en déficience physique de Québec (IRDPO) 3- Centre interdisciplinaire de recherche en réadaptation

et intégration sociale (CIRRIIS) / Université Laval

Abstract: "OBJECTIVES. The pilot study aimed at determining the effects of peripheral magnetic theta burst stimulation (pTBS) on motor control of ankle dorsiflexors in children with cerebral palsy (CP). We specifically tested 1) the influence of pTBS on cortical motor excitability related to ankle dorsiflexors, and 2) if cortical influences are accompanied by improvement of spasticity, lower limb function and mobility. METHODS. Three CP children (2 boys; mean age: 8 years 7 months, SD: 9 months) were enrolled in 5 stimulation sessions. Sham vs. continuous pTBS (inhibitory effects expected) were applied on the tibial nerve for reducing spasticity of plantar flexors whereas sham vs. intermittent pTBS (facilitatory effects expected) were used to enhance motor control of the tibialis anterior muscle (TA). Active and passive ranges of motion (ROM), spasticity and transcranial magnetic stimulation (TMS) outcomes of the TA (active motor threshold, amplitudes of motor evoked potentials, short intracortical motor inhibition) were measured pre- and post-pTBS. Functional performance (standardized clinical assessments for gait and balance) was assessed by a blinded physical therapist at baseline, at last session, and at follow-up (2 and 6 weeks after the last session). RESULTS. Data is under blinded analysis. First results tend to show marginal improvements of motor cortex excitability, spasticity and active and passive ROM with real stimulation when compared to sham. CONCLUSIONS. This study will improve our understanding of cerebral functioning in CP and will bring about ancillary insights on the efficacy of peripheral neurostimulation for reducing spasticity and disability in CP children. "

Funding: Canada Foundation for Innovation, Natural Sciences and Engineering Research Council of Canada, Fonds de la Recherche en Santé du Québec

Title: The role of the MYD88-dependent inflammatory pathway in motor and cognitive behaviors

Authors: Manon Le Bel¹, Janelle Drouin-Ouellet², Mélanie Bousquet³, Karl Gue⁴, Gibrat Claire⁵, Saint-Pierre Martine⁶, Francesca Cicchetti⁷

Affiliation: 1-7 Centre de Recherche du CHUL (CHUQ)

Abstract: Neuroinflammation appears to play a central role in several neurodegenerative diseases such as Parkinson's disease, but the mechanisms of action remain elusive. Here, we investigated the role of inflammation to dopamine vulnerability using depleted mice for MyD88, a protein implicated in the cascade of events leading to the innate immune response driven by the Toll-like receptors. Four to six month-old mice (C57BL/6 and MyD88 KO) were assessed for motor and

cognitive behaviors, striatal and prefrontal cortex biochemical profiles. Our preliminary results show that MyD88 KO mice have reduced spontaneous locomotor activity ($p < 0,001$) and altered performances in place preference ($p < 0,01$) as well as Barnes maze test ($p < 0,01$) compared to controls. These results suggest that these mice are hypolocomotive, present some components of anxious behaviour and affectation of cognitive tasks. Additional behavioral assessments demonstrated that motor coordination and balance (rotarod and beam traversal), muscular tone (inverted grid), memory and exploratory behaviours (object recognition and T-water maze) were not affected in these mice. HPLC analyses revealed elevated levels of dopamine ($p < 0,05$) and decreased levels of GABA ($p < 0,05$) in the striatum of MyD88KO mice compared to controls. Moreover, increased levels of dopamine ($p < 0,05$) and DOPAC ($p < 0,05$) were observed in the prefrontal cortex of these animals. These preliminary observations suggest a link between inflammation, behavior and neurotransmitter alterations in MyD88 KO mice. Better understanding of these interactions will help unravel the impact of inflammation on more vulnerable neurotransmitter systems such as DA, and shed light onto some of the pathogenic mechanisms underlying Parkinson's disease.

Funding: Canadian Institute of health research (CIHR) Canada Foundation for Innovation (CFI)

Title: Levodopa induces the phosphorylation of the cytoskeletal-associated proteins Tau in the MPTP mouse model of Parkinson's Disease

Authors: Christine Robitaille¹, Fahd Awada², Laure Chagniel³, Michel Cyr⁴

Affiliation: 1,3,4 Université du Québec à Trois-Rivières 2- Université de Montréal

Abstract: Parkinson's disease is associated with severe degeneration of nigrostriatal dopamine producing neurons. Pharmacological dopamine replacement with its synthesis precursor, levodopa, remains the most effective treatment for Parkinsonism. However, long term treatment with levodopa leads to the emergence of abnormal involuntary movements known as dyskinesia. This secondary effect of levodopa appears to be triggered by an overactivation of the direct striato-nigral output pathway, known to express high levels of dopamine D1 receptors in the striatum. Recently, we have demonstrated in cell cultures model and in rats striatum slices that activation of D1 receptors could regulate functions of structural proteins such as the microtubule associated protein tau. Whether dysfunctions in cytoskeletal-associated proteins are associated to levodopa in vivo is unknown. This study investigated, in striatal neurons of MPTP mice (30mg/Kg,

once daily, s.c., 5 days) receiving or not levodopa therapy (25mg/kg, twice daily, i.p., 5 injections), the expression and phosphorylation levels of tau using the western blot technique. Our results demonstrated that, although MPTP treatment was without significant effect in mice, levodopa had a profound influence on the levels of tau phosphorylated at serines 199/202 or 214 in these mice. Interestingly, this outcome was observed one hour after the last levodopa injection whereas there were no effect in animals sacrificed 24 hours post treatment. These findings demonstrated a tonic and direct effect of levodopa therapy on the levels of phosphorylated tau in depleted mice, suggesting that alterations in cytoskeletal constituents could be associated with dopamine replacement therapies in Parkinson's disease patients.

Funding: This work was supported by the NSERC.

Title: Hippocampal Neural Circuit Alterations in a Rat Model of Epileptogenesis Induced by Atypical Febrile Seizure

Authors: Patricia N. Awad¹, Bidisha Chattopadhyaya², Nathalie Sanon³, Elie Baho⁴, Lionel Carmant⁵, Graziella Di Cristo⁶

Affiliation: 1-6 CHU Sainte-Justine / Université de Montréal

Abstract: Febrile seizures affect 5% of children. About 20% of these children develop atypical febrile seizures with an increased risk of developing epilepsy. The presence of a cerebral malformation predisposes the development of both atypical hyperthermic seizures and temporal lobe epilepsy. A rodent model of dual pathology was established by combining a cortical freeze lesion at post-natal day 1(P1) and hyperthermia-induced seizure at P10 (LH rats). 86% of the LH male rats develop epilepsy. The goal of this study is to determine the synaptic alterations present in LH rats before the onset of spontaneous seizures. We quantified by Western Blot the expression of GABAergic enzymes and KCC2 cotransporter. GAD enzymes were increased 50% at P10 and P20. Moreover, we found a three-fold increase of KCC2 levels at P10 in lesioned rats, which was only maintained until P20 if the rat also had a hyperthermic seizure (50% increase in LH rats). We also found a shift of GABA reversal potential (EGABA) to more hyperpolarized potentials, consistent with an increase in GABAergic activity. Finally, using diolistic labeling we observed a striking reduction in spine density in basal dendrites of CA1 neurons. Recently, it has been suggested that KCC2 may play a role in spine formation, thus we are currently investigating whether the observed increase in KCC2 expression levels is responsible for the spine density

reduction. All together, these results suggest the presence of specific alterations in the model that occur early on, and could point to a time window for therapeutic intervention.

Funding: Savoy Foundation

Title: Spatial and temporal spinal glial responses in STZ-induced diabetic rats: correlation to neuropathic pain

Authors: Satyanarayana Padi¹, Xinag Shi², Ji Zhang³

Affiliation: 1-3 Alan Edwards Centre for Research on Pain

Abstract: Diabetic neuropathic pain is one of the most severe complications whose underlying mechanisms are not clear. Since spinal glia play an important role in the pathogenesis of trauma associated neuropathic pain, we seek to investigate the reaction of spinal microglia and astrocytes to persistent hyperglycemia and the correlation of such changes to the development of neuropathic pain. Diabetes was induced by streptozotocin. Rats started to exhibit mechanical allodynia and thermal hyperalgesia at 2 weeks, reaching the lowest levels at 1 month, and maintained neuropathic pain state for 3 months after induction. Diabetic rats showed a significant increase in the number of Iba-1+ cells in dorsal horn and a remarkable down-regulation of GFAP and GS expressed in astrocytes in both dorsal and ventral horns. While total Cx43ir is increased, the dephosphorylated Cx43 which is responsible for the opening of gap junctions was reduced. In general, such glial response was first observed at 1 month in lumbar spinal cord and progressively spread into cervical levels at later time point. ATF3 expression in both DRG sensory and spinal cord motor neurons was almost undetectable even 3 months after diabetes induction. These results indicated that spinal microglia and astrocytes respond differently to persistent hyperglycemia. While spinal microglia became activated in dorsal horns, astrocytes demonstrated a significant reduction on the expression of several important proteins. The spatial and temporal pattern of spinal microglial activation to hyperglycemia closely corresponds to the appearance of symptoms starting in the feet and spread up the hands in diabetic patients.

Funding: CIHR AND CIHR-Neuroinflammation grants

Title: Optical spinal cord injury using a pulsed near-infrared laser to investigate mechanisms of myelinated fiber degeneration.

Authors: David Stirling¹, Karen Cummins², Craig Brideau³, Peter Stys⁴

Affiliation: 1-4 University of Calgary/ Hotchkiss Brain Institute

Abstract: Spinal cord injury (SCI) induces delayed degeneration of myelinated fibers, however, the mechanisms remain poorly understood. To spatially and temporally synchronize injury, we developed an optical SCI model to ablate live dorsal column axons and documented changes in both axon and myelin as the lesion evolved over time. Acutely, only severed YFP+ axons formed swollen endbulbs both proximal and distal to injury, and retracted within enlarged myelin tubes. Remote to the lesion site myelin ballooned and separated from the axonal cylinder. Subsequently, we distinguished two main forms of axonal retraction/dieback both proximal and distal to the injury: i. slow sigmoidal retraction, characterized by the slow corkscrew coiling of the axon within areas of ballooned myelin, ii. Acute Wallerian degeneration, characterized by rapid spheroid formation and fragmentation of the axon. Importantly, low Ca^{2+} aCSF, or interference with intra-axonal Ca^{2+} store release using ryanodine and 2-APB, significantly reduced proximal axonal retraction. To further probe acute axon and myelin changes following injury, we combined polarization-dependent two-photon and spectral microscopy, and documented spectral shifts of lipophilic fluorescent dyes within distal endbulbs as the first putative pathologic divergence between the proximal and distal axonal stump. In addition, spectral shifts and polarization-dependent changes were detected discretely within myelin that ballooned around normal or swollen axons separated by periaxonal fluid (fluorescein) accumulation. We conclude that optical SCI, combined with spectral and polarization-dependent two-photon microscopy, is a powerful model to examine mechanisms of axonal degeneration with great spatio-temporal precision.

Funding: CIHR, AHFMR, CRC, Paralyzed Veterans of America Research Foundation

Title: The Machado-Joseph Disease Associated mutant form of ataxin-3 promotes clearance of parkin through the autophagy pathway

Authors: Thomas Durcan¹, Maria Kontogiannea², Edward Fon³

Affiliation: 1-3 Montreal Neurological Institute

Abstract: Machado-Joseph disease (MJD) is the most common dominant inherited ataxia worldwide, caused by an unstable CAG trinucleotide expansion mutation resulting in an expanded polyglutamine tract within ataxin-3. Interestingly, MJD often presents with clinical and neuropathological symptoms of Parkinson disease (PD), raising the possibility that ataxin-3 may interact with a PD-associated protein. One such candidate was parkin, with mutations in the

parkin gene accounting for ~50% of early onset PD cases. Using a combination of binding assays and immunoprecipitations, we demonstrate a direct interaction between parkin and ataxin-3. This interaction is direct and bimodal, involving a Ubl: UIM and RING: Josephin interactions between parkin and ataxin-3. Although many DUBs regulate the stability of their respective E3 partner, wild-type ataxin-3 had no effect on parkin stability. Remarkably, a decrease in parkin levels was only observed in transgenic mice over expressing the MJD-associated mutant form of ataxin-3. These findings were confirmed in HEK293T cells using cycloheximide pulse chases. Moreover, the mutant ataxin-3 targeted parkin for clearance through the autophagy pathway. Furthermore, parkin co-localizes with fragmented mitochondria, resulting in their clearance followed by the subsequent clearance of GFP-parkin aggregates. Taken together, we propose a novel mechanism to explain the parkinsonian symptoms often observed in MJD, whereby the expanded ataxin-3 elicits mitochondrial damage to the mitochondria, which in turn promotes clearance of the damaged mitochondria and parkin through the autophagy pathway.

Funding: Parkinson's disease Foundation and National Ataxia Foundation.

Title: A subset of nociceptive specific spinothalamic tract neurons are responsible for normal and pathological pain signaling

Authors: Guillaume Lavertu¹, Sylvain Côté², Yves De Koninck³

Affiliation: 1-3 Université Laval

Abstract: Which spinal projection neurons are responsible for relaying pain remains controversial. The two main ascending pathways relaying pain signals supraspinally are in laminae I and V. Lamina I appears essentially nociceptive and thermoceptive specific. In lamina V, however, it has been proposed that wide dynamic range neurons (WDR), onto which both innocuous and noxious input converge, are best suited to encode and relay pain signals because their dynamic range reflects better that of pain sensation in humans. Direct testing of this hypothesis is lacking however because a correlation between pain behaviours and the response properties of deep spinal projection neurons has not been conducted. We tested this by quantifying and manipulating behavioural and neuronal nociceptive thresholds in normal and pathological pain conditions. We found that nociceptive withdrawal threshold in adult rats matches that of nociceptive specific (NS) deep spinothalamic tract (STT) neurons and that the latter display greater encoding capacity in the painful regime than WDR-STT neurons.

Furthermore, the change in withdrawal threshold of rats with nerve injury matched that of NS-STT neurons, while WDR-STT neurons showed no change in threshold nor maximal response to noxious stimuli. Blocking local spinal inhibition with strychnine and bicuculline, known to cause allodynia-like behaviour, caused a selective shift in NS-STT neuron threshold without altering any parameters of WDR-STT neuron response profiles. Our findings reveal that, contrary to accepted thought, deep NS-STT neurons appear better suited for pain signaling than WDR-STT neurons and that NS-STT neurons may be the appropriate target for pain therapeutics.

Funding: Funded by CIHR and FRSQ.

Title: Frontal substrates of cognitive performance heterogeneity during aging

Authors: Cyril Bories¹, Zoé Husson², Matthieu Guitton³, Yves De Koninck⁴

Affiliation: 1-4 Université Laval

Abstract: Normal aging is associated with a variable lowering of cognitive and non-cognitive functions. These non-predictive declines represent widely shared concerns and a major public health issue. Hence, identifying their substrates in the aging brain is one of the main challenges in biomedical sciences. Although widely investigated in humans, the frontal dysfunctions and their correlates remain relatively understudied in animal models of aging. Here, we aimed to identify potential substrates of age-associated deficits in 3-27 month-old Fisher rats. We combined, in the same animals, novelty recognition and exploratory behavioral tasks with assessment of structural and functional aspects of prefrontal synaptic activity. We found an increase in heterogeneity of cognitive performance with aging, culminating in a clear bimodal distribution in aged rats (>23 month-old) for exploratory behaviors. Comparison of behavioral performances with synaptic activities revealed a correlation between GABAA receptor-mediated synaptic activity and occurrence of both stereotyped exploratory behavior and novelty-related memory deficits. On the other hand, we observed a specific increase in AMPA receptor mediated synaptic activity in aged animals with preserved exploratory behavior. Moreover, the ratio of expression of excitatory to inhibitory postsynaptic markers also correlated with stereotyped exploratory behavior. Finally, we found that the synaptic cell-adhesion proteins Neuroligin 2 and Neuroligin 1, known to be implicated into synaptic identity determination, could play an unexpected role in the regulation of the synaptic balance in aged population. Thus, our results reveal that a dualism in the evolution of

synaptic balance could underlie the variable behavioral performances observed during aging.

Funding: funded by CIHR and FRSQ

Title: Unilateral brain cooling as a method for assessing neuroprotection and post-ischemic plasticity after global ischemia

Authors: Greg Silasi¹, Fred Colbourne²

Affiliation: 1-2 University of Alberta

Abstract: "Therapeutic hypothermia provides unrivaled neuroprotection in animal models of global ischemia, however to achieve permanent protection the treatment must be prolonged (1-2 days). Other forms of stroke, such as focal ischemia or hemorrhage, may require even longer cooling to minimize swelling or continued neuronal death. In the current experiments we evaluated the effects of protracted cooling (up to 1 week) on neuroprotection and synaptic plasticity within the hippocampus after global ischemia. We first adapted a focal cooling system to decrease hippocampal temperature to $\sim 32^{\circ}\text{C}$ in one hemisphere, while leaving the contralateral side normothermic. Focal cooling substantially reduced CA1 neuronal injury when treatment began 1 hr after global ischemia. In a second experiment we evaluated whether the timing and duration of unilateral brain cooling influences neuroprotection and post-ischemic plasticity such as synaptogenesis, neurogenesis and neurotrophin signaling. Following 8 minutes of global ischemia rats were either left normothermic, or cooled on post-ischemic days 1-2, 1-4, 2-4 or 1-7. All cooling protocols, except for the day 2-4 cooling, were significantly neuroprotective for CA1 neurons. Immunohistochemical labeling showed that synaptophysin and BDNF expression in the mossy fiber bundle was not influenced by hypothermia. In addition, the rate of hippocampal neurogenesis, as assessed by Ki67/DCX labeling, was also not influenced by hypothermia. Together, our results indicate that delayed focal brain cooling is neuroprotective after global ischemia. Furthermore, hypothermia prolonged for up to 1 week does not negatively impact post-ischemic plasticity."

Funding: CIHR, Heart and Stroke Foundation of Canada

Title: Possible Implication of Dopamine in Electroretinogram Anomalies Reported in Psychiatric Disorders

Authors: Joëlle Lavoie¹, Jean-Martin Beaulieu², Marc Hébert³

Affiliation: 1-3 Centre de recherche Université Laval Robert-Giffard

Abstract: "Electroretinogram (ERG) anomalies have been reported in patients with seasonal affective disorder (SAD) and in young offspring at high risk (HR) to develop schizophrenia or bipolar disorder. Since dopamine dysfunction is hypothesized to be at the origin of many psychiatric disorders, it is believed that these ERG anomalies could be associated with a dopaminergic disruption. In this study, we investigated if a dopaminergic dysfunction at the levels of D1 or D2 receptors or in the expression of central dopamine could impact the ERG. ERGs were performed on anaesthetized (ketamine-xylazine) dopamine transporter knockout mice (DAT-KO) in which a five-fold increase in extracellular dopamine is observed and in D1 or D2 receptor knockout mice (D1R-KO and D2R-KO). Photopic and scotopic luminance response function protocols were used from which two parameters were derived, namely Vmax and logK interpreted as maximal function and sensitivity of the retina, respectively. Homozygous (HO) knockout mice were paired with wildtype (WT) mice. A decrease in scotopic logK was observed in DAT-KO mice (WT = -1.91 log units, HO = -1.78 log units; P = 0.0213). D1R-KO mice showed a significant decrease of Vmax in both the photopic (WT = 340.54 μ V, HO = 267.05 μ V; P = 0.0023) and scotopic ERGs (WT = 491.42 μ V, HO = 393.43 μ V; P < 0.0001). No change was found in the D2R-KO mice. These data support the hypothesis that dopamine disruption could be at the origin of the ERG anomalies observed in some psychiatric disorders. "

Funding:

Title: Dose-related effects of chronic resveratrol pretreatment on angiogenesis following global cerebral ischemia.

Authors: Catrinel Girbovan¹, Megan Dunbar², H el ene Plamondon³

Affiliation: 1-3 University of Ottawa

Abstract: "Angiogenesis, or the formation of new blood vessels, is an essential element of brain development and health. Resveratrol (RSV) is a naturally occurring polyphenol phytoalexin mainly found in grape seeds and skins but also in a variety of other botanical species including berries and peanuts. Various studies indicated that RSV has an array of beneficial health effects including

anti-aging, anti-cancerous, neuroprotective actions and the ability to promote neurogenesis. According to the neurovascular niche hypothesis, it is likely that the ability of RSV to induce neurogenesis be linked to stimulation of angiogenesis. Thus, the present study examined the effects of 21-day resveratrol pre-treatment (1 or 10 mg/kg dose; i.p.) on angiogenesis in the DG, CA1 and CA3 layers of the hippocampus as well as the septal area following 10 min global ischemia. Endothelial cell adhesion molecule-1 (CD-31)-labelling of blood vessels was used to evaluate angiogenesis. Our findings indicated significant increase in CD-31 immunoreactivity expression in RSV-treated ischemic compared to sham animals and saline-treated controls 7 days post injury. The effect was most profound in the higher dose groups but expression was broad and not restricted to areas most affected by ischemic injury (i.e., CA1 layer), suggesting complex relationships between angiogenesis and neuronal injury post stroke."

Funding: NSERC grant to HP

Title: Detection of O6- and N7-methylguanine adducts in brain tumour tissues obtained from glioblastoma multiforme patients treated with temozolomide

Authors: Emad Seyed Sadr¹, Alain Tessier², Mohamad Seyed Sadr³, Jad Alshami⁴, Mitsuhiro Anan⁵, Carmen Sabau⁶, Rolando Del Maestro⁷

Affiliation: 1, 3-7 McGill University 2- Concordia University

Abstract: The current standard of care for glioblastoma multiforme includes surgery, radiotherapy and chemotherapeutic agents such as temozolomide (TMZ), a DNA methylating compound. The cytotoxic effects of TMZ have been linked to guanine methylation at the N7 and O6 positions. Methyl guanine adducts are not currently used as markers of TMZ efficacy and previous attempts at elucidating guanine methylation at the O6 position relied on methyl adduct-correcting proteins. Using mass spectrometry, we have evaluated an analytical assay to directly detect both N7- and O6-methylguanine adducts from genomic DNA following TMZ treatment. N7- and O6-methylguanine adducts were successfully detected in genomic DNA extracted from TMZ-treated cell lines and brain tumour tissue samples from patients treated with TMZ pre-operatively. The detection limits observed with mass spectrometry were below 1 fmoles for N7-methylguanine and O6-methylguanine. This technique is useful in evaluating guanine methylation and could potentially provide rapid information about patient response and outcome.

Funding: Brain Tumour Foundation of Canada, Di Giovanni Brain Tumour Fund

Title: Reduced CRF expression in discrete brain regions at long reperfusion intervals following global ischemia in rats

Authors: Patricia Barra de la Tremblaye¹, Marc R. Milot², H el ene Plamondon³

Affiliation: 1-3 University of Ottawa

Abstract: We have demonstrated time-dependent changes in CRH concentrations in 10 brain regions including hippocampal, parahippocampal and hypothalamic regions, the amygdala and frontal cortex, at 4, 24 and 72 h following ischemia in rats and increased in vivo CRH release at the central nucleus of the amygdala (CeA) in the hours following global ischemia (Khan et al., 2004). Of interest, losses of CRF-positive neurons were recently demonstrated in the CeA 6 weeks following neonatal hypoxia-ischemia (Carty et al., 2010). The present study aimed to investigate long-term effects of global ischemia on brain CRF-immunoreactivity (CRF-ir) in rats that underwent a 10-minute global ischemia and were perfused 30 days following ischemic injury. CRF-ir was assessed in the hippocampal CA1 and CA3 pyramidal layers, the CeA and the paraventricular nucleus of the hypothalamus (PVN). Our findings revealed reduced CRF immunoreactivity in the PVN and CeA in ischemic compared to sham-operated rats. We observed no significant difference in CRF expression in hippocampal CA1 and CA3 layers between rat groups. Reduced PVN and amygdalar CRF expression could play a role in emotional memory impairment (e.g. reduced contextual fear response) observed at similar time intervals following global ischemia in rats (Barra de la Tremblaye and Plamondon, 2011).

Funding: NCERC grant to HP

Title: Immediate pregabalin treatment after spinal cord injury prevents the development of mechanical allodynia.

Authors: Daniel Marsh¹, Jason Meisner², Christine Short³

Affiliation: 1,2 Dalhousie University 3- QEII Health Sciences Centre

Abstract: "Treatment strategies to ameliorate, or abrogate, the development of spinal cord injury (SCI)-induced neuropathic pain (NP) are in demand as NP is a frequent outcome after SCI and is often refractory to standard treatment. The gabapentinoid drug, pregabalin, has proved efficacious in diminishing established post-SCI NP, but has not been tested as a preemptive agent. An Infinite Horizon computer-controlled impactor was used to apply a 50 kilodyne contusion at T11 to model SCI-induced NP. FVB mice were administered pregabalin (10 mg/kg, s.c.) twice daily for two

weeks beginning within hours, or 1 week following SCI, and compared to saline-treated mice. Mechanical allodynia was determined by measuring 50% withdrawal thresholds prior to SCI, and for 2-6 weeks post SCI. Saline-treated mice, and 1 week delay pregabalin-treated mice demonstrated reduced withdrawal thresholds indicative of SCI-induced NP. In contrast, immediate pregabalin-treated mice exhibited withdrawal thresholds not significantly different from baseline values of uninjured mice. The lumbar expression of GFAP or Cav α 2 δ -1 protein was not altered by SCI or pregabalin treatment. In contrast, the microglial marker CD11b was increased following SCI. This increase in CD11b was diminished in animals receiving pregabalin immediately post-SCI. Peri-lesion and lumbar expression of thrombospondin-1 was transiently increased following SCI, peaking 24-72 hours after SCI. Thrombospondin-1 is a synaptogenic extracellular matrix protein and competes with pregabalin for high affinity binding to the Cav α 2 δ 1 receptor. These findings suggest that pregabalin administration, soon after SCI, may serve as an intervention and block the SCI-induced development of below-lesion mechanical allodynia and neuropathic pain. "

Funding: Capital District Health Research Fund, Nova Scotia Health Research Foundation

Title: Is Altered Lipid Regulation Involved in Age Related Deficits in Adult Neurogenesis

Authors: Laura Hamilton¹, Nita Avrith², Anne Aumont³, Frédéric Calon⁴, Karl Fernandes⁵

Affiliation: 1-3, 5 Université de Montreal 4- Université Laval

Abstract: Neurogenesis is an important source of neural plasticity that is down-regulated between early and late adulthood. With aging and metabolic disorders, lipid accumulations are observed in a variety of tissues, however, the impact of altered lipid regulation on stem cell behaviour is not well understood. In the present study, we aim to understand if changes in lipid regulation within the CNS are involved in age-associated decreases in neural stem cell activity, neurogenesis and cell replacement in the subventricular zone (SVZ). We have observed that during aging there are dramatic increases in lipid droplet number and size within the ependymal cells of the SVZ. Ependymal cells direct CSF flow and secrete factors that are critical for the maintenance of the SVZ stem cell niche. Thus, perturbation of the ependymal cells could have a significant impact on the stem cell niche and its activity. We are currently developing novel in vitro assays to evaluate the effect of increased local lipid accumulations on ependymal cell function. We are also using colony-forming "neurosphere" cultures of SVZ stem cells to assay the

impact of increased circulating lipids on neural stem cell proliferation, self-renewal and differentiation. Finally, we are establishing a complementary animal model in which lipids accumulate within the SVZ of young adult mice. Together, these in vitro and in vivo models will enable us to identify the role of lipids in regulating stem cell behaviour and provide important clues as to how lipids may be involved in regulating neurogenesis under normal and pathological conditions.

Funding: Supported by: the Canadian Institutes of Health Research

Title: MicroRNAs as modulators of tau alternative splicing in sporadic progressive supranuclear palsy

Authors: Pascal Smith¹, Sébastien Hébert²

Affiliation: 1-2 Département de psychiatrie et de neurosciences / Université Laval

Abstract: "OBJECTIVES : Tauopathies represent a large class of neurological and movement disorders characterized by abnormal intracellular deposits of the microtubule associated protein tau. It's now well established that mis-splicing of tau exon 10, causing an imbalance between three-repeat (3R) and four-repeat (4R) tau isoforms, can cause disease; however, the underlying mechanisms affecting tau splicing in neurons remain poorly understood. The small noncoding microRNAs (miRNAs), known for their critical role in posttranscriptional gene expression regulation, are increasingly acknowledged as important regulators of alternative splicing. The objective of this study was to identify miRNAs that could regulate tau exon 10 splicing, and consequently tau isoform abundance. METHODS : We generated a list of potential miRNAs predicted to target known tau splicing factors. Neuro2a cells were transfected with candidate microRNAs, and tau splicing was analyzed by Western blot and PCR. Brain tissues were analyzed to confirm our in vitro experiments. RESULTS : Overexpression of miR-132 decreased the overall 4R-tau (+ exon 10) vs. 3R-tau (-exon 10) ratios in cells. We show that miR-132 directly targets the neuronal splicing factor PTBP2/nPTB, which equally modulated 4R:3R-tau ratios. In patients suffering from sporadic progressive supranuclear palsy, a major 4R-tau tauopathy, we observed significant changes in miR-132 as well as PTBP2 expression levels. CONCLUSIONS : Our results suggest that miR-132 is a modulator of tau exon 10 splicing through PTBP2 modulation in a subset of tauopathies. "

Funding: French National Research Agency, Canadian NSERC Individual Discovery 092850 grant,

Research on Alzheimer's Disease and Related Disorders Québec-France-Canada Collaboration grant

Title: Augmenting collateral blood flow during stroke via transient aortic occlusion

Authors: Ian Winship¹, Glenn Armitage², Gomathi Ramakrishnan³, Kathryn Todd⁴, Ashfaq Shuaib⁵

Affiliation: 1-5 University of Alberta

Abstract: Stroke, brain dysfunction caused by an interruption in blood flow to the brain, is the third leading cause of death and the leading cause of chronic disability in Canada. Focal ischemic stroke, caused by occlusion of an artery carrying blood to a particular region of the brain, leads to neuronal death and functional impairment dependent on the size and location of the ischemic territory. During an acute ischemic event, "collateral circulation" may provide an alternative route for blood flow to reach ischemic tissue. In particular, anastomatic connections between the anterior and middle cerebral arteries are opened by middle cerebral artery occlusion (MCAo) and may allow partial reperfusion of ischemic territories. It has been suggested that partial occlusion of the suprarenal abdominal aorta may increase CBF and reduce ischemic tissue by redistribution of the cardiac output. Here, we used laser speckle contrast imaging to map dynamic changes in collateral blood flow to ischemic territories after thromboembolic middle cerebral artery occlusion in rats treated with transient aortic occlusion. Our data showed that transient aortic occlusion increases blood flow to ischemic territories through anastomoses between the ACA and MCA and prevented the collapse of the distal MCA branches during the acute period or stroke. Given the high failure rate of current stroke therapies, new approaches are urgently needed and understanding the mechanisms of transient aortic occlusion during acute ischemia will speed the translation of this transformative new strategy to the clinic.

Funding: CIHR, AHFMR, Alberta Innovates Health Solutions, Heart and Stroke Foundation, University Hospital Foundation, Faculty of Medicine and Dentistry

Title: Hypoxia-induced co-activation of NMDAR and pannexin1 are involved in the anoxic depolarization of hippocampal pyramidal neurons

Authors: Nicholas Weilingner¹, Peter L. Tang², Roger Thompson³

Affiliation: 1-3 University of Calgary, Hotchkiss Brain Institute

Abstract: "Vascular diseases can cause acute and chronic oxygen deprivation (hypoxia) and lead

to central nervous system dysfunction. Pyramidal neurons in the CA1 region of the hippocampus are particularly susceptible to hypoxia, and respond initially with an “anoxic depolarization” (AD), characterized by the activation of multiphasic inward currents that are strongly correlated with neuronal death and dysfunction. The initial phase typically exhibits high-frequency mini excitatory post-synaptic potentials (mEPSP) prior to a substantial (nA) current that partially recovers by approximately 40%. We hypothesized that the AD is mediated in part by pannexin-1 (Panx1) because Panx1 channels are opened by ischemia (Thompson et al. 2006), and N-methyl-D-aspartate receptor (NMDAR) activation (Thompson et al. 2008) - two key components of the AD. Using whole-cell patch clamp recordings we activated the AD with hypoxia in the presence or absence of a variety of Panx1 blockers (10panx, probenecid, and an intracellular Panx1 antibody). Panx1 block attenuated the net AD current and promoted recovery to the pre-hypoxic baseline. APV (a competitive NMDAR antagonist) had similar effect as the Panx1 blockers; while MK801 (a NMDAR open channel blocker) or internally applied BAPTA did not affect the peak AD or AD recovery. In addition, NR2B and Panx1 co-immunoprecipitate, suggesting that a direct interaction between NMDARs and Panx1 may be key to the mechanism of activation. These data suggest that agonist binding to the NMDA receptor activates Panx1 during the AD and, interestingly, this appears to occur independently of intracellular Ca²⁺ or ion conductance by the NMDAR."

Funding: CIHR, AHFMR, Canadian Stroke Network

Title: Anti-depressive effects of sleep deprivation require the astrocyte-dependent sleep-homeostat

Authors: Dustin Hines¹, L. Ian Schmitt², Rochelle Hines³, Philip Haydon⁴

Affiliation: 1-4 Tufts Medical

Abstract: Approximately one in 10 Canadians will suffer from major depression at least once in their lifetime. Current treatments take weeks for clinical efficacy, limiting the ability to bring instant relief to suicidal patients. One non-pharmacological intervention that rapidly alleviates symptoms of depression is sleep deprivation (SD). In contrast to pharmacological agents, SD produces a rapid improvement in mood. The mechanism by which SD can improve depressive symptoms remains unknown. We have recently discovered that astrocytes regulate responses to SD including changes in NREM activity, as well as increased sleep time following SD. Via conditional expression of a dominant negative SNARE domain within astrocytes (dnSNARE),

extracellular adenosine levels are reduced, leading to a reduction in the pressure to sleep. We have asked whether this astrocytic modulation of the sleep homeostat contributes to the anti-depressive effects of SD. A total night of SD in mice reduces depressive-like behaviors as measured by immobility time in both the tail suspension test and forced swim task. Inhibition of the glial sleep homeostat in dnSNARE mice prevents the change of immobility time induced by SD, suggesting that the SNARE-sensitive accumulation of adenosine mediates the anti-depressive-like actions of SD. This hypothesis is supported by the observation that the beneficial effects of SD are prevented in A1R^{-/-} mice, and in mice treated with the adenosine receptor antagonist cyclopentyltheophylline. These data suggest that the beneficial effects of SD are mediated by a glial-dependent adenosine pathway, and provide a unique opportunity to develop novel, fast acting therapies for depression.

Funding: Heart and Stroke Foundation of Canada & National Institutes of Health

Title: What determines whether murine ependymal stem cells are activated during pathologies of the adult spinal cord?

Authors: Stéfanny Beaudoin¹, Laura Hamilton², Christian Dugas³, Anne Aumont⁴, Isabelle Pineau⁵, Steve Lacroix⁶, Karl Fernandes⁷

Affiliation: 1-4,7 Université de Montréal 5,6 Université Laval

Abstract: A subpopulation of ependymal cells in the adult spinal cord, while normally quiescent, displays neural stem cell properties post-traumatic injury. This makes ependymal stem cells a potential target for promoting spinal cord repair. In the present study, we aim to establish what circumstances, and in response to what types of spinal cord pathologies, ependymal stem cells become activated. We have studied temporal and spatial parameters of ependymal stem cell activation in response to three models of spinal cord pathologies: i) Spinal cord contusion injury using the Infinite Horizon Impactor on minimal setting to preserve the central canal. ii) EAE, the multi-focal demyelinating model of Multiple Sclerosis resulting in autoimmune-directed demyelination in the CNS of affected mice and undergoes both demyelinating and remyelinating phases. iii) LPC-induced (chemical) demyelination of local axons. Preliminary results reveal that these three experimental models differ in overall patterns of ependymal stem cell activation. The magnitude, spatial extent, and kinetics of stem cell proliferation as well as expression of stem cell markers appear to be regulated according to an injury-type specific manner. Moreover, factors

such as central canal disruption, demyelination, and inflammation are likely key determinants of the acquisition/expression of neural stem cell properties by ependymal cells. In ongoing experiments, we are further defining molecular mechanisms involved in ependymal stem cell activation during spinal cord pathologies. Eventually, modulation of these mechanisms may enable us to harness endogenous ependymal stem cells to promote spinal cord repair by enhancing tissue preservation or replacing damaged spinal cord cell types.

Funding: Multiple Sclerosis Society of Canada, Canadian Institutes of Health Research (CIHR)

Title: Identification of a novel JNK-interacting protein as a potential therapeutic target for ischemic stroke

Authors: Guang Yang¹, Max Cynader²

Affiliation: 1,2 Brain Research Centre, University of British Columbia

Abstract: Clinical applications of neuroprotection strategies in ischemic stroke have been disappointing due to problems of efficacy and side effects. We have identified a novel interacting protein (NIP) of c-jun N-terminal kinase (JNK) as a key player in acute ischemic brain injury. NIP interacts with JNK and activates downstream neuronal cell death signals in response to pathological stressors that include excitotoxicity. We have developed novel peptides targeting a previous unknown JNK-interacting motif on NIP that block the enhancement of the NIP-JNK interaction induced by excitotoxicity and selectively abolish the activation of JNK isoforms 2 and 3, while leaving JNK1 unaffected. Application of these peptides successfully blocks JNK activation and neuronal cell death pathways, protects cultured neurons from excitotoxicity, and dramatically reduces brain damage and behavioural deficits in a rat model of focal ischemic stroke. The combination of strong neuroprotective efficacy and the potential advantages of isoform- and scenario-selective inhibition of JNK in reducing side effects suggest that targeting this novel interaction of NIP and JNK may be of therapeutic value for ischemic stroke and other neurological diseases.

Funding: CIHR

Title: Calcineurin and protein phosphatase inhibitor-2 mediate fast axonal transport defects induced by amyloid beta oligomers in cultured hippocampal neurons

Authors: Kathlyn Gan¹, Emily Fan², Michael Silverman³

Affiliation: 1-3 Simon Fraser University

Abstract: "Disruption of fast axonal transport (FAT) is an early pathological event in several neurodegenerative disorders, including Alzheimer's disease (AD). Soluble amyloid beta oligomers (A β O), a causative agent of AD, induce hyperphosphorylation of the axonal microtubule-associated protein, tau; however, it is controversial how this modification disrupts transport. We have previously shown that A β O impede FAT of dense core vesicles (DCVs) in cultured neurons through a NMDA receptor-dependent mechanism that is mediated by a tau kinase, glycogen synthase kinase 3 beta (GSK3 β). Notably, this occurs without concomitant microtubule destabilization and tau hyperphosphorylation at residues responsible for microtubule binding (Ser262, Thr231). Alternatively, transport defects may result from dysregulation of signaling cascades that activate GSK3 β and inhibit motor proteins. GSK3 β is activated by protein phosphatase 1 (PP1) through reduction of autoinhibitory phosphorylation. PP1, in turn, is inhibited by protein phosphatase inhibitor-2 (I-2) and activated by calcineurin, a calcium/calmodulin-dependent phosphatase that is upregulated in the presence of A β O. To determine whether these regulators of PP1 mediate A β O-induced transport defects, we overexpressed I-2 and inhibited calcineurin using FK506 in cultured mouse hippocampal neurons. We subsequently assessed DCV transport using live imaging of fluorescently-tagged brain-derived neurotrophic factor (BDNF) and assayed tau hyperphosphorylation at Ser262 and Thr231 by immunocytochemistry. Both I-2 overexpression and calcineurin inhibition rescued A β O-induced transport defects. Furthermore, calcineurin inhibition reversed these defects with no significant change in tau hyperphosphorylation at Ser262 and Thr231. Our results indicate that PP1 dysregulation, via calcineurin and I-2, mediates A β O-induced FAT disruption independently of tau hyperphosphorylation at microtubule binding sites."

Funding: CIHR-Institute of Neurosciences, Mental Health and Addiction

Title: Levodopa induces the phosphorylation of the cytoskeletal-associated proteins Tau in the MPTP mouse model of Parkinson's Disease

Authors: Christine Robitaille¹, Fahd Awada², Laure Chagniel³

Affiliation: 1,3 Université du Québec à Trois-Rivières 2- Université de Montréal

Abstract: Parkinson's disease is associated with severe degeneration of nigrostriatal dopamine

producing neurons. Pharmacological dopamine replacement with its synthesis precursor, levodopa, remains the most effective treatment for Parkinsonism. However, long term treatment with levodopa leads to the emergence of abnormal involuntary movements known as dyskinesia. This secondary effect of levodopa appears to be triggered by an overactivation of the direct striato-nigral output pathway, known to express high levels of dopamine D1 receptors in the striatum. Recently, we have demonstrated in cell cultures model and in rats striatum slices that activation of D1 receptors could regulate functions of structural proteins such as the microtubule associated protein tau. Whether dysfunctions in cytoskeletal-associated proteins are associated to levodopa in vivo is unknown. This study investigated, in striatal neurons of MPTP mice (30mg/Kg, once daily, s.c., 5 days) receiving or not levodopa therapy (25mg/kg, twice daily, i.p., 5 injections), the expression and phosphorylation levels of tau using the western blot technique. Our results demonstrated that, although MPTP treatment was without significant effect in mice, levodopa had a profound influence on the levels of tau phosphorylated at serines 199/202 or 214 in these mice. Interestingly, this outcome was observed one hour after the last levodopa injection whereas there were no effect in animals sacrificed 24 hours post treatment. These findings demonstrated a tonic and direct effect of levodopa therapy on the levels of phosphorylated tau in depleted mice, suggesting that alterations in cytoskeletal constituents could be associated with dopamine replacement therapies in Parkinson's disease patients.

Funding: This work was supported by the NSERC.

Title: Defining a role for parkin in mitochondrion-to-lysosome vesicular trafficking

Authors: Gian-Luca McLelland¹, Vincent Soubannier², Heidi McBride³, Edward Fon⁴

Affiliation: 1,4 Montreal Neurological Institute 2,3 University of Ottawa Heart Institute

Abstract: A common cellular symptom of sporadic Parkinson's disease (PD) is mitochondrial dysfunction. Specific deficits in mitochondrial respiration in the substantia nigra have been observed in PD patient brains, and mitochondrial toxins have been shown to induce parkinsonism in rodents. Recently, parkin, a PD-associated protein, has been shown to participate in the degradation of respiration-incompetent mitochondria through the autophagic pathway upon gross depolarization of the mitochondrial membrane potential. Here, we describe the involvement of parkin in a novel vesicular pathway regulating mitochondrial quality control. Upon generation of reactive oxygen species specifically within mitochondria, parkin induces the formation of

mitochondria-derived vesicles (MDVs), which bud off mitochondria in a manner independent of Drp1, the major mitochondrial fission protein. Parkin colocalizes with a specific subset of cargo-selective MDVs, which contain markers of the mitochondrial matrix and respiratory chain, but are deficient for the outer membrane protein TOM20. These MDVs ultimately target to lysosomes for degradation. Since several PD-linked parkin mutations abrogate this process, we hypothesize that loss of parkin-dependent MDV formation may impair the ability of mitochondria to selectively degrade oxidized and dysfunctional proteins, which in turn may be detrimental to the mitochondrial reticulum in long-lived cells such as neurons.

Funding: Fonds de la recherche en santé du Québec, Parkinson Society Canada, Canadian Institutes of Health Research, Canadian Association for Neuroscience

Title: Effects of ultrasound-delivered immunotherapy on the brain of a mouse model of Alzheimer's disease

Authors: Jessica Jordao¹, Emmanuel Thévenot², JoAnne McLaurin³, Kullervo Hynynen⁴, Isabelle Aubert⁵

Affiliation: 1,2,4,5- Sunnybrook Research Institute 3- University of Toronto

Abstract: Alzheimer's disease (AD) is characterized by pathological hallmarks including plaques composed of amyloid-beta (Aβ) peptides and neuronal cell death. Neurodegeneration is progressive and leads to severe cognitive deficits. Evidence that Aβ is neurotoxic has led to several therapeutic approaches that aim to reduce Aβ load in the brain of AD patients. A major limiting factor of Aβ therapeutic agents is that their bioavailability in the brain is restricted by the blood-brain-barrier (BBB). Innovative technology capable of enhancing the permeability of the BBB, thereby allowing entry of potential therapeutics into the brain, shows great promise in circumventing this problem. The use of low-intensity focused ultrasound in the presence of a contrast agent was shown to cause localized and transient permeability of the BBB. Here, we demonstrate that a low dose of anti-Aβ antibody administered intravenously can be delivered to the brain of a transgenic mouse model of AD using MRI-guided focused ultrasound. The rate of ultrasound-induced BBB permeability was found to be proportional to endogenous IgM levels entering the brain. Efficacy of ultrasound-delivered immunotherapy was evaluated by the stereological quantification of Aβ plaques 4 days following a single treatment and it was found that Aβ burden was significantly reduced in treated animals. Synaptophysin levels were not

significantly changed, suggesting that ultrasound does not trigger neuronal damage. In conclusion, the delivery of antibody by MRI-guided ultrasound shows promise in reducing plaque load within a short survival period and at a low antibody dosage.

Funding: CIHR (JJ, JM, IA), OMHF (IA), NIH (KH)

Title: Investigating the role of the GABAA alpha2 subunit in models of addiction

Authors: Rochelle Hines¹, Dustin Hines², Stephen Moss³

Affiliation: 1-3 Tufts Medical

Abstract: Addiction is a chronic disease involving multiple brain circuits including reward, motivation, and memory pathways. Dysfunction in these circuits leads to biological, psychological, and social disruptions, costing North America an estimated \$400 billion per year. Although common pathways and mechanisms have been discovered, addiction comprises a complex group of disorders, and has been related to both genetic and environmental risk factors. Genetic studies conducted in families with multiple addicts have revealed critical targets for alcoholism and addiction. In particular, linkage and association studies have identified single-nucleotide polymorphisms in the gene encoding the GABAA alpha2 subunit (GABRA2) to be significantly associated with alcoholism and addiction to other drugs of abuse. We have developed a mouse model with a mutation in the intracellular loop of Gabra2 (Gabra2-1) to study the mechanisms by which alpha2 contributes to addiction. Gabra2-1 mice show increases in the expression of alpha2 in the nucleus accumbens and frontal cortex. In contrast to littermate controls, heterozygous and homozygous Gabra2-1 mutants do not show increases in voluntary drinking behavior using the intermittent access paradigm. Gabra2-1 mice also do not develop conditioned place preference to alcohol, demonstrating that Gabra2 may play a critical role in the rewarding effects of alcohol. Conditioned place preference and self administration of other drugs of abuse are currently being examined to determine if this mutation in alpha2 contributes generally to addictive behavior and circuitry, providing novel insights into the pathways mediating alcoholism and addiction.

Funding: Canadian Institutes of Health Research & National Institutes of Health

Title: The Parkinson's Disease-Linked Protein PINK1 Is Imported into Mitochondria and Cleaved to Regulate its Protective Function

Authors: Andrew Greene¹, Edward Fon²

Affiliation: 1,2 McGill University

Abstract: Mutations in PTEN-induced kinase 1 (PINK1) are the second most common cause of recessively-inherited Parkinson's disease (PD), a disorder characterized by neuronal loss that is thought to be at least partly due to mitochondrial defects. The PINK1 protein has an N-terminal mitochondrial targeting sequence and has recently been shown to selectively accumulate on depolarized mitochondria and recruit the E3 ligase Parkin to induce autophagy of the defective organelles. However, the mechanism by which mitochondrial membrane potential regulates PINK1 levels is only just now being elucidated. We hypothesized that PINK1 is imported into healthy, polarized mitochondria and destabilized by an intramitochondrial protease, but accumulates on the outer surface of depolarized mitochondria due to the well-established membrane potential-dependence of protein import. To test this, we undertook an RNA interference screen of all known mitochondrial proteases. PINK1 accumulated in mitochondrial fractions upon knockdown of the mitochondrial processing peptidase (MPP), the presenilin-associated rhomboid-like protease (PARL), the m-AAA protease, and the protease ClpXP. We show that PINK1 is cleaved in at least two steps, first by MPP, then by PARL and AFG3L2, to generate a cleavage product that is rapidly exported from mitochondria and degraded by the proteasome. PINK1 import and proteolytic destabilization thus act together to maintain low levels of the protein on healthy mitochondria, so that PINK1 may rapidly accumulate on mitochondria that become depolarized and induce their clearance.

Funding:

Funding: generously provided by the Canadian Institutes of Health Research (CIHR) and the Fonds de la recherche en santé (FRSQ)

Title: Impaired physiological responses and delayed axonal regeneration following nerve crush in mice overexpressing normal or ALS-linked TDP-43

Authors: Jean-Nicolas Audet¹, Vivek Swarup², Jean-Pierre Julien³

Affiliation: 1-2 Centre de Recherche du CHUQ

Abstract: "Tar DNA Binding protein 43 (TDP-43) is a protein encoded by the gene TARDBP in humans. Recent studies have demonstrated an involvement of this protein in patients suffering fronto-temporal dementia (FTLD-U) and amyotrophic lateral sclerosis (ALS). Indeed, while TDP-43 is normally a nuclear protein, cytosolic inclusions were found in most cases of FTLD-U and ALS.

To understand the mechanisms leading to pathologies involving TDP-43, lesions of the sciatic nerve were performed in mice overexpressing normal (WT) or mutant (G348C) TDP-43. Phenotypic and histological analyses were performed at different time points until 28 days after injury. After 28 days, whereas control mice had regained most of their normal mobility; transgenic mice were still noticeably paralyzed at the injured limb. Analysis of the sciatic nerve 11 days after nerve crush showed that the number of regenerating axons in the distal portion of the lesion was considerably reduced in transgenic mice. Their caliber was also slightly lower compared to normal mice. In addition, astrogliosis and microgliosis were maintained much longer in mice overexpressing WT or mutant TDP-43. Our results suggest that TDP-43 expression, especially the mutant form, is associated with axonal growth impairment following injury."

Funding: Canada Research Chair in Neurodegeneration, Canadian Institutes of Health Research

Title: COMPARISON OF THE EFFECTS OF PHENELZINE AND ITS METABOLITE PEH ON INHIBITION OF MAO-A, MAO-B, AND PRIMARY AMINE OXIDASE.

Authors: Dmitriy Matveychuk¹, Erin MacKenzie², Aldo Olivieri³, Andrew Holt⁴, Glen Baker⁵

Affiliation: 1,2,5 Neurochemical Research Unit, Department of Psychiatry, University of Alberta
3,4 Department of Pharmacology, University of Alberta

Abstract: "The antidepressant drug phenelzine (PLZ) and its metabolite beta-phenylethylidenehydrazine (PEH) have both been shown to protect neurons against toxic damage in an animal model of stroke. Both drugs inhibit GABA-transaminase (thus elevating brain GABA levels) and are known to sequester the reactive aldehyde formaldehyde. PLZ is a known substrate for, as well as a non-selective irreversible inhibitor of, monoamine oxidase (MAO), while the actions of PEH on MAO have not been extensively investigated. We have now studied the effects of PLZ and PEH as inhibitors of human MAO-A, MAO-B, and primary amine oxidase (PrAO; formerly semicarbazide-sensitive amine oxidase, SSAO) using spectrophotometric and radioligand enzyme assays. PLZ was shown to be a relatively potent inhibitor of both MAO-A and MAO-B enzymes, with respective IC₅₀ values of 30 nM and 375 nM. In contrast, PEH was a much weaker inhibitor, with respective IC₅₀ values of 129 μM and 173 μM. Both PLZ and PEH were relatively potent inhibitors of PrAO, with IC₅₀ values of 4.2 nM and 5.9 nM, respectively. As PrAO is overexpressed in Alzheimer's disease and produces toxic formaldehyde, inhibition of this enzyme could prove to have a protective effect against neurodegeneration. Although PLZ has neuroprotective properties,

there is the potential, like other drugs that inhibit MAO-A irreversibly, for a severe interaction with tyramine-containing foods. PEH does not significantly inhibit MAO-A but appears to share the neuroprotective qualities of PLZ and thus may prove to be an exciting new drug in its own right."

Funding: Funds provided by CIHR and AHFMR.

Title: Endogenous cIAP1 protects retinal ganglion cells from NMDA-induced death

Authors: Nicolas Unsain¹, Barbara Morquette², Ariel Wilson³, Adriana Di Polo⁴, Phil A Barker⁵

Affiliation: 1,5 Montreal Neurological Institute. Mcgill University 2-4 Universite de Montreal

Abstract: "Retinal ganglion cell (RGC) death is a hallmark of sight-threatening neurodegenerative diseases including glaucoma. We have previously shown that glutamate-dependent TNF α production can promote RGC demise through a non-cell autonomous mechanism and are presently attempting to dissect signalling pathways that contribute to this neuronal loss. Cellular inhibitor of apoptosis protein 1 (cIAP1) plays a critical role regulating TNF α production and signaling and previous studies have established that retinal expression of cIAP1 decreases with age. Therefore, in this study we addressed the role of cIAP1 in RGC survival in eyes that were exposed to elevated levels of NMDA. We found that genetically modified animals lacking cIAP1 expression showed a significant decrease in RGC survival compared to normal control animals. In separate experiments, we used SMAC mimetic compounds to deplete cIAP1 levels in the eye and report that this also increased the death of RGC after NMDA challenge. Thus, chronic or acute depletion of cIAP1 sensitizes RGC to NMDA-induced death."

Funding: Emerging Team Grant from the Canadian Institute of Health Research (CIHR) to PAB, ADP and Dr. Derek Bowie (McGill)

Title: Enkephalin and neurotensin promoters interact with the transcription factor Nur77 in the mouse striatum.

Authors: David Voyer¹, Emmanuelle Bourhis², Claude Rouillard³, Daniel Levesque⁴

Affiliation: 1,2,4 Université de Montréal 3- Université Laval

Abstract: Nur77 (NGFI-B, NR4A1) is a transcription factor of the orphan nuclear receptor family that is mainly expressed in brain areas receiving dopamine inputs. Treatment with dopaminergic drugs (antipsychotic and antiparkinsonian drugs) strongly modulated Nur77 mRNA levels, while genetic ablation of Nur77 (knockout) alters dopamine-mediated effects. In addition, we observed

that dopaminergic drug-induced neuropeptides enkephalin and neurotensin expression is altered in Nur77 knockout mouse striatum. Since neuropeptides are well known neuromodulators of the dopamine system, we hypothesized that Nur77 might alter dopamine-mediated effects through the modulation of neuropeptide expression. To explore this possibility, we seek for putative NGFI-B (Nur77) responsive elements (NBRE) in neuropeptide gene promoters and evaluate the interaction of Nur77 with those putative response elements using electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) with a selective Nur77 antibody. We identified multiple totally or partially conserved putative NBRE sequences in the preproenkephalin and proneurotensin gene proximal promoters, but none in the prodynorphin gene promoter. We observed gel retardation of labeled oligonucleotides containing putative NBRE sequences with in vitro synthesized Nur77 protein and preproenkephalin as well as proneurotensin gene promoter sequences. Most interestingly, interactions between the transcription factor and neuropeptide gene promoters were confirmed using ChIP from striatal tissues using a selective Nur77 antibody. These results indicate that Nur77 can interact with a subset of neuropeptide gene promoters in the striatum. It suggests that dopaminergic drug-induced Nur77 participates in the induction of neuropeptide genes in the striatum.

Funding: Supported by, Canadian Institute for Health Research, Faculty of Pharmacy and the Fonds de la Recherche en Santé du Québec.

Title: Sexual behaviour in tachykinin knockout mice

Authors: Joana Dida

Affiliation: 1- University of Toronto

Abstract: Tachykinin peptides influence complex social behaviours in mice. We investigated whether removal of genes for TAC1-encoded peptides substance P (SP) and neurokinin A (NKA) and the TAC4-encoded peptide hemokinin-1 (HK-1) as well as their preferred receptor neurokinin-1 (NK-1R) influence sexual behaviours by using tachykinin knockout mice. TAC1^{-/-}, TAC4^{-/-} and TACR1^{-/-} mice were compared to C57BL/6 wild type mice. To exclude the possibility of compensatory mechanisms such as SP/NKA compensating for the lack of HK-1 and vice versa, we generated TAC1/4^{-/-} “double knockout mice”, deficient for both TAC1 and TAC4-encoded peptides. To test whether mutant mice respond to social odours of the opposite sex in a similar fashion, we used the female urine sniffing test. We performed the olfactory

habituation/dishabituation test to test whether an animal can smell and is able to differentiate odours. Ultrasonic vocalizations (USVs) were investigated in male-female mating studies. Male mice deficient for the NK-1R, but not for its ligands SP/NKA and/or HK-1, exhibited an impairment of olfactory investigation of female urine. The decreased olfactory investigation of female urine in TACR1-/- mice was not due to an inability to smell or distinguish different odours as they showed similar patterns of olfactory habituation/dishabituation as wild-types. Compared to controls, male TACR1-/- mice displayed significantly reduced vocalizations and sexual behaviours towards females. NK-1R signalling facilitates olfactory investigation of female urine and sexual behaviour including increased USVs. The lack of change in these behaviours in mice deficient for SP/NKA and/or HK-1 suggests that NKB may play a role in these pathways.

Funding: OMHF grant to Dr. John Yeomans CIHR grant to Dr. Christopher Paige

Title: Live Imaging of immune response in pre-onset ALS

Authors: Mathieu Gravel¹, Essam Abdelhamid², Jasna Kriz³

Affiliation: 1-3 Université Laval

Abstract: Although major clinical symptoms in Amyotrophic Lateral Sclerosis (ALS) disease arise from the degeneration and death of motor neurons, recent results from transgenic animal models suggest involvement of a “non-cell autonomous” mechanism. Thus excitotoxicity from glial cells dysfunction and inflammatory processes associated with ALS are likely to contribute in the neurodegenerative onset and propagation process. Microglial activation in response to inflammation is associated with significant induction of several Toll-like receptors (TLRs). In an attempt to better understand the events of early pathogenesis leading to neuronal death, we generated double transgenic mice from the SOD1G93A mouse and the TLR2-Luc-GFP reporter mouse, a transgenic mouse model bearing the dual reporter system luciferase and green fluorescent protein under the transcription control of the murine TLR2 promoter. These double transgenic mice and littermates controls were then monitored using in-vivo biophotonic/bioluminescent imaging to assess the microglial response over the course of the disease. In addition to the longitudinal imaging, these mice were treated with lipopolysaccharide to induce a strong microglial response. Following activation with LPS, the analysis of the TLR2 induction from the spinal cord, brain and olfactory bulb all suggested a sub-optimal microglial response in the SOD1G93A mice when compared to wild-type controls. Combined with immuno-

fluorescent labeling, in-situ hybridization and cell culture experiments, our results suggest a defective microglial response.

Funding: Canadian Institutes of Health Research ALS Society of Canada

Title: Exploring new drugs which can affect the viability and transformation of childhood medulloblastomas

Authors: Norbert F Ajeawung¹, Helena Michielin Pavel², Robert Faure³, Joshi Harish⁴, Donald Poirier⁵, Deepak Kamnasaran⁶

Affiliation: 1-3, 5, 6 Université Laval 4- Emory University

Abstract: BACKGROUND AND OBJECTIVES: Medulloblastoma is one of the most common malignant tumour of the central nervous system in newborn infants and children, accounting for about 15-20% of pediatric brain tumors. Despite current diagnostic and therapeutic advances, the morbidity and mortality rates still remain high. Furthermore, children who survive medulloblastoma are at risk of long-term sequelae related to the neurological effects of the tumor and treatments from surgery, radiotherapy and chemotherapy. Therefore, it is of great importance to identify new anticancer drugs, which can assist in improving the survival of children and with minimal or no side-effects. OBJECTIVES: Our primary objective was to identify the efficacy of new structural classes of drugs belonging to a family of steroid biogenesis inhibitors, the peroxovanadium superfamily and derivatives of active compounds found in opium. RESULTS: Our data so far demonstrated that the panel of new structural classes of drugs examined can significantly affect the viability and transformation of the D324 medulloblastoma pre-clinical cell line model. Most importantly, the toxicity of our drugs is almost devoid on our non-transformed control cell lines. Current work continues to explore the efficacy of these drugs on additional malignant medulloblastoma pre-clinical cell line models, in conjunction with complementing our in-vitro findings with in-vivo tumor assays. CONCLUSIONS This body of work is novel and highly significant in our effort to discover improved treatments for childhood brain tumors.

Funding: Fondation des étoiles, Canadian Foundation for Innovation (Leaders Opportunity Funds) and Fonds de la recherche en santé du Québec

Title: Potential of adult human skin-derived precursor cells for BDNF, GDNF and dopamine expression: implications for cell-based therapy

Authors: Remi Parenteau-Bareil¹, Caroline Auclair-Daigle², Janelle Drouin-Ouellet³, Francesca Cicchetti⁴, François Berthod⁵

Affiliation: 1,2,5 Centre LOEX de l'Université Laval 3,4 CRCHUL (CHUQ)

Abstract: Introduction: Skin-derived precursor cells (SKPs) have shown therapeutic potential for autologous cell-based treatments of neurological diseases. Objective: We aimed to investigate the potential of adult human SKPs to produce and release brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF) and dopamine (DA), to develop new therapeutic strategies for Parkinson's disease (PD). Methods: SKPs were obtained after enzymatic digestion of skin biopsies. Cells were cultured as floating spheres in medium containing epidermal growth, leukemia inhibitory and basic fibroblast growth factors. After expansion, spheres were triturated and seeded on poly-d-lysine/laminin coated flasks and cover slips. Dopaminergic-like cells were obtained after three weeks of differentiation in various cocktails of morphogens and neurotrophins. DAergic-like cells were analyzed for generic and dopaminergic neuronal markers expression such as tyrosine hydroxylase (TH) using immunofluorescence and flow cytometry techniques. RT-PCR analysis was used to assess Nurr-1 expression, a dopaminergic phenotype triggering gene. BDNF, GDNF and DA secretion by differentiated cells was quantified by ELISA. Results: TH expression was assessed by immunofluorescence and flow cytometry (33% of differentiated SKPs expressing TH after 21 days of differentiation (D21). Vesicular monoamine transporter-2 and dopamine transporter were also detected by immunofluorescence. BDNF, GDNF and dopamine (~440 pg/ml, ~1800 pg/ml and ~12 ng/ml at D21) were secreted by differentiated SKPs. Finally, Nurr-1 expression was also observed by RT-PCR. Conclusion: Adult human SKPs have the capacity to differentiate into DA-like neurons and to secrete growth factors. These results are promising for the development of alternative cell-based treatments for PD.

Funding: Canadian Institutes of Health Research

Title: Behavioral recovery after immediate inhibition of the contralesional hemisphere following ischemic lesions in rats

Authors: Babak Khoshkrood Mansoori¹, Leah Rose Feldman², Stephan Quessy³, Numa Dancause⁴,

Affiliation: 1-4 University of Montreal

Abstract: "Objective: Recent studies in humans have provided evidence that inhibition of the contralesional hemisphere can improve motor function after stroke. To date, the effect of

continuous inhibition initiated immediately after stroke has never been tested. Therefore, we evaluated the effect of sustained inhibition of the contralesional hemisphere initiated immediately after the lesion on behavioral recovery in a rat model of ischemic stroke. **Methods:** In 8 female adult rats, ischemic lesions were induced with 6 microinjections of endothelin-1 (400pmol/ μ l, 2 μ l, 0.4 μ l/minute) in the caudal forelimb area (CFA) and one microinjection in the dorsal striatum of the left hemisphere. Four rats were immediately implanted with a muscimol osmotic pump (10mM, 0.5 μ l/hr, for 14 days) in contralesional M1 CFA. The remaining 4 rats were sham-operated with a metal rod of similar diameter as the osmotic pump cannula implanted in the contralesional CFA. Recovery of hand function was evaluated weekly over a period of 8 post-operative weeks with the Montoya staircase test. **Results:** Pre-operative staircase data were identical in both groups of rats for both forelimbs. For the impaired forelimb, each group showed significant deficit from its baseline in the first week after surgery (Tukey HSD; $p < 0.05$). When comparing the two groups, although there was no difference in the first 3 weeks after lesion, in weeks 4 to 8, the experimental group showed significantly better recovery (t-test; $p < 0.05$). **Conclusion:** Our preliminary data suggest that inhibition of the contralesional cortex initiated immediately after an ischemic lesion can increase functional recovery in adult rats. "

Funding: BKM: Bourse de Recrutement de Departement de Physiologie LRF: Canadian Stroke Network ND & SQ: Power Corporation Chair

Title: The Role of OPA1 in Mitochondrial Function during Starvation

Authors: David Patten¹, Marc Germain², Karine Pilon-Larose³, Ruth Slack⁴

Affiliation: 1-4 University of Ottawa

Abstract: The cell possesses different mechanisms to adapt to changes in nutrient availability, one of which is autophagy. Another such mechanism is the proposed hypothesis that changes in mitochondrial ultrastructure regulates mitochondrial oxidative phosphorylation by limiting diffusion of specific metabolites to the electron transport chain. Until recently, a molecular tool to investigate this hypothesis has been lacking. OPA1, the primary gene responsible for dominant optic atrophy, is classically known as a mitochondrial GTPase responsible for inner membrane fusion but can also regulate cristae structure. It has been previously demonstrated that cristae structure is maintained by OPA1 complexes and that these complexes disassemble during cell death, releasing pro-apoptotic factors. Whether or not OPA1 complexes respond to different

energetic states and help to regulate OXPHOS through cristae modulation is the focus of this work. In isolated liver mitochondria we demonstrate that Complex I and Complex II substrates reduce the levels of OPA1 complexes, in line with previous reports showing enlarged cristae under these same conditions. These complexes are very dynamic and reversible, in agreement with Hakenbrock's original findings on cristae architecture. Interestingly, the mitochondrial uncoupler CCCP had no effect on OPA1 complexes under these conditions. Consistent with these results, intact cells starved by various means show an increase in OPA1 complexes directly preceding instances of increased autophagy (as analyzed by p62 degradation). Studies showing the direct contribution of OPA1 complexes on mitochondrial OXPHOS and protein interactors required for OPA1 oligomerization are currently ongoing.

Funding: Supported by CIHR to RSS

Title: The role of the histamine H1 receptor in rats' anxiety-related behaviours in two animal models of anxiety

Authors: San-San Chee¹, Janet Menard²

Affiliation: 1-2 Queen's University

Abstract: "Histaminergic neurons are localized in the tuberomammillary nucleus of the posterior hypothalamus (TM), where they project to almost all regions of the brain. Histamine exerts its effects via four receptor subtypes: H1, H2, H3, and H4. The neural histaminergic system regulates various physiological processes including arousal, homeostasis, pain, and learning. More recent research suggests that histamine may also regulate anxiety; for example, destruction of the rat TM reduces defensive behaviours in the elevated plus maze (EPM). Interestingly, the TM shares bidirectional connections with the lateral septum (LS), an area well implicated in anxiety. Lesions or pharmacological perturbations of the LS decrease anxiety responses in several animal models, including the EPM and the shock probe burying test (SPBT). Prior work in our laboratory found that bilateral infusions of histamine (0.5-µg/side) reduced anxiety-related behaviours in two animal models of anxiety, the EPM and novelty-induced suppression of feeding paradigm (NISF), while having no effects in a third model, the SPBT. These results suggest that histamine, when infused into the LS, is anxiolytic, and this effect is test-specific. In the current study, pre-treatment with the H1 receptor antagonist pyrilamine (10-µg/side) blocked the anxiolytic effects of histamine in the NISF but not in the EPM, indicating that the H¹ receptor regulates rats'

neophagic responses in the NISF but not their defensive behaviours in the EPM. "

Funding: NSERC

Title: Peripheral neurostimulation combined to motor training in chronic low back pain : influence on cortical motor excitability and anticipatory postural adjustment

Authors: Hugo Massé-Alarie¹, Véronique Flamand², Hélène Moffet³, Cyril Schneider⁴

Affiliation: 1,2,4 Centre de recherche du CHUQ - Neurosciences, 3- Centre interdisciplinaire de recherche en réadaptation et intégration sociale (CIRRS), Québec

Abstract: "Objective(s): Chronic low back pain (CLBP) is associated to a faulty volitional activation of the transversus abdominis muscle (TrA). An underlying mechanism could be a maladaptive reorganization of TrA motor cortical representation (M1) and a correlated delay of TrA activation during anticipatory postural adjustment (APA). Training at TrA hollowing seems to improve this dysfunction. Also, new evidences suggest that repetitive magnetic stimulation of nerves provides a therapeutic window to improve motor control. Our study thus combined peripheral neurostimulation and TrA hollowing training for further improving TrA activation and normalizing M1 excitability. Methods: Sixteen CLBP were randomized in two groups [Sham stimulation + TrA hollowing training] (ST) and [Neurostimulation + Training] (NT). The effects were tested on M1 excitability and TrA anticipatory activation before and after Sham/Neurostimulation alone and after ST/NT combination. Pre/post-experiment evaluation was administrated by a blinded physical therapist (functional/beliefs questionnaires, physical examination). Results: NT group presented a marginal improvement of M1 excitability (135% vs -48% for ST) and TrA anticipatory activation (65% vs 26% for ST). ANOVA detected a triple Group x Isometric x Time interaction ($F_{2,4}=281,54$; $p \leq 0,0001$; Isometric was a pre-experiment evaluation of TrA activation disorder) with M1 excitability and TrA anticipatory activation both improved post-NT in patients presenting TrA activation disorder at enrollment. Conclusions: Post-neurostimulation increase of M1 excitability may have facilitated motor training during TrA hollowing thus leading to normalize TrA anticipatory activation patterns. These preliminary data also suggest that intervention success could be predicted by clinical testing of the initial ability to contract TrA. "

Funding: FRSQ

Title: Antipsychotic drug-induced tardive dyskinesia in a primate model: role of the transcription factor Nur77

Authors: Daniel Levesque¹, Souha Mahmoudi², Marie-Thérèse Parent³, Pierre J. Blanchet⁴

Affiliation: 1,2 University of Montreal, Faculty of Pharmacy 3,4 University of Montreal, Faculty of Dentistry, Stomatology department

Abstract: Tardive dyskinesia (TD) is a motor complication arising in patients chronically exposed to antipsychotic drugs. Despite the fact that new generations of atypical antipsychotic drugs has a lower propensity to generate TD, increasing clinical conditions in which antipsychotic drugs are chronically prescribed, in particularly vulnerable individuals such as elderly and bipolar patients, will inevitably has for consequence that TD is not going to disappear. The pathophysiology of TD remains elusive and therapeutics difficult, so experimental modeling is more important than ever. We have exposed young adult capuchin (*Cebus apella*) monkeys to prolonged treatments with haloperidol (6-18 months, N=9) or clozapine (6 months, N=6). A group of untreated animals (N=6) was used as controls. An Abnormal Involuntary Movements (AIMs) scale was used for TD rating. Four out of 9 haloperidol-treated animals developed TD. No significant TD was observed in the clozapine group. The abnormal purposeless movements were similar to those found in humans and typically stereotyped in nature. Variable orofacial dyskinetic movements were seen, including forehead contractions, chewing movements, tongue protrusions, and lip retraction. Brain post mortem analysis of the expression of Nur77, a transcription factor of the nuclear receptor family closely associated with dopamine-mediated effects, was performed using in situ hybridization. Haloperidol strongly up-regulated Nur77 mRNA levels in the caudate-putamen, whereas clozapine tended to reduce striatal Nur77 expression. Interestingly, within haloperidol-treated animals, only non dyskinetic animals showed increased Nur77 expression. This suggests that Nur77 expression might be associated with a reduced risk to TD.

Funding: This work was supported by the Canadian Institutes for Health Research (CIHR).

Title: PERIPHERAL IMMUNITY OF THE TRIPLE TRANSGENIC MOUSE MODEL OF ALZHEIMER DISEASE

Authors: Isabelle St-Amour¹, Isabelle Paré², Cassandra Ringuette-Goulet³, Renée Bazin⁴, Frédéric Calon⁵

Affiliation: 1,5 CRCHUL 2-4 Héma-Québec

Abstract: "Immunologic abnormalities have been described in peripheral blood and central nervous system of patients suffering from Alzheimer disease (AD), yet their role in the development of the pathology remains largely unknown. The triple transgenic mouse (3xTg-AD) is used to reproduce the neuropathological hallmarks of AD which are A β (amyloid plaques) and tau (neurofibrillary tangles) pathologies and other AD features. In this study, we investigated important immunologic parameters in the brain, the spleen and the peripheral blood of 9, 13 or 16 month-old 3xTg-AD mice using multiplex ELISA and cytometric analysis, compared to age-matched non-transgenic littermates (NonTg). Significant differences in cytokine concentrations were observed before the modulations of cell populations. Specifically, we found significant ($p < 0.05$) increases of serum GM-CSF (3.5X), TNF- α (14X), IL-5 (8.8X) and IL-12 (8.7X) in 9 month-old 3xTg-AD mice and of IL-2 (13.2X) and TNF- α (4.4X) in 13 month-old 3xTg-AD mice compared to NonTg. On the other hand, a decrease in peripheral B (-42% and -36%, $p < 0.05$) and T lymphocytes (-53% and -40%, $p < 0.001$) associated with increased activation was observed in 13 and 16 month-old 3xTg-AD mice but not at 9 month. Moreover, higher IL-3 (2X, $p < 0.001$), MIP-1 α (2.6X, $p < 0.05$) and IFN γ (2.2X, $p < 0.02$) concentrations were observed in the parieto-temporal cerebral cortex of 16 month-old 3xTg-AD mice compared to NonTg. Collectively, these results suggest that the 3xTg-AD model reproduces age-dependent immunologic modifications observed in human AD patients and represents a suitable model to study the role of immune system defects in the pathogenesis of AD."

Funding: Fondation Héma-Québec, IRSC, CRSNG, FQRNT, Talecris

Title: Magnetic stimulation of nerves in chronic stroke: an exploratory TMS protocol on the changes of sensorimotor function

Authors: Louis-David Beaulieu¹, Brenda Brouwer², Cyril Schneider³

Affiliation: 1-3 Université Laval

Abstract: "Objective(s): To elucidate the neurophysiological and clinical effects of a specific repetitive magnetic stimulation protocol, intermittent theta-burst stimulation of the peripheral nervous system (iTBS), in healthy individuals and patients with chronic stroke. Specific objectives are: (1) to measure whether iTBS applied to the common peroneal nerve (CPN of the dorsiflexor muscles, as tibialis anterior-TA) influence the primary motor cortex (M1) excitability; (2) to determine whether M1 changes, if any, are paralleled by improvement of foot function in

stroke subjects; and (3) to test if clinical improvement is carried-over to walking ability. We expect the application of iPTBS to influence M1 excitability associated with TA, increase muscle strength and improve locomotor patterns. Methods: Sixteen subjects with chronic stroke and 16 healthy controls will be allocated to two groups: (A) iPTBS to CPN; (B) sham iPTBS to CPN. The double-blinded study will evaluate each subject for clinical (spasticity, dorsi/plantarflexion range of motion, TA strength), neurophysiology (corticomotor and intracortical excitability, TA reaction time testing) and mobility measures (muscle activation pattern during gait) in 2 sessions: (1) at baseline without iPTBS; (2) pre/post-iPTBS or pre/post-sham. Only neurophysiological testing will be conducted in controls. Results: Descriptive statistics and ANOVA (repeated measures, post-hoc tests) will help detect between-protocol and between-group differences and Spearman/Pearson coefficients will test for relationships between neurophysiological and clinical measures. Conclusions: This original study with healthy and stroke subjects will determine further the neurological effects of iPTBS and provide indication about its potential benefits as an adjunct to rehabilitation therapy. "

Funding:

Title: Antipsychotic drug-induced tardive dyskinesia in a primate model: role of the transcription factor Nur77

Authors: Souha Mahmoudi¹, Marie-Thérèse Parent², Daniel Levesque³

Affiliation: 1,3 Faculty of Pharmacy, University of Montreal 2- Faculty of Dentistry, Stomatology Dept., University of Montreal

Abstract: Tardive dyskinesia (TD) is a motor complication arising in patients chronically exposed to antipsychotic drugs. Despite the fact that new generations of atypical antipsychotic drugs has a lower propensity to generate TD, increasing clinical conditions in which antipsychotic drugs are chronically prescribed, in particularly vulnerable individuals such as elderly and bipolar patients, will inevitably has for consequence that TD is not going to disappear. The pathophysiology of TD remains elusive and therapeutics difficult, so experimental modeling is more important than ever. We have exposed young adult capuchin (*Cebus apella*) monkeys to prolonged treatments with haloperidol (6-18 months, N=9) or clozapine (6 months, N=6). A group of untreated animals (N=6) was used as controls. An Abnormal Involuntary Movements (AIMs) scale was used for TD rating. Four out of 9 haloperidol-treated animals developed TD. No significant TD was observed in the

clozapine group. The abnormal purposeless movements were similar to those found in humans and typically stereotyped in nature. Variable orofacial dyskinetic movements were seen, including forehead contractions, chewing movements, tongue protrusions, and lip retraction. Brain post mortem analysis of the expression of Nur77, a transcription factor of the nuclear receptor family closely associated with dopamine-mediated effects, was performed using in situ hybridization. Haloperidol strongly up-regulated Nur77 mRNA levels in the caudate-putamen, whereas clozapine tended to reduce striatal Nur77 expression. Interestingly, within haloperidol-treated animals, only non dyskinetic animals showed increased Nur77 expression. This suggests that Nur77 expression might be associated with a reduced risk to TD.

Funding: This work was supported by the Canadian Institutes for Health Research (CIHR).

Title: Depletion of cholinergic neurons in the nucleus accumbens: potential animal model of schizophrenia.

Authors: François Laplante¹, Ron Sullivan²

Affiliation: 1-2 McGill University

Abstract: Studies of post mortem schizophrenic brains have revealed a loss of cholinergic interneurons, most pronounced in the ventral striatum. We proposed that this local cholinergic deficit leads to dysfunction of both mesolimbic and mesocortical dopamine (DA) systems of relevance to schizophrenic symptomatology. We have reproduced this pathological feature in rats and investigated on the functional and behavioral consequences of such lesions. The microinjection of a novel saporin immunotoxin into the nucleus accumbens (N. Acc) of adult rats, reduced the number of cholinergic neurons in N. Acc by 40-50 %. Such lesions resulted in a heightened response to the locomotor activating effects of amphetamine. Lesioned rats also showed impaired sensorimotor gating (prepulse inhibition of the acoustic startle response) which was reversed by the antipsychotic haloperidol, suggesting a postsynaptic upregulation of DAergic mechanisms in the N.Acc. In addition, lesioned rats displayed working memory deficits. Some rats were implanted with voltammetric recording electrodes in the N.Acc and prefrontal cortex to examine the increases in in vivo extracellular DA release in response to a brief tail pinch stress. Lesioned rats showed a significantly reduced activation of both mesolimbic and mesocortical DA system compared to controls. Taken together, the data suggest that reduction in the density of cholinergic neurons in the N.Acc produces behavioral alterations relevant to schizophrenic

symptomatology but also triggers deficits in prefrontally-mediated function known to be under mesocortical DAergic regulation. This raises the possibility that ventral striatal cholinergic deficits may be causally linked to cortical/subcortical functional imbalances proposed to exist in schizophrenia.

Funding: Supported by CIHR, FRSQ and NARSAD.

Title: Alteration of network oscillations in Schizophrenia mouse model

Authors: Frederic Manseau¹, Lalit Srivastava², Sylvain Williams³

Affiliation: 1-3 Douglas Mental Health University Institute

Abstract: "Rhythmic oscillations engaging coordinated activity of large neuronal ensembles are increasingly recognized as a central mechanism for encoding and transferring information in the brain. Cognitive disruption in schizophrenia is associated with altered patterns of brain rhythms and may be related to changes in dynamic interactions within neuronal networks. Mice harboring a null mutation for dysbindin-1 (a top candidate gene with reduced expression in schizophrenia) show deficits in object recognition and social interaction. To test the hypothesis that dysbindin-1 knockout (dby-KO) animals may show alterations of network oscillations, we used the complete in-vitro mouse hippocampus; a preparation developed in our laboratory, which displays powerful field oscillations in the theta (2-12 Hz) and gamma frequency bands (25-55 and 150-300 Hz; slow-gamma (SG) and fast-gamma (FG) respectively). We performed multiple local field potential recordings of spontaneous activity from the subiculum; the major output region of the hippocampus. Consistent with our previous studies, the amplitude of gamma oscillations was modulated by theta band activity, and FG oscillations were highly coherent within distances of over hundreds of $\sim\mu\text{m}$ in the subiculum. Our preliminary results suggest however that the coupling between theta and gamma may be altered in dby-KO mice compared to WT (n = 5 and 6 respectively; Modulation Index: 0.26 \pm 0.02 vs. 9.24 \pm 0.93 for SG; 3.4 \pm 0.35 vs. 12.09 \pm 1.03 for FG; P<0.001). The decrease in coherence of FG recorded at progressively more distant sites (75-600 $\sim\mu\text{m}$) within subiculum was not significantly different between dby-KO and WT mice. These results suggest that dysbindin-1 may regulate hippocampal oscillations."

Funding:

Title: The effect of Alzheimer's dementia on the volumes of the Left Hippocampus

Authors: Ammar Mahmood¹

Affiliation: 1- Simon Fraser University

Abstract: "The incidence of neurodegenerative diseases such as dementias, in particular, Alzheimer's, have steadily increased in the western societies over the last few decades. Though the individual illness may vary in severity, these dementias typically constitute a heavy burden and decreased quality of life for those affected. There is thus an urgent need to develop biomarkers that can assist in early identification of the presence of dementia as well the type of dementia. Volumetric size of certain brain components is an obvious biomarker to study and correlate to presence of neurodegenerative disease and its progression. Medical resonance (MR) imaging is frequently employed to acquire the structural imagery of brain tissues for use in quantifying volumes of brain tissue structures. In particular, in Alzheimer's, based on pathology, volumes of regions such as the hippocampus and ventricles are expected to alter and correlate with progression of the disease. In this paper, I will be analyzing volumes of the left hippocampus from a set of 486 subjects with mild and severe Alzheimer's taken from the Alzheimer disease neuroimaging initiative (ADNI) to assess their ability to inform about the presence of dementia as compared to normal healthy age-matched controls. Hypothesis: Volume of the hippocampus would be correlated with disease status; with subjects having severe Alzheimer's having smaller hippocampi than those with mild Alzheimer's, who in turn would have smaller hippocampi than age-matched control subjects. "

Funding:

Title: MCL-1 is a stress sensor that regulates autophagy in a developmentally regulated manner.

Authors: Marc Germain¹, Angela Nguyen², Nicole LeGrand³, Nicole Arbour⁴, Jacqueline Vanderluit⁵, David Park⁶, Joseph T. Opferman⁷, Ruth Slack⁸

Affiliation: 1-6,8 University of Ottawa 7- St. Jude Children's Research Hospital

Abstract: Apoptosis has an important role during development to regulate cell number. In differentiated cells, however, activation of autophagy has a critical role by enabling cells to remain functional following stress. In this study, we show that the antiapoptotic BCL-2 homologue MCL-1 has a key role in controlling both processes in a developmentally regulated manner. Specifically, MCL-1 degradation is an early event not only following induction of apoptosis, but also under

nutrient deprivation conditions where MCL-1 levels regulate activation of autophagy. Furthermore, deletion of MCL-1 in cortical neurons of transgenic mice activates a robust autophagic response. This autophagic response can, however, be converted to apoptosis by either reducing the levels of the autophagy regulator Beclin-1, or by a concomitant activation of BAX. Our results define a pathway whereby MCL-1 has a key role in determining cell fate, by coordinately regulating apoptosis and autophagy.

Funding: Supported by a grant from HSFC to RSS. MG is the recipient of research fellowships from the HSFC and PSC.

Title: Regulation of autophagy by the BCL2 homologues MCL1 and BAX.

Authors: Larisa Romanova¹, Marc Germain², Angela P. Nguyen³, Ruth S. Slack⁴

Affiliation: 1-4 University of Ottawa

Abstract: "Under starvation conditions, autophagy becomes activated and allows for recycling of nutrients and subsequent cell survival. In some circumstances however, autophagy can contribute to cell death. This occurs for example following ischemic stroke, where neurons are acutely deprived of oxygen and nutrients, activating both autophagy and classical cell death pathways such as apoptosis and necrosis. At the molecular level, the BCL2 family of proteins regulate both apoptosis and autophagy. MCL1, an anti-apoptotic BCL2 homologue, is degraded not only during apoptosis, but also following induction of autophagy. We have recently shown that MCL1 is a key regulator of both apoptosis and autophagy, and that this is regulated in a developmentally regulated manner. Specifically, we generated MCL1 conditional knockout mice by using the CaMKIIa promoter. These animals show massive neuronal cell loss in cortical layers along the corpus callosum and reduced number of cells at the sensory-motor cortex and hippocampus. By investigating further, we found that post-mitotic neurons have a signs of increased autophagy with limited activation of apoptotic machinery. To further understand the neuronal cell death, MCL1 conditional knockout mice were crossed with BAX knockout animals, BAX being a pro-apoptotic BCL2 homologue. MCL1; BAX double knockout mice live longer while deletion of BAX partially rescues the neuronal cell loss. We are currently addressing the role of BAX in the regulation of autophagy."

Funding: Supported by HSFO and CIHR grants to RSS2. LR3 is supported by University of Ottawa Excellence scholarship and CIHR scholarship.

Poster Category D : Sensory and Motor Systems

Title: Salicylate-induced tinnitus triggers serotonergic-dependant anxiety

Authors: Christine Marquilly¹, Milomir Stefanovic², Matthieu Guitton³

Affiliation: 1-3 Faculty of Pharmacy, Laval University

Abstract: Tinnitus, the perception of sound in the absence of external acoustic stimulation, severely alters the quality of life of sufferers. Over the years, clinical and animal studies have reported a correlation between occurrence of tinnitus and high levels of anxiety. Our results show that salicylate-induced tinnitus in mice cause anxiety-like behaviors similar to anxiety induced by anxiogenic agents. Dose-response analysis of the development of anxiety-like behavior under salicylate treatment shows that anxiety appears only following a threshold related to the actual occurrence of tinnitus. In addition, anxiety built over time following a similar temporal pattern that the ones observed using behavioral models of tinnitus, thus reinforcing the notion that salicylate-induced anxiety is actually linked to salicylate-induced tinnitus. Surprisingly, the co-injection of a serotonergic agent with salicylate did not exacerbate anxiety levels. In contrast, such treatment abolished anxiety-like behaviors, thus suggesting that salicylate-induced tinnitus-induced anxiety is mediated, at least partially, by the serotonergic system.

Funding: This work has been supported by the CIHR.

Title: Recovery of hindlimb locomotion involves spontaneous compensatory changes within the spinal locomotor circuitry after incomplete spinal cord injury in the cat

Authors: Marina Martinez¹, Hugo Delivet-Mongrain², Hugues Leblond³, Serge Rossignol⁴

Affiliation: 1-4 Université de Montréal

Abstract: After incomplete spinal cord injury (SCI), compensatory changes occur throughout the whole neuraxis including the spinal cord below the lesion as suggested by previous experiments using a dual spinal lesion paradigm. Indeed, after a lateral spinal hemisection at T10-11 and locomotor training for 2-14 weeks, cats were completely spinalized at T13. Trained cats expressed bilateral spinal hindlimb locomotion as soon as 24 hours after spinalization, a process which normally takes 2-3 weeks when a complete spinalization is performed without the prior hemisection. To study the importance of timing and locomotor training after hemisection, the interval between the two SCI was fixed to 3 weeks and cats were not treadmill-trained but were

recorded only once a week. After the unilateral hemisection, compensatory left/right asymmetries were observed in several kinematic parameters and in the coupling of flexor and extensor muscles. As early as 24 hours after the complete spinalization, all cats except two were able to re-express hindlimb locomotion either bilaterally (n=6) or unilaterally on the side of the previous hemisection (n=3). The asymmetries previously observed after hemisection disappeared or were even reversed after spinalization. The early expression of hindlimb locomotion after the complete spinalization suggests that the spinal cord has been changed by new dynamic interactions between the spinal and supraspinal structures during the hemispinal period. Such recovery can be obtained without specific locomotor training even when the delay between the two lesions is as short as 3 weeks suggesting potent intrinsic spinal mechanisms of recovery.

Funding: IRSC, Equipe Multidisciplinaire en Réadaptation Locomotrice (EMRL)

Title: Chronic treatment with MPEP, a metabotropic glutamate receptor type 5 antagonist, prevents the development of levodopa-induced dyskinesias in de novo parkinsonian monkeys

Authors: Nicolas Morin¹, Laurent Grégoire², Baltazar Gomez-Mancilla³, Fabrizio Gasparini⁴, Thérèse Di Paolo⁵

Affiliation: 1,2,5 Molecular Endocrinology and Genomic Research Center Université Laval 3,4- Novartis Institute for BioMedical Research

Abstract: Brain glutamate overactivity is well documented in PD and antiglutamatergic drugs have been proposed to relieve PD symptoms and decrease dyskinesias. Metabotropic glutamate receptors are topics of recent interest in PD. This study sought to prevent the development of levodopa-induced dyskinesias with the addition of the metabotropic glutamate receptors type 5 (mGluR5) antagonist MPEP in monkeys with a 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) lesion to model Parkinson disease (PD). These MPTP monkeys were naive to dopaminergic drug treatments. Eight L-Dopa ovariectomized female *Macaca fascicularis* MPTP monkeys were treated once daily for one month with L-Dopa or with L-Dopa and MPEP (10 mg/kg, given 15 minutes prior to L-Dopa). Motor behavior was measured for all the duration of the L-Dopa motor effect and during one month. The antiparkinsonian activity of MPTP monkeys treated with MPEP and L-Dopa was generally maintained during the month as measured with locomotion and antiparkinsonian scores as compared to L-Dopa alone. The mean dyskinesia score significantly increased over a month in the levodopa alone treated group, by contrast it was lower

by 76%, 63%, 67% and 78% in levodopa+MPEP treated MPTP monkeys respectively from week 1 to week 4. The duration of the levodopa effect decreased in the L-Dopa alone treated group modeling wearing-off while the decrease was not significant in presence of MPEP. This study showed a beneficial chronic antidyskinetic effect of blocking mGluR5 in L-Dopa-treated MPTP monkeys thus supporting the therapeutic use of an mGluR5 antagonist to maintain normal brain glutamate neurotransmission in PD and prevent dyskinesias.

Funding: TDP: CIHR. NM:Parkinson Society Canada. F. Gasparini and B. Gomez-Mancilla are employees of Novartis

Title: Chronic treatment with MPEP, a metabotropic glutamate receptor type 5 antagonist, prevents the development of levodopa-induced dyskinesias in de novo parkinsonian monkeys

Authors: Nicolas Morin¹, Laurent Grégoire², Baltazar Gomez-Mancilla³, Fabrizio Gasparini⁴, Thérèse Di Paolo⁵

Affiliation: 1,2,5 Molecular Endocrinology and Genomic Research Center Université Laval 3,4 Novartis Institute for BioMedical Research

Abstract: Brain glutamate overactivity is well documented in PD and antiglutamatergic drugs have been proposed to relieve PD symptoms and decrease dyskinesias. Metabotropic glutamate receptors are topics of recent interest in PD. This study sought to prevent the development of levodopa-induced dyskinesias with the addition of the metabotropic glutamate receptors type 5 (mGluR5) antagonist MPEP in monkeys with a 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) lesion to model Parkinson disease (PD). These MPTP monkeys were naive to dopaminergic drug treatments. Eight L-Dopa ovariectomized female *Macaca fascicularis* MPTP monkeys were treated once daily for one month with L-Dopa or with L-Dopa and MPEP (10 mg/kg, given 15 minutes prior to L-Dopa). Motor behavior was measured for all the duration of the L-Dopa motor effect and during one month. The antiparkinsonian activity of MPTP monkeys treated with MPEP and L-Dopa was maintained during the month as measured with locomotion and antiparkinsonian scores as compared to L-Dopa alone. The mean dyskinesia score significantly increased over a month in the levodopa alone treated group, by contrast it was lower by 76%, 63%, 67% and 78% in levodopa+MPEP treated MPTP monkeys respectively from week 1 to week 4. The duration of the levodopa effect decreased in the L-Dopa alone treated group modeling wearing-off while the decrease was not significant in presence of MPEP. This study

showed a beneficial chronic antidyskinetic effect of blocking mGluR5 in L-Dopa-treated MPTP monkeys thus supporting the therapeutic use of an mGluR5 antagonist to maintain normal brain glutamate neurotransmission in PD and prevent dyskinesias.

Funding: TDP: CIHR. NM: Parkinson Society Canada. F. Gasparini and B. Gomez-Mancilla are employees of Novartis

Title: Homeostatic synaptic plasticity in the developing zebrafish spinal cord

Authors: Laura Knogler¹, Meijiang Liao², Pierre Drapeau³

Affiliation: 1-3 University of Montreal

Abstract: Homeostatic synaptic plasticity has been proposed to stabilize network activity levels and maintain neuronal firing within a set range. It has been shown in several in vitro preparations that global alterations in network activity induce compensatory homeostatic changes, but little is known as to whether homeostatic mechanisms can be induced cell-autonomously, and whether just one, a few, or all synapses of a neuron change in response to synapse-specific activity perturbations in vivo. We used zebrafish embryos expressing botulinum toxin (BoTox) mRNA, a neurotoxin that upon translation prevents exocytosis, thereby blocking chemical neurotransmission. In preliminary work we found that upon global BoTox expression following mRNA injection, the majority of fish were not swimming by hatching at 3dpf, a behaviour that appears in uninjected controls by 2dpf. Touching the BoTox-expressing fish on the tail, which normally provoked a robust escape response, elicited this response only occasionally. Spontaneous network activity was significantly reduced as measured by patch-clamp electrophysiology and stereotypical network activity (swimming-related rhythms) appeared immature or not present. We are examining the impact of this treatment on synaptic scaling (spontaneous events) and in addition are expressing BoTox in subsets of neurons to test whether all (global) remaining synapse scale or not (local scaling).

Funding: This work is funded by a NSERC PGS-D studentship (LDK) and CIHR and GRSNC grants (PD)

Title: Blocking nerve growth factor degradation in the rat hind paw skin leads to hypersensitivity to noxious stimuli and to sympathetic fibre sprouting

Authors: Geraldine Longo¹, Maria Osikowicz², Claudio Cuello³, Alfredo Ribeiro-da-Silva⁴

Affiliation: 1-4 McGill University

Abstract: Our laboratory previously identified a sympathetic fibre sprouting into the upper dermis of the hind paw skin in a chronic inflammation model. In this ectopic territory, sympathetic fibres wrapped around peptidergic nociceptive afferents. This fibre sprouting was interpreted as nerve growth factor (NGF)-mediated, since both sympathetic and peptidergic nociceptive fibres are dependent on NGF for trophic support. Recently, NGF was shown to be released from cells as a precursor (proNGF) together with the enzymatic cascade leading to its conversion into the mature (active) form (mNGF) and to its degradation (Bruno and Cuello, PNAS 2006). Matrix metalloproteinase (MMP)-9 was found to be the enzyme which degrades mNGF. In the current study, we hypothesized that inhibition of MMP-9 in naïve animals would prevent the degradation of endogenous mNGF, leading to sympathetic fibre sprouting in the skin. Therefore, an MMP-9 inhibitor was repeatedly injected subcutaneously into the glabrous skin of the hind paw. The MMP-9 inhibitor administration led to hypersensitivity to both mechanical and thermal stimulation, similarly to that observed in the chronic inflammation model. We observed an invasion of sympathetic fibres into the upper dermis of MMP-9 inhibitor-treated animals. A significant increase in mNGF and decrease in MMP-9 protein levels in the treated group was also detected. We confirmed by zymography that administration of the inhibitor reduced the MMP-9 enzymatic activity. Our data indicates that inhibition mNGF degradation is sufficient to induce abnormal fibre sprouting and lowered pain thresholds.

Funding: Funded by CIHR, the Louise and Alan Edwards Foundation and MITACS Accelerate Program

Title: Development and plasticity of glomerular maps in the zebrafish olfactory system

Authors: Oliver Braubach¹

Affiliation: 1- Yale University

Abstract: "The zebrafish olfactory system mediates behavioral responses to food, habitat, conspecifics and predators. Odors that initiate such behaviors are diverse and we asked if they are processed in separate neural pathways, beginning with molecularly distinct sensory neurons and their axonal projections to the olfactory bulbs. We therefore characterized neural pathways in the zebrafish olfactory system throughout the development of this animal. Using immunohistochemical probes for G-protein subunits and calcium binding proteins, we first

identified distinct types of olfactory sensory neurons and traced their axonal projections to specific glomeruli, which are spheroidal synaptic processing units that organize and relay sensory information in the olfactory bulbs. In mature zebrafish, we identified 25 large and anatomically distinct glomeruli; these units were innervated by putative pheromone-responsive sensory neurons, and resembled specialized glomeruli known in other animals. Furthermore, large glomeruli already formed 48-72 hours after fertilization and persisted in stable configurations afterwards, suggesting that they comprise specialized and developmentally stable neural pathways, possibly involved in mediating innate olfactory behaviors such as attraction to food and conspecific odors. In contrast, most glomeruli (~115) were comparatively small and arranged with varying distributions in coarsely circumscribed regions of the olfactory bulbs. The development of these latter units could be modified by sensory experience, suggesting that they comprise plastic olfactory pathways involved in the acquisition of learned olfactory behaviors during postembryonic development. Collectively our results suggest that innate and learned olfactory behaviors are represented in distinct olfactory pathways that differ in neurochemistry and anatomy."

Funding: CIHR and NSERC (A.F) NSERC (R.P.C) NSHRF (O.B)

Title: Morphological classification of layer V pyramidal neurons that project onto V1 using principal component analysis and cluster analysis.

Authors: Marie-Eve Laramée¹, Stéphanie Prince², Kathleen Rockland³, Gilles Bronchti⁴, Denis Boire⁵

Affiliation: 1,2,4,5 Université du Québec à Trois-Rivières, Chimie-Biologie 3- Picower Institute for Learning and Memory, RIKEN-MIT Center for Neural Circuit Genetics, MIT

Abstract: INTRODUCTION: Only few immunohistochemical markers are useful for distinguishing subtypes of pyramidal neurons (Molnar and Cheung 2006). Some authors therefore attempted to classify the pyramidal neurons using their dendritic arborization. Morphologically distinct neuronal types can be further confirmed according to their axonal target (Larsen et al., 2007), the sensory modality (Benavides-Piccione et al., 2005) and hierarchical level (Elston and Rosa 2000) of the cortical area in which they are found and whether they are involved in callosal connections (Solloway et al., 2002). In this study, we performed complete reconstructions of dendritic arbors of layer V pyramidal neurons that project onto the primary visual cortex (V1) from the primary

somatosensory (S1) and auditory (A1) cortices and from the medial (V2M) and lateral (V2L) parts of the secondary visual cortex, from both hemispheres. **METHODS:** AdSynEGFP retrograde tracer (Tomioka 06) was pressure injected in V1 of five C57BL/6 mice. After immunohistochemical processing, 63 retrogradely labeled neurons were reconstructed and morphological analysis was carried out using NeuroLucida (MicroBrightField). **RESULTS:** Principal component analysis and cluster analysis showed a continuous range of pyramidal cell morphologies that project onto V1. No distinct groups were found. Therefore, dendritic morphology of neurons that project onto V1 is not determined by the sensory modality, the hierarchical level or callosal connection.

CONCLUSION: This result might indicate that the terminal target is the determinant factor for dendritic organization or, alternately, that the dendritic arbor is not sufficient to discriminate morphological classes of pyramidal neurons.

Funding: NSERC and FRSQ grants to DB and GB NSERC fellowship to MEL CFI grant to DB

Title: Development and plasticity of glomerular maps in the zebrafish olfactory system

Authors: Oliver Braubach¹, Nobuhiko Miyasaka², Tetsuya Koide³, Yoshihiro Yoshihara⁴, Roger Croll⁵, Alan Fine⁶

Affiliation: 1- Yale University 2-4 RIKEN Brain Science Institute 5,6 Dalhousie University

Abstract: "The zebrafish olfactory system mediates behavioral responses to food, habitat, conspecifics and predators. Odors that initiate such behaviors are diverse and we asked if they are processed in separate neural pathways, beginning with molecularly distinct sensory neurons and their axonal projections to the olfactory bulbs. We therefore characterized neural pathways in the zebrafish olfactory system throughout the development of this animal. Using immunohistochemical probes for G-protein subunits and calcium binding proteins, we first identified distinct types of olfactory sensory neurons and traced their axonal projections to specific glomeruli, which are spheroidal synaptic processing units that organize and relay sensory information in the olfactory bulbs. In mature zebrafish, we identified 25 large and anatomically distinct glomeruli; these units were innervated by putative pheromone-responsive sensory neurons, and resembled specialized glomeruli known in other animals. Furthermore, large glomeruli already formed 48-72 hours after fertilization and persisted in stable configurations afterwards, suggesting that they comprise specialized and developmentally stable neural pathways, possibly involved in mediating innate olfactory behaviors such as attraction to food and

conspicuous odors. In contrast, most glomeruli (~115) were comparatively small and arranged with varying distributions in coarsely circumscribed regions of the olfactory bulbs. The development of these latter units could be modified by sensory experience, suggesting that they comprise plastic olfactory pathways involved in the acquisition of learned olfactory behaviors during postembryonic development. Collectively our results suggest that innate and learned olfactory behaviors are represented in distinct olfactory pathways that differ in neurochemistry and anatomy."

Funding: CIHR, NSERC (A.F) NSERC (R.P.C) NSHRF (O.B)

Title: Variations in MT activity cause variations in motion detection performance.

Authors: Jackson Smith¹, Chang'an Zhan², Erik Cook³

Affiliation: 1,3 McGill University 2- Southern Medical University

Abstract: "When asked to report the perception of an identical stimulus on separate occasions, a subject may give opposite answers. Trial-to-trial noise in the firing rate of sensory neurones is known to covary with these fluctuations in perceptual performance. Recent studies suggest that this covariation is epiphenomenal; that is, sensory neurones receive top-down, neuromodulatory feedback related to attention or decision bias such that their correlation with behaviour is non-causal. However, a framework in which sensory neural fluctuations directly cause fluctuations in behaviour is a simple alternative explanation. We recorded from middle temporal neurones in monkey subjects performing a motion detection task. Emphasis was placed upon a causal link by presenting very brief motion pulse signals at random times and locations. Neural-behaviour covariations changed over time, appearing just before motion pulse onset and peaking shortly afterwards. They were modulated by the location of the motion pulse and by whether one pulse or two simultaneous pulses were shown. A feed-forward model that causally linked sensory stochasticity with variations in detection performance did an excellent job emulating both the observed changes in behaviour and the evolution of neural-behaviour covariations over time and between different stimulus conditions."

Funding: CIHR and NSERC

Title: Stimulation-Induced Seizures Reduce Hyperpolarization-Activated Cation Current (I_h) in Layer 5 Pyramidal Cells From Sensorimotor Cortex

Authors: Jeffery Boychuk¹, Quentin Pittman², Cam Teskey³

Affiliation: 1-3 University of Calgary

Abstract: "Behavioral seizures can be induced experimentally by stimulating the corpus callosum (CC) to generate epileptiform discharges in sensorimotor cortex. Studies using intracortical microstimulation (ICMS) find that experimental seizures result in larger movement representations, decreased movement thresholds and more multiple responses at individual sites. While these abnormal cortical movement representations indicate altered neuronal properties within sensorimotor cortex, there is limited information on the locus and nature of these changes. Here, slice electrophysiology was used to investigate how seizures alter the properties of individual pyramidal cells from layer 5 of sensorimotor cortex because their activation is the central component of ICMS-derived movements. Rodents were implanted with stimulating electrodes in the CC at postnatal day 29-32. Three days later animals were given four stimulation sessions (two/day) to induce seizures. Two days following the final seizure session whole-cell configuration recording techniques were performed on brain slices from sensorimotor cortex. Results show that pyramidal cells from animals given seizures exhibit reductions in hyperpolarization activated cationic current (I_h). In voltage clamp, I_h was identified by the difference in current responses in the presence of an I_h blocker (ZD7288 15 μM, 20 min) from those before its application. Reduced I_h was indicated by a decrease in the amplitude of ZD7288-sensitive currents (peak-steady state) generated by hyperpolarizing steps. Ongoing work is aimed at understanding how the reduction of I_h following seizures contributes to motor map changes."

Funding: Canadian Institutes of Health Research (CIHR), Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: A Bayesian ideal observer model for tactile spatial perception

Authors: Michael Wong¹, Daniel Goldreich²

Affiliation: 1-2 McMaster University

Abstract: "Is human tactile spatial perception optimal? We constructed a Bayesian ideal observer that performs optimally on tactile spatial tasks, and compared the performance of the ideal observer to that of humans tested previously on the same tasks. We used a continuum mechanics model (Phillips & Johnson, 1981) to simulate the firing rates of a population of slowly adapting type-1 primary afferents (SA1) in response to a square wave grating indented statically for 1s into

the skin. We introduced firing rate noise typical of either SA1s (low variability) or primary somatosensory cortical neurons (high variability, Poisson). The ideal observer optimally decoded these firing rates using Bayesian model comparison to perform grating orientation discrimination and grating detection tasks. When we fed the ideal observer SA1 firing rates, its performance overwhelmingly surpassed human perception; when we fed it cortical firing rates, its performance worsened considerably but still surpassed human perception. Thus, human tactile spatial perception is suboptimal in that humans do not make full use of the information carried by either primary afferents or primary somatosensory cortical neurons. When we fed the ideal observer only the action potentials evoked during a short (e.g., 200ms) initial portion of the stimulus, its performance approached human levels. Thus, humans may perceive suboptimally because they do not integrate sensorineural information over the entire stimulus-presentation period. Alternatively, humans may derive tactile spatial inferences from the activity of higher-order cortical neurons, whose receptive fields may be larger, and/or firing rates more variable, than those of primary somatosensory cortical neurons. "

Funding: Natural Sciences and Engineering Research Council of Canada.

Title: A Functional Main Olfactory System is Necessary and Sufficient for the Initiation and Maintenance of Maternal Behavior in *Mus musculus*

Authors: Kyle Roddick¹, Heather Schellinck²

Affiliation: 1-2 Dalhousie University

Abstract: "It is now evident that both the main olfactory system (MOS) and the accessory olfactory system (AOS) may be activated by general and pheromonal odorants. Moreover, it has been demonstrated that the MOS is required to activate the AOS to initiate male aggression and puberty acceleration in mice. It is not clear, however, if the main olfactory system may act independently or whether action of both the main and accessory olfactory systems are required for specific behavioral responses to occur. We report here the results of a double dissociation study designed to examine how olfactory cues from the MOS and AOS modulate maternal behaviour in mice. Ablation of the main olfactory epithelium with ZnSO₄ significantly reduced pup survival. In addition, ANOVA revealed that pup growth, nest quantity and quality, nursing behavior, and pup retrieval were significantly impaired in ZnSO₄ treated mice compared with mice in which the AOS had been deactivated by surgical removal of the vomeronasal organ. Mice

with both a nonfunctional MOS and a nonfunctional AOS also had significant deficits in all maternal behaviors; control mice with sham inactivation of both systems showed no significant deficits. These results suggest that the main olfactory system is both necessary and sufficient for the expression of maternal care whereas the accessory olfactory system does not appear to be essential for such behavior."

Funding: NSERC

Title: A Bayesian ideal observer model for human vibrotactile perception

Authors: Arindam Bhattacharjee¹

Affiliation: 1- McMaster University

Abstract: Accurate perception requires efficient decoding of stimulus-evoked sensorineural activity. Here we present a Bayesian ideal observer model that optimally decodes neural population responses to low frequency (5-40 Hz) vibrotactile stimuli. Fed simulated firing rates calculated from response properties of primate rapidly adapting type-1 (RA1) afferents (Johnson 1974), the ideal observer makes probabilistic inferences about stimulus amplitude and frequency. We incorporated known densities of RA1 afferents reported for human fingertip, and introduced variability into the stimulus-evoked firing rates to simulate responses in the periphery (low-variability RA1 noise) or primary somatosensory cortex (high-variability Poisson noise). Implementing two-interval forced-choice procedures, we quantified the model's performance on vibrotactile threshold detection (TD), amplitude discrimination (AD), and frequency discrimination (FD) tasks. On all tasks, the model perceived more accurately at the peripheral than cortical level, suggesting that cortical response variability impairs perception. Nevertheless, the model's performance at the cortical level far surpassed human perception, suggesting that humans fail to utilize all information carried in cortical firing rates. While quantitatively the model outperforms humans, qualitatively the performance of the model is similar to that of humans. For example, the model's performance follows a psychometric function such that the probability of correct responses increases monotonically with stimulus amplitude (TD task), or with the difference in amplitudes (AD task) or frequencies (FD task) between two stimuli. The model's performance sets a benchmark in vibrotactile perception against which to compare human performance in order to gain insights into the decoding efficiency of the human somatosensory

system.

Funding: Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Measuring tactile spatial acuity: a simple but rigorous alternative to the flawed two-point test.

Authors: Jonathan Tong¹, Daniel Goldreich²

Affiliation: 1-2 McMaster University

Abstract: Weber long ago measured tactile spatial resolution as the distance between two contacts to the skin necessary to evoke the sensation of distinct points (Weber, 1835). The classic two-point task remains popular to this day, particularly in clinical settings: calipers of increasing tip-separation are applied to the skin until the patient responds “two.” Contrary to the belief that this task rigorously measures spatial acuity, critics argue that task performance depends upon a subjective criterion for responding “two” (Craig and Johnson, 2000). To prevent criterion effects, some investigators use a two-interval forced-choice (2IFC) procedure: stimulating the skin with both one and two points, in random order, and asking the subject which interval contained two. Unfortunately, this version of the task may provide a non-spatial magnitude cue (Johnson and Phillips, 1981) arising from surround suppression: two closely spaced points elicit fewer action potentials than does a single point of equal indentation (Vega-Bermudez and Johnson, 1999). Here we compare the 2IFC two-point task to an equally simple alternative task, 2IFC horizontal-vertical (HV) discrimination, in which the subject is stimulated with two points, once along the skin’s medial-lateral axis (horizontally) and once along the proximal-distal axis (vertically). We assess both tasks on the fingertip, finger base, palm, and forearm, and we correlate task performance with an anatomical measure associated with receptive field spacing (Peters et al. 2009). Preliminary data indicate that HV discrimination, unlike the two-point task, provides a rigorous measure of tactile spatial acuity.

Funding: The study was funded by an NSERC grant.

Title: Neuroendocrine control of decision making during larval escape response: Genes, brain, and behavior.

Authors: Markus Klose¹, Andrew Giles², Catharine Rankin³, Doug Allan⁴

Affiliation: 1-4 UBC

Abstract: We recently developed a behavioural recording system to simultaneously monitor large numbers of larvae responding to a stressful light stimuli. This new system allows high-throughput analysis of an array of behavioral parameters at sub-second time resolutions, increasing data collection 100 fold. We examined the role of the *fmr* gene in regulating the larval escape response. Expression of *fmr* has been detected in only 44 neurons, all located in the central nervous system. The 6 Tv neurons, each of which project to the neurohaemal organs, reveal by far the strongest expression of *fmr*. This expression is regulated by retrograde BMP signaling. We exploited the BMP pathway to ubiquitously ablate *fmr* expression and then restore it in the Tv neurons. We found that the expression of *fmr* in the 6 Tv neurons regulates steady-state locomotor speed, while expression of *fmr* in the remaining 38 neurons regulates the decision to either increase or decrease crawling speed during sustained exposure to intense light. Behavioural, electro-, and opto-physiological data support the hypothesis that the main product of the *fmr* gene, the neuropeptide DPKQDFMRamide, is released into the hemolymph and activates two G-protein coupled receptors, the FR (Fmr Receptor) and the DmsR-2 (Drosophila myosuppressin Receptor-2). Activation of this receptor complex facilitates Ca²⁺-induced Ca²⁺ release from the ER, activating CaMKII to enhance transmitter release.

Funding: EJLB & CIHR

Title: Influence of the pulvinar on striate and extrastriate cortices revealed by electrical stimulation and voltage sensitive dye recording

Authors: Christian Casanova¹, Matthieu Vanni²

Affiliation: 1-2 Université de Montréal

Abstract: It is well known that the primary visual cortex received its main thalamic drive from the lateral geniculate nucleus (LGN) through layer IV. In contrast, projections from the pulvinar end for the most part in layer I, suggesting that pulvinar exerts a diffuse modulatory influence on activity of the primary visual cortex (PVC). If that is the case, one would expect the spatio-temporal responses evoked by activation of these two pathways to be different. We investigated this issue by measuring the spatiotemporal dynamics of voltage sensitive dyes activation in the visual cortex following thalamic electrical stimulation in two different species: cats and tree shrews. Animals were anesthetized with halothane. RH1691 dye was used to stain the cortex. Stimulating electrodes were placed in the LGN and pulvinar. The electrical stimulation of the LGN

induced a biphasic response comprising a fast positive component followed by a slow one in delimited portions of PVC. These responses were followed by activation, with increased delays, in extrastriate and contralateral cortices. The profile of the second slow component was strongly dependent on the strength of the stimulation and was generally stronger in extrastriate cortex. Stimulation of pulvinar induced weak and diffuse responses in PVC but provoked strong activations in extrastriate areas. In most cases, the co-stimulation of pulvinar and LGN strongly reduced the LGN-induced responses. These data suggest that the pulvinar exerts a modulatory influence on the processing of LGN inputs in PVC while it may directly contribute to extrastriate cortex functioning by providing driver signals.

Funding: Supp by CIHR and NIH (NEI)

Title: Orientation specificity of contrast adaptation in mouse primary visual cortex

Authors: Nathan Crowder¹, Aaron Stroud², Emily LeDue³

Affiliation: 1-3 Psychology Department, Dalhousie University

Abstract: "Contrast adaptation in the visual cortex allows visual neurons to maintain optimal stimulus sensitivity under changing viewing conditions. Previous studies examining orientation specificity of contrast adaptation in cats have demonstrated two types of adaptation in the primary visual cortex (V1): 1) Non-oriented adaption, where neurons adapt to stimuli of all orientations; and 2) Orientation-specific adaption, where neurons only adapt to stimuli matching the cell's preferred orientation. It has been proposed that local intracortical networks formed between neighboring V1 neurons may mediate this aspect of contrast adaptation. The orientation specificity of contrast adaptation is hypothesized to relate to a neuron's position within the pinwheel orientation map within cat V1: cells which show non-oriented contrast adaptation are thought to occur near pinwheel centers, while cells with orientation-specific contrast adaptation should be found in iso-orientation domains. Mice and rats lack cortical orientation columns, therefore if local networks in V1 dictate the orientation specificity of contrast adaptation in these species, all V1 neurons should exhibit non-oriented contrast adaptation. We recorded from mouse V1 neurons, and compared the amount of adaptation produced by gratings at both preferred and orthogonal orientations. We found that the majority of cells demonstrate non-oriented contrast adaptation, supporting the hypothesis that local intracortical networks are involved in orientation

specificity of contrast adaptation in the striate cortex."

Funding: Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Trading speed and accuracy by coding time: a coupled-circuit cortical model

Authors: Dominic Standage¹, Michael Dorris²

Affiliation: 1-2 Queen's University

Abstract: Our actions take place in space and time, but despite the role of time in decision theory and the growing acknowledgement that the encoding of time is as crucial as the encoding of space, few studies have considered the interactions between spatial and temporal codes in perceptual decisions. The speed-accuracy trade-off (SAT) provides a window into spatiotemporal interactions. Our working hypothesis is that temporal coding controls the SAT by gain modulation (Standage et al, Front Comput Neurosci, 2011). Here, we propose that local cortical circuits are inherently suited to coding space and time for decisions, supporting a framework of distributing temporal processing. We couple two generic local-circuit models, each consisting of pyramidal cells and inhibitory interneurons, connected by AMPA, NMDA and GABA synapses. One circuit codes time by ramping activity, seen in cortex during tasks with a timing requirement. The other makes decisions in a simulated perceptual task. The networks are identical, except the timing network is modulated by mesocortical dopamine, consistent with pharmacological studies indicating a role for dopamine in interval timing. A simple learning rule is sufficient for the timing network to quickly learn to produce new interval estimates, which show signature characteristics of estimates by experimental subjects. To trade speed and accuracy, the network simply learns longer or shorter intervals, driving the rate of decisions downstream by gain modulation. In terms of cortical function, these results should be expected of a generally uniform structure that evolved to provide a model for action in a spatiotemporal world.

Funding: Canadian Institutes of Health Research

Title: The anatomical locus of visual adaptation

Authors: Susan Boehnke¹, David Berg², Laurent Itti³, Douglas Munoz⁴

Affiliation: 1,4 Queen's University 2,3- University of Southern California

Abstract: "When stimuli are repeatedly flashed into the receptive field of visual neurons in the superior colliculus (SC), the neural response to subsequent stimuli is reduced in magnitude and

delayed in onset, i.e. adaptation is observed. These repetition effects occur in a rate-dependant fashion - the faster the presentation rate, the greater the effect. Although they are also observed in visual cortical areas such as FEF and V4, it is not known if the effects are due to early processing in the retina or thalamus that is simply inherited by cortex and colliculus, or due to processing in cortex and/or the SC itself. To determine this we exploited the fact that binocularity does not emerge until V1 and we used ferro-electric shutter goggles to alternate visual stimuli between the eyes to dissociate the relative contribution of monocular and binocular mechanisms in producing the repetition effects. Reduction in magnitude and increases in latency of SC visual responses was observed if sequences of 7 stimuli (50ms duration, 200ms interval) were presented to both eyes, were alternated between the eyes (1/2 presentation rate to monocular channels, full rate to binocular channels), or presented to either eye alone. There was some recovery of onset latency with alternation between the eyes, but not of response magnitude. This suggests that some of the increase in response onset latency with repetition occurs in monocular channels (i.e. retina, thalamus, or input to V1); while the decrease in response magnitude occurs mostly in the binocular neurons of V1 or beyond."

Funding: CIHR and National Science Foundation (Collaborative Research in Computational Neuroscience)

Title: Peripheral nerve injury is accompanied by region-specific changes in global methylation in the brain

Authors: Maral Tajerian¹, Sebastian Alvarado², Magali Millecamps³, Moshe Szyf⁴, Laura Stone⁵

Affiliation: 1,3,5- Alan Edwards Center for Research on Pain 2,4- McGill University

Abstract: Background Chronic neuropathy is accompanied by severe pain and physical disability. Chronic pain is associated with mood disorders, cognitive impairment, and alterations in cortical structure and function. Chronic pain results in long-term biological changes that are difficult to reverse, including pathological changes in gene expression. Epigenetic modifications such as DNA methylation are involved in long-term regulation of gene expression. The role of epigenetic modulation in the development and maintenance of chronic pain is currently unexplored. Objective Identify changes in global genome-wide DNA methylation in the brain that are induced by chronic neuropathy. Methods Neuropathic pain was induced in 3-month old male CD1 mice using the Spared Nerve Injury (SNI) model. Animals were tested for hypersensitivity to

mechanical and cold stimuli and for motor impairment using the von Frey, acetone and rotarod tests, respectively. Animals were sacrificed and DNA was extracted from the thalamus, amygdala, prefrontal and visual cortices. The luminometric methylation assay was used to measure global genome-wide DNA methylation. Results Following SNI surgery, animals develop hypersensitivity to cold and mechanical allodynia and motor dysfunction. Chronic neuropathic pain is accompanied by a bilateral decrease in global methylation in the prefrontal cortex and amygdala but not in the thalamus. Conclusions Peripheral nerve injury induces region-specific changes in global methylation in the brain. Future studies will identify specific genes whose promoters are differentially methylated in chronic pain conditions. Changes in DNA methylation could be an important factor in the development and maintenance of chronic pain.

Funding: Supported by grants from the FRSQ and CIHR.

Title: Unilateral subthalamotomy allows a reduction of levodopa in MPTP monkeys

Authors: Vincent Jourdain¹, Laurent Grégoire², Martin Parent³, Thérèse Di Paolo⁴

Affiliation: 1,2,4- Centre de recherche du CHUQ, CHUL 3- Centre de Recherche Université Laval Robert-Giffard

Abstract: The subthalamic nucleus (STN) is a major driving force of the basal ganglia and its neurons are overactive in Parkinson's disease (PD). Chronic treatment of PD with levodopa induces dyskinesias (LID). Subthalamotomy leads to improvement of motor symptoms in PD patients but its mechanisms remain largely unknown. In this study, an optimal dose of levodopa, defined as the highest dose inducing low or no dyskinesia, was established in three female MPTP-lesioned monkeys. A suboptimal dose was defined as 60 percent of the optimal dose of levodopa. MPTP monkeys underwent a unilateral subthalamotomy by stereotactic injection of ibotenic acid. Parkinsonian disability and dyskinesias were measured at baseline and following administration of optimal and suboptimal doses of levodopa, before and after the surgery. Each dose was tested three to four times at 2-3 days interval. Subthalamotomy had no effect on parkinsonian disability at baseline and with the optimal dose, but increased the response to low-dose of levodopa. Following surgery, the parkinsonian scores with suboptimal doses were similar to pre-operative optimal dose measures, suggesting a ceiling effect. STN lesion increased dyskinesias in response to suboptimal and optimal doses of levodopa. The duration of response to levodopa increased by 20 to 25% with the suboptimal dose, whereas no changes were observed with the optimal dose. The

delay of response to levodopa remained unchanged following surgery. Subthalamotomy allowed a 40% reduction of the dose of levodopa in dyskinetic MPTP monkeys with a similar beneficial antiparkinsonian effect and increased the duration of the lower levodopa dose.

Title: Size and growth of primary sensory cortices in neonatally enucleated and congenitally anophthalmic mice.

Authors: Ian Massé¹, Sonia Guillemette², Marie-Ève Laramée³, Gilles Bronchti⁴, Denis Boire⁵

Affiliation: 1-5 Université du Québec à Trois-Rivières

Abstract: Introduction: Previous studies show auditory and somatosensory activity in the primary visual cortex (V1) in anophthalmic (An) ZRDCT but not in enucleated and intact C57BL/6 mice. ZRDCT/An mice have smaller visual and somatosensory cortices and a larger auditory cortex compared to both groups of C57BL/6 mice. In this study we set out to determine whether these differences are a consequence of vision loss or features of the different mouse strains. Methods: The volume, area and thickness of the primary visual, auditory and somatosensory cortices and the volumes of the specific thalamic nuclei were measured in intact and in enucleated C57BL/6 as well as in ZRDCT/An mice. In order to test for strain differences, the same measurements were taken in fourth generation backcrossed intact, enucleated and anophthalmic C57BL/6 X ZRDCT/An hybrid mice. The size of these cortices was also measured in P5 hybrids to assess their growth rates. Results: In hybrid mice, the visual cortex and LGNd are smaller in blind compared to intact cases. The other differences between ZRDCT and C57BL/6 mice are no longer present in hybrids. This suggests that they are due to strain features and not blindness. The growth curve of V1 is steeper in the anophthalmic than in enucleated hybrid mice and similar to that of intact hybrid mice. This could indicate that crossmodal sensory activity could contribute to the development of V1.

Funding: NSERC to GB and DB CFI to DB.

Title: Changes in tactile spatial acuity over development: from pre-pubescence into adulthood.

Authors: Daniel Goldreich¹

Affiliation: 1- McMaster University

Abstract: "The question of whether, and if so how, tactile spatial acuity (TSA) changes during

childhood has been largely disregarded in the literature. Only two groups have investigated this question, with equivocal results [1,2,3]. We predicted that children would have better TSA than adults by virtue of their diminutive digits [4]. Under the reasonable assumption that receptor number is fixed from early childhood into adulthood, the smaller fingertips of children may possess finer TSA than adult fingertips because tactile receptors in the skin might necessarily become more sparsely distributed as the fingertips grow (maintaining adequate receptive field coverage). Indeed, tactile receptors appear fully mature in function by ages 5-7 years [4,5]. Apart from physical changes in the periphery, age-related cortical maturation (e.g. myelination) may also influence acuity [1,2,3]. We are performing a cross-sectional study to track the developmental course of TSA in children (ages 6-16 years), using our fully-automated tactile testing apparatus [6]. In addition, we are measuring several physical properties of the fingertip to determine whether these influence TSA. These candidate variables are: surface temperature, hydration, elasticity and sebum content of the skin, and fingertip surface area, volume and sweat pore spacing. Preliminary results suggest that acuity indeed worsens with age; however, more data are needed to make stronger conclusions about the effects on TSA of age, finger size and the additional candidate variables we are measuring. "

Funding: NSERC

Title: The priming of adult incision-induced hyperalgesia by prior neonatal incision is maintained by central neuroimmune activity.

Authors: Simon Beggs¹, Suellen Walker², Maria Fitzgerald³, Gillian Currie⁴

Affiliation: 1- Neuroscience, Physiology and Pharmacology, Hospital for Sick Children, Toronto
2,4- Program in Neurosciences & Mental Health, Hospital for sick children, Toronto
3- Portex Unit: Pain Research UK

Abstract: Tissue injury during a critical neonatal period produces long-term alterations in sensory processing and enhances sensitivity to repeat injury in later life. Using the plantar hindpaw incision model in the rat pup, we evaluated the response to incision in adulthood to determine if prior neonatal injury alters the degree of postoperative hyperalgesia and spinal microglial activity. Hyperalgesia was quantified by behavioural thresholds and recording flexion reflex EMG responses to hindpaw mechanical stimuli. Microglial proliferation in the dorsal horn was determined by Iba1 immunoreactivity following incision or tibial nerve electrical stimulation

in adults with and without prior neonatal incision. The effects of systemic and spinal minocycline were compared across groups. Reflex sensitivity was greater in the repeat versus single incision groups. Iba1 immunoreactivity in the dorsal horn increased following adult single incision but was apparent earlier and more widespread after repeat incision. Pre-treatment with intrathecal minocycline blocked hyperalgesia in the repeat incision group but had no effect 24 hours following single incision. Intraperitoneal minocycline reduced hyperalgesia in both single and repeat incision groups. Neonatal incision is associated with enhanced responses to subsequent injury that persist into adulthood. Changes in the time course and degree of microglial proliferation in the spinal cord may contribute to the long-term enhancement of responses to repeat surgery. Effects of intrathecal minocycline in the repeat incision group suggest centrally-mediated inhibition of microglial function, whereas systemic minocycline may have peripheral anti-inflammatory effects. These studies have implications for perioperative outcomes in children and adults with prior neonatal surgery.

Funding: "IASP Early Career Grant (SW); MRC (MF), CIHR (GC, SB)"

Title: Changes in tactile spatial acuity over development: from pre-pubescence into adulthood.

Authors: Ryan Peters¹, Daniel Goldreich²

Affiliation: 1-2 McMaster University

Abstract: "The question of whether, and if so how, tactile spatial acuity (TSA) changes during childhood has been largely disregarded in the literature. Only two groups have investigated this question, with equivocal results [1,2,3]. We predicted that children would have better TSA than adults by virtue of their diminutive digits [4]. Under the reasonable assumption that receptor number is fixed from early childhood into adulthood, the smaller fingertips of children may possess finer TSA than adult fingertips because tactile receptors in the skin might necessarily become more sparsely distributed as the fingertips grow (maintaining adequate receptive field coverage). Indeed, tactile receptors appear fully mature in function by ages 5-7 years [5]. Apart from physical changes in the periphery, age-related cortical maturation (e.g. myelination) may also influence acuity [1,2,3]. We are performing a cross-sectional study to track the developmental course of TSA in children (ages 6-16 years), using our fully-automated tactile testing apparatus [6]. In addition, we are measuring several physical properties of the fingertip to determine whether these influence TSA. These candidate variables are: surface temperature, hydration,

elasticity and sebum content of the skin, and fingertip surface area, volume and sweat pore spacing. Preliminary results suggest that acuity indeed worsens with age; however, more data are needed to make stronger conclusions about the effects on TSA of age, finger size and the additional candidate variables we are measuring. "

Funding: NSERC

Title: Investigation of cytoskeletal proteins in neurons of the cat lateral geniculate nucleus

Authors: Emily LeDue¹, Nathan Crowder², Kevin Duffy³

Affiliation: 1-3 Dalhousie University

Abstract: "Cytoskeletal elements, such as neurofilament and the actin-binding protein spectrin, maintain the structural integrity neurons. Three neurofilament subunits (heavy: NF-H; medium: NF-M; or light: NF-L) form heteropolymers that act as an intracellular scaffold to maintain neuron shape. The largest neurons in the cat dorsal lateral geniculate nucleus (dLGN), which compose the Y cell class, are distinguishable by their inclusion of the NF-H subunit. This observation raises the possibility that other classes of dLGN neurons can also be distinguished by their cytoskeletal content. In this study of the dLGN we examined the relationship between neuron size and cytoskeletal composition. We found that antibodies for each neurofilament subunit labeled neurons of approximately equal size, and these labeled cells were large compared to the general population. Spectrin labeling was evident in most dLGN neurons, and was not related to neuron size. Double labeling for neurofilament and GABA revealed largely non-overlapping populations. The very small degree of overlap between neurons reactive for GABA and neurofilament is compelling evidence that neurofilament is predominant in projection neurons. The small population of GABAergic neurofilament positive cells raises the possibility that there exists a subset of inhibitory projection neurons within the dLGN. "

Funding: This research was supported by a grant from NSERC to NAC and grants from NSERC and CFI to KRD.

Title: Making perceptual decisions with visual and reward information

Authors: Celina Nahanni¹, Michael Warfe², Michael Dorris³, Martin Pare⁴

Affiliation: 1-4 Queen's University

Abstract: "The neural process of decision-making is thought to integrate both external (sensory)

and internal (reward) information. Here we studied the interplay between these signals in monkeys trained to report with a saccadic eye movement the most salient item among several visual stimuli. Two monkeys performed a visual search task, in which we manipulated both the target-distracter discriminability (difference in luminance) and the reward magnitude for correct targeting; in each block, reward outcome differed only at a specific ('tagged') stimulus position. We found that the impact of reward magnitude on discrimination augmented with decreasing target discriminability; namely, reward increased the probability of response toward the tagged position and reduced the latency of these responses. To characterize the interaction between signals of visual discriminability and reward magnitude, we implemented a model based on Signal Detection Theory (SDT). The observed effect of target discriminability was modeled with Gaussian distributions representing neuronal responses to target and distractor stimuli, and the separation between these distributions fitted to match response probability. When reward was associated with the target, and when target discriminability -augmented, the separation between target and distractor representations increased accordingly. Conversely, when reward was associated with a distractor, the proportion of errors increased was captured by the reduced separation between target and distractor representations. This could be conceived as reward introducing an additive response bias, proportional to the reward magnitude. This bias could operate outside of the integration process of decision-making. "

Funding: CIHR

Title: Trade-offs in neural performance following metabolic stress involves the AMPK pathway

Authors: Tomas Money¹, Micheal Sproule², R Melrdrum Robertson³

Affiliation: 1-3 Queen's University

Abstract: Nervous systems are energetically expensive to maintain, and there is now good evidence that circuits can be optimized for efficiency depending on the real-time demands and constraints of the animal's environment. We have tested the hypothesis that severe metabolic stress alters the properties of neural circuits in ways that reduce the energetic demand. The locust, *Locusta migratoria*, was used to study recovery of neural activity following an anoxic coma. Neural function was monitored using the visual looming detector circuit, namely the descending contralateral movement detector (DCMD). We show that the observed anoxic effects on measures of metabolic rate, flight steering behaviour, and AP properties are modifiable through modulation

of the AMPK metabolic pathway. We suggest this is evidence of a coordinated cellular mechanism to reduce neural energetic demand following a severe metabolic stress.

Funding: Funded by NSERC

Title: Bimanual stroke assessment using an intuitive robotic task

Authors: Carl Jackson¹, Stephen Scott²

Affiliation: 1-2 Queen's University

Abstract: Clinical assessment of sensory, motor and cognitive function often relies on subjective measures that are prone to floor and ceiling effects and use coarse scales to ensure good inter-rater reliability. We are exploring the potential of robotic technology for clinical assessment and have previously built a suite of tasks to objectively quantify sensorimotor function of each limb. However, many daily activities require us to coordinate our hands together to perform them and these bimanual skills require an intact corpus callosum and brain areas not probed in our unimanual tasks. Here we present preliminary results from a newly developed bimanual coordination task. Participants used the KINARM exoskeleton to support a virtual bar with a hand at each end. The bar had a ball balanced on top of it, and participants used the bar to move the ball around the screen to hit and stabilize at targets. They performed the task with three levels of difficulty: the ball was stuck in place and did not move relative to the bar, the ball moved on the bar only as a function of bar tilt, or the ball was given virtual rolling physics. Behavioural data generated metrics quantifying task-level performance (e.g. number of targets hit), basic motor control (e.g. deviation from an ideal path) and bimanual coordination (e.g. correlation between the movements of each hand). Preliminary data show that stroke patients differ greatly from healthy controls on some or all of these criteria, depending on the nature and severity of the stroke.

Funding: NSERC CREATE & ORF-RE

Title: Two types of local circuit inhibitory interneurons control vibrissal inputs in the brainstem

Authors: Marie-Andree Bellavance¹, Martin Deschenes²

Affiliation: 1-2 Centre de recherche Universite Laval Robert-Giffard

Abstract: The brainstem trigeminal complex is the first relay station in the vibrissal sensory system. The trigeminal subnucleus interpolaris (SpVi), densely populated by inhibitory interneurons, was proposed to play a central role in the inhibitory gating of vibrissal inputs.

However, the cellular organization of SpVi interneurons and their role in the local circuit dialogue remain largely unknown and, therefore, were examined here. Whole-cell patch-clamp recordings were obtained from interneurons and projection cells in SpVi of brainstem slices of GAD67-GFP mice. Synaptic responses were evoked by local stimulation of the trigeminal tract. Recorded neurons were filled with biocytin and reconstructed anatomically. Based on membrane properties, two distinct types of interneurons were identified: (1) those with a relatively fast firing rate and fast afterhyperpolarisation (AHP) and (2) those with two AHP components, an I_h current and a rebound depolarization or spike. These two types of interneurons demonstrated different properties of excitatory synapses, with the first one displaying short-term depression, and the second one showing no change at all stimulation frequencies (20-200 Hz). The two types of interneurons were differentially recruited during repetitive activity, resulting in temporally distinct dynamics of disynaptic inhibition in projection cells. The spike probability and the integration time window in both projection cells and interneurons were tightly controlled by GABAergic inhibition. Our data reveal the existence of two local inhibitory circuits within SpVi, which may provide temporally distinct modes of inhibition. By detecting different features of afferent activity, these inhibitory circuits may be involved in selection of vibrissal sensory information.

Funding: CIHR, NSERC, CRCN

Title: The dorsal raphe nucleus in the rat: A single-axon tracing study of its efferent projections.

Authors: Dave Gagnon¹, Martin Parent²

Affiliation: 1-2 Centre de Recherche Universite Laval Robert-Giffard

Abstract: This study aimed at providing the first detailed description, at the single cell level, of the efferent projections of the dorsal raphe nucleus (DRN) in rodents. We used electrophysiological guidance to microiontophoretically labeled DRN neurons with biotinylated dextran amine. These neurons were easily recognizable by their highly regular (2-3 Hz) spontaneous firing patterns. Somatodendritic domains and axon projections of labeled neurons were reconstructed individually in three dimensions from serial sagittal sections using a computerized image analysis system. Typically, DRN neurons have a medium size cell body (15-20 μm) from which emerge 3-5 primary dendrites, mainly oriented along the anteroposterior axis. The axon arches rostroventrally and traverses the central portion of the midbrain tegmentum. It emits a major

collateral that passes through the ventral tegmental area and the supramammillary nucleus where it displays axon varicosities en passant before terminating in the mammillary bodies. The main axon travels within the medial forebrain bundle and gives off a major collateral that heads toward the central medial thalamic nucleus and the lateral habenula. It then courses through the ventral pallidum and the substantia innominata, where it exhibits axon varicosities, before entering the ventral striatum to arborize within the nucleus accumbens. Other labeled axons continue their course dorsally, run through the corpus callosum and innervate the dorsal striatum together with a wide area of the cortex. These results provide the first direct evidence that DRN neurons are endowed with a highly collateralized axon and form a widely distributed network that covers much of the forebrain.

Funding:

Title: Peripheral inflammatory mechanical hyperalgesia is mediated by metallothionein 2

Authors: Nader Ghasemlou¹, Christian von Hehn², Enrique Cobos³, Isaac Chiu⁴, Shane Cronin⁵, Chi Him Ma⁶, Christian Brenneis⁷, Clifford Woolf⁸

Affiliation: 1-8 Harvard Medical School and Children's Hospital Boston

Abstract: "Peripheral inflammation contributes to pain hypersensitivity through multiple mechanisms, including peripheral and central sensitization. The relative contribution of the cell types and mediators responsible remains unclear, with secreted inflammatory factors able to alter the pain response by acting on peripheral nerve terminals. We now show that the metallothionein (MT) 1/2 proteins are upregulated and secreted by fibroblasts after peripheral inflammation induced by intraplantar injection of complete Freund's adjuvant (CFA). MT1/2 were secreted by fibroblasts in vitro when stimulated with IL-1beta, one of the first cytokines upregulated after injury. Calcium imaging of cultured DRG neurons showed that MT2, but not MT1, has a direct effect on sensory neurons. Interestingly, the calcium fluxes observed occur only in a subset of neurons. In vivo analysis of metallothionein function, by direct intraplantar injection, revealed a dose-dependent effect for MT2 in the generation of acute pain and mechanical hypersensitivity lasting for 6h after injection with no change in thermal sensitivity; there was no effect for MT1. Using the CFA model of inflammatory pain, we observed a lack of mechanical hypersensitivity in MT1/2 null mice, while thermal hypersensitivity was unaltered. Furthermore, a function-blocking antibody against MT1/2 was able to mitigate mechanical hypersensitivity in wildtype mice after

CFA-induced inflammation. Our results show that endogenously produced MT2 secreted by activated fibroblasts, acts directly on peripheral nerve terminals to mediate mechanical hypersensitivity after peripheral inflammation. The use of pharmacological inhibitors of MT2 may represent a novel therapeutic tool to alleviate mechanical pain brought on by peripheral inflammatory pain."

Funding: This work was supported by NIH Grant NS039518 (CJW) and a post-doctoral fellowship from the FRSQ (NG).

Title: Synchronous neuronal activity between the striatum and cerebellum during oscillatory states

Authors: Jonathan Bourget-Murray¹, Jennifer Robinson², Richard Courtemanche³

Affiliation: 1-3 Concordia University

Abstract: "Local field potential (LFP) oscillations around 7 Hz are present in the cerebellum and basal ganglia, and those appear modulated during movement preparation. These could serve to bind sensorimotor activity. Anatomical studies have found multiple pathways linking the cerebellar cortex and striatum, including subcortical pathways. In the experiments described here, we test their basic synchrony patterns. In the unrestrained rat, we simultaneously recorded from two bilateral dorsolateral striatum sites, simultaneously with two bilateral cerebellar paramedian lobule sites, using a modified Harlan headstage with multiple moveable electrodes. Each of the four sites had two recording electrodes ($\sim 1\text{ M}\Omega$ each), which were recorded for over 3 weeks (total 8 electrodes). These electrodes can record both the LFP signal (0.5-125 Hz) and spiking from isolated neurons (600-6000 Hz). Rats were left unrestrained, and were more often quiet, yet could explore their environment which consisted in a dark behavioral arena. This arrangement permitted to explore patterns of oscillatory synchronization, between the striatum and cerebellum at different orientations. Our results show that same-location sites have a high crosscorrelation coefficient (ipsi/cerebellum = 0.84, ipsi/striatum = 0.89), that contralateral sites are a bit lower (contra/cerebellum = 0.68, contra/striatum = 0.79), and finally that cerebellar/striatal cross-correlation coefficients have lower values (ipsi = 0.47, contra = 0.42). A one-way ANOVA reveals a main effect of comparison ($p < 0.01$). These patterns of synchrony will be further analyzed to determine the changes due to anesthesia/behavior."

Funding: NSERC - Natural Sciences and Engineering Research Council of Canada

Title: Validation of peripheral electrical nerve stimulation (PENS) procedures using an implanted stimulation and recording device

Authors: Ian Swan¹, Robert Somogyi-Csizmazia², Kerry Delaney³

Affiliation: 1-3 University of Victoria

Abstract: The primary objective of this research is to test whether somatosensory stimulation, in the form of peripheral electrical nerve stimulation (PENS), can enhance functional recovery following ischemic damage to the forelimb sensory cortex in mice. The conceptual basis for this hypothesis derives from the idea that PENS will enhance the temporal precision of sensory information processing in surviving cortical regions and that improved sensory processing will lead to greater recovery of tactile sensation /function that is required for improved motor function after stroke. The first stage of this project involves validating the ability of PENS procedures to elicit appropriate somatosensory stimulation. Using a custom built implantable stimulation and neurophysiological recording device we can electrically stimulate the forepaw of a mouse and record the subsequent evoked response potentials (ERPs) generated by the PENS. Electrophysiological data recorded with this device shows that PENS (sub-motor threshold) reliably produces an evoked response potential (ERP) over the forepaw region of the somatosensory cortex. This finding is supported by IOS data which shows a distinct alteration in the hemodynamic state over the same region of the somatosensory cortex following similar forepaw electrical stimulation procedures. The combined stimulation and recording capabilities of this device appear suitable for the proposed future experiments investigating the therapeutic affects of PENS following stroke.

Funding: Project funded by Heart and Stroke Foundation of BC/Yukon.

Poster Category E : Homeostatic and Neuroendocrine Systems

Title: The antagonists of the Na/K ATPase regulate the activity of the NaX channel in the rat MnPO neurons

Authors: Emmanuelle Berret¹, Benjamin Nehmé², Didier Mougnot³

Affiliation: 1-3 CHUL and Université Laval

Abstract: "The median preoptic nucleus (MnPO) holds a strategic position in the hypothalamus, having direct access to the cerebrospinal fluid (LCR) ion composition. This confers to the MnPO neurons an important role in the hydromineral homeostasis regulation by acting as central detectors of extracellular Na⁺ ([Na⁺]_{out}). The mechanism underlying Na⁺ detection involves the atypical Na⁺-channel, NaX, which is functionally expressed in the MnPO neurons. The study purpose was to determine the intrinsic characteristics of this channel and its possible regulation by other membrane proteins involved in the cellular Na⁺ homeostasis, like the Na/K-ATPase. The patch-clamp technique has been used in the whole-cell configuration to record the Na⁺ leak current in dissociated MnPO neurons in control condition and in the presence of ouabain and strophanthidin, two antagonists of the $\alpha 1$ and $\alpha 3$ subunits of Na/K-ATPase, respectively. Simultaneous immuno-histochemical labellings of Na/K-ATPase and NaX were also performed. The anatomical data showed a co-expression of the $\alpha 1$ and $\alpha 3$ subunits with the NaX channel in the MnPO neurons. The pharmacological study showed that the Na⁺ leak current amplitude was reduced up to 55% by 30 μ M strophanthidin and 50 μ M ouabain, respectively. The dose-response curves showed that strophanthidin (EC₅₀=9,8 μ M) had a greater inhibitory effect than ouabain (EC₅₀=23,2 μ M) for a similar concentration, and concomitant application did not provide further inhibition of the Na⁺ current. Our results show a fine regulation of the intracellular [Na⁺] in the MnPO neurons. The Na⁺ influx carried by the NaX channel is directly regulated by the $\alpha 1$ subunit of the Na/K-ATPase "

Funding: provided by CIHR.

Title: Differential expression of the NaX channel (SCN7a) in the brain of the rat and mouse.

Authors: Didier Mougnot¹, Benjamin Nehmé²

Affiliation: 1,2 Centre de recherche du CHUQ (CHUL) et axe de Neurosciences, Université Laval

Abstract: The Na⁺ leak channel NaX is a primary determinant of the Na⁺ detection in the lamina

terminalis. However, the distribution pattern and the cellular phenotypes expressing this channel remain to be determined in both the rat and mouse brain to clarify the mechanisms of Na⁺ detection in rodents. Here, we combined NaX immunofluorescence with fluorescent markers of specific cellular phenotypes to identify the cellular populations expressing the NaX channel throughout the rat and mouse brain. RT-PCR validated the NaX protein in all the regions of interest. In the rat and mouse, NaX was found in the magnocellular neurons immunoreactive to oxytocin and vasopressin in the supraoptic and paraventricular nuclei, in neurons (NeuN-ir) of the area postrema, septum, thalamus and of the hypoglossal nucleus. NaX was present in the tanycytes (vimentin-ir) of the median eminence. The NaX expression showed discrepancies among structures and cellular subtypes in the rat and mouse. NaX was expressed in neurons and tanycytes of the subfornical organ and vascular organ of the lamina terminalis of the rat, whereas NaX was restricted to tanycytes in the mouse. NaX was specifically expressed in the mouse hippocampus and Purkinje cells and in the median preoptic nucleus of the rat. Our anatomical study extends our knowledges on the implication of the NaX channel in controlling the hydromineral balance of the rodents. Moreover, identifying the NaX channel in extrahypothalamic regions further suggest the involvement of this atypical Na⁺ channel in various physiological functions.

Funding: Supported by the CIHR (MPO#178002).

Title: Differential expression of the NaX channel (SCN7a) in the brain of the rat and mouse.

Authors: Didier Mougnot¹, Benjamin Nehmé², Méline Henry³

Affiliation: 1-3 Centre de recherche du CHUQ (CHUL) et axe de Neurosciences, Université Laval

Abstract: The Na⁺ leak channel NaX is a primary determinant of the Na⁺ detection in the lamina terminalis. However, the distribution pattern and the cellular phenotypes expressing this channel remain to be determined in both the rat and mouse brain to clarify the mechanisms of Na⁺ detection in rodents. Here, we combined NaX immunofluorescence with fluorescent markers of specific cellular phenotypes to identify the cellular populations expressing the NaX channel throughout the rat and mouse brain. RT-PCR validated the NaX protein in all the regions of interest. In the rat and mouse, NaX was found in the magnocellular neurons immunoreactive to oxytocin and vasopressin in the supraoptic and paraventricular nuclei, in neurons (NeuN-ir) of the area postrema, septum, thalamus and of the hypoglossal nucleus. NaX was present in the tanycytes

(vimentin-ir) of the median eminence. The NaX expression showed discrepancies among structures and cellular subtypes in the rat and mouse. NaX was expressed in neurons and tanycytes of the subfornical organ and vascular organ of the lamina terminalis of the rat, whereas NaX was restricted to tanycytes in the mouse. NaX was specifically expressed in the mouse hippocampus and Purkinje cells and in the median preoptic nucleus of the rat. Our anatomical study extends our knowledge on the implication of the NaX channel in controlling the hydromineral balance of the rodents. Moreover, identifying the NaX channel in extrahypothalamic regions further suggest the involvement of this atypical Na⁺ channel in various physiological functions.

Funding: Supported by CIHR (MPO#178002)

Title: Cholecystokinin induces c-Fos expression in the subfornical organ

Authors: Al-Shaimaa Ahmed¹

Affiliation: 1- Hotchkiss Brain Institute, Department of Physiology and Pharmacology, University of Calgary

Abstract: "Introduction: Cholecystokinin (CCK) is a gut hormone secreted in response to feeding. It activates the nucleus of the solitary tract (NTS) and area postrema (AP) via afferent vagal fibres. The subfornical organ (SFO) is a circumventricular organ that is implicated in the control of energy balance and fluid homeostasis. Using the marker c-Fos, we studied the effect of a hypophagic dose of CCK on neuronal activation in SFO. Experiment: Male Sprague-Dawley rats, adapted to handling, were fasted before receiving an i.p. injection of vehicle or the CCK -1 receptor antagonist devazepide (1 mg/kg). Thirty min later, rats received vehicle or CCK (16ug/kg). After 90 min, rats were euthanized. Brains were dissected and processed for c-Fos immunoreactivity and immunopositive nuclei were counted. Results: CCK caused a substantial increase in c-Fos immunoreactivity in AP (102.0 ± 12.5 cells) and NTS (259.2 ± 20.8 cells) compared to vehicle (AP: 16.0 ± 5.5; NTS: 47.5 ± 2.5 cells). This was significantly (p<0.05) reduced in rats pretreated with devazepide (AP: 28 ± 3.5; NTS: 171 ± 17.0 cells), which also attenuated CCK-induced hypophagia. In SFO, CCK induced c-Fos immunoreactivity (CCK: 40.5 ± 10.6; vehicle: 6.6 ± 2.7 cells). However, c-Fos immunoreactivity after administration of devazepide alone or with CCK was comparable to CCK-treated rats (59.6 ± 11.6 and 40.5 ± 2.5; respectively). Conclusion: These data suggest that CCK activates neurons in SFO, but not through the CCK-1 receptor. Functional studies are ongoing

to identify the role of SFO in the anorexigenic actions of CCK. "

Funding: Achievers in Medical Science (AIMS)Scholarship CIHR, Neurobiology of Obesity

Title: Polydendrocytes - A novel proliferative niche in the adult mouse hypothalamus?

Authors: Sarah Robins¹, Maia Kokoeva²

Affiliation: 1-2 McGill University

Abstract: Recently, evidence has accumulated supporting the existence of a neural stem cell niche in the hypothalamus of adult rodents. However, this niche remains poorly characterised, and the identity of the proliferating cell types remains to be proved. Here, we present evidence that NG2+ polydendrocytes are a substantial source of proliferating cells in this region. Elsewhere in the brain, polydendrocytes are being intensively researched as possible neural stem cells, but their function in the hypothalamus remains unexplored. We investigate the cell cycle kinetics of polydendrocytes using intracerebroventricular infusions of the cell proliferation markers BrdU and EdU, and show that a subset of the population can undergo self-renewing divisions. In addition, we use NG2creERT:Tomato mice to track the fate of the progeny of dividing polydendrocytes. Co-localisation of tomato reporter protein with markers of mature cell types such as HuC/D (neurons), APC (oligodendrocytes) and GFAP (astrocytes) allows us to establish which neural lineages are derived from hypothalamic polydendrocytes. Hypothalamic neurogenesis has previously been suggested to play a role in maintaining energy homeostasis. We wish to determine whether polydendrocytes are required for this function, by searching for evidence of energy dysregulation when polydendrocytes are removed. Ablation is achieved by using mitotic inhibitors or transgenic mouse models. Our work to date suggests that polydendrocytes may form a novel neurogenic niche in the hypothalamus, and that further work in this area is vital to understand how plastic changes within the hypothalamus act to regulate energy expenditure.

Funding: Funded by the CIHR

Title: Oxytocin influences the excitability of neurons in the nucleus of the solitary tract

Authors: Andrea Mimee¹, Alastair Ferguson²

Affiliation: 1-2 Queen's University

Abstract: Nesfatin-1 has been identified as one of the most potent centrally acting anorexigenic

factors, and it is expressed in the nucleus of the solitary tract (NTS), a medullary structure with critical roles in the regulation of feeding. Evidence suggests nesfatin-1 requires functional oxytocin signaling to exert its actions, and thus in this study we investigated how oxytocin may contribute to nesfatin-1 signaling in the NTS. We have previously shown nesfatin-1 elicits depolarizing and hyperpolarizing effects in NTS neurons. Here, we initially examined the effects of oxytocin on the excitability of NTS neurons using whole-cell current clamp recordings from rat NTS neurons in slice preparation. Bath applied 10nM oxytocin influenced 75% of cells, with 67% of responsive cells exhibiting hyperpolarizations ($n=16$, $-6.9 \pm 1.0\text{mV}$) and 33% of responsive cells depolarizations ($n=8$, $7.5 \pm 1.5\text{mV}$). We next pre-treated NTS slices with an oxytocin receptor antagonist (OVT) and a vasopressin V1 receptor antagonist (V1-R ant), and bath applied nesfatin-1 to determine whether it signals through the oxytocin receptor. Pre-treatment of slices with $1\text{-}\mu\text{M}$ OVT and V1-R ant did not abolish nesfatin-1 effects, as 10nM nesfatin-1 affected 54% of cells tested (13/24), with depolarizations representing 77% of observed effects ($n=10$, $4.6 \pm 0.4\text{mV}$) and hyperpolarizations 23% of observed effects ($n=3$, $-3.7 \pm 0.5\text{mV}$). Our findings identify the NTS as a site in which oxytocin acts to influence the excitability of neurons with potential effects on food intake, and suggest the nesfatin-1 receptor, which remains unknown, is neither an oxytocin nor vasopressin V1 receptor.

Funding: CIHR, NSERC, and FQRNT

Title: Resilience and vulnerability to chronic unpredictable stress: implication of endorphins.

Authors: Patrick Bérubé¹, Jean-François Poulin², Caroline Gagnon³, Sylvie Laforest⁴, Guy Drolet⁵,

Affiliation: 1-5 Université Laval, Centre de recherche du CHUQ

Abstract: "The present study assessed the involvement of enkephalin (ENK) and dynorphin (DYN) neuropeptides in behavioral adaptation following 21 days of chronic unpredictable stress (CUS) procedure in Sprague Dawley rats. Social interaction (SI; done 3 days after CUS), elevated plus-maze (EPM; done 4 days after CUS), sucrose consumption and sucrose preference (1% sucrose solution; 4h period; at the end of CUS) tests were used in k-means clustering in order to split resilient and vulnerable individuals from stressed group. All rats were perfused 1 week after the last stress session. In situ hybridization for ENK and DYN were performed to characterize changes in mRNA expression in the amygdaloid complex, striatum, nucleus accumbens (NAc) and prefrontal cortex. 21 days of CUS decreased the duration of SI ($p<0.001$), weight gain ($p<0.001$)

and increase basal corticosterone levels ($p=0.006$) in stressed ($n=49$) when compared to unstressed individuals ($n=25$). Vulnerable had lower SI ($p<0.001$), lower open arms time ratio ($p<0.001$) and total arms distance ($p<0.004$) in the EPM than resilient animals. ENK is increased in dorsolateral striatum and in posterior part of the basolateral amygdalar nucleus while DYN expression is increased in medial orbitofrontal ($p=0.022$), infralimbic cortex ($p=0.036$) and in medial ($p<0.001$) and dorsal ($p<0.001$) part of the NAc (shell). In contrast, there was no difference for ENK and DYN levels between resilient and unstressed individuals. These results have broad implications for understanding the functional role of opioids neurotransmission in resilience to repeated unpredictable psychogenic stress. "

Funding: Canadian Institutes of Health Research

Title: Apelin-13 decreases blood pressure through modulating the excitability of neurons in the subfornical organ

Authors: Pauline Smith¹, Li Dai², Alastair Ferguson³

Affiliation: 1-3 Queen's University

Abstract: Apelin, an adipocyte derived hormone, acts in the central nervous system (CNS) to influence body fluid balance, cardiovascular regulation, and energy homeostasis. The subfornical organ (SFO), a circumventricular structure which lacks the normal blood brain barrier (BBB), is classically known for its roles in the control of fluid balance and cardiovascular regulation and has recently been implicated in energy homeostasis. The lack of the BBB and presence of the apelin receptor uniquely positions the SFO to detect the presence of circulating apelin and relay this information, via established efferent pathways, to hypothalamic autonomic nuclei. We have used both in vivo (microinjection) and in vitro (whole cell current clamp recording) techniques to investigate the cardiovascular consequences of apelin receptor activation in response to administration of apelin-13. Microinjection of apelin-13 ($0.5\text{-}\mu\text{l } 10^{-7}\text{M}$) into the SFO resulted in a decrease in blood pressure (mean AUC= $-1612.7 \pm 359.8\text{mmHg}\cdot\text{sec}$) without influencing heart rate. Current clamp recordings from dissociated SFO neurons revealed that apelin-13 (10^{-8}M) influenced the excitability of 74% of SFO neurons tested (14/19). The majority of responsive cells were depolarized by apelin-13 ($6.2 \pm 1.6\text{mV}$, $n=10$) while the remaining cells hyperpolarized ($-7.6 \pm 1.3\text{mV}$). These results suggest that apelin-13 acts in the SFO to control BP and that such effects may occur as a direct result of the ability of this adipokine to modulate the excitability of neurons

in the SFO. These data support a role for the SFO as a CNS site at which apelin may act to influence central autonomic processing.

Funding: Canadian Institutes of Health Research

Title: Multiple sodium depletions induced a salt sensitization in a subpopulation of Sprague-Dawley rats: A differential role for mu-opioid receptor (mu-OR) expression in the ventral pallidum.

Authors: Alexis Gobeil Simard¹, Caroline Gagnon², Sylvie Laforest³, Didier Mouginot⁴, Guy Drolet⁵

Affiliation: 1-5 Université Laval, Neurosciences - Centre de recherche du CHUQ(CHUL)

Abstract: Salt overconsumption is not due to a survival requisite. In the present study, we sought to determine whether repeated sodium depletion induces a Na⁺ sensitization by modulating the mu-opioid receptor (mu-OR) expression in the ventral pallidum (VP). 46 young male Sprague-Dawley rats were submitted to one sodium depletion-repletion episode per week, for 3 consecutive weeks. Each episode consisted in two subcutaneous injections of furosemide (FURO) or saline (CTL). Every week, daily spontaneous intake of 1.8% NaCl solution were recorded before sodium depletion, and need-induced Na⁺ intake was also measured after each sodium depletion. Rats were sacrificed after the 3rd sodium repletion and in situ hybridization procedure (ISH) was performed to evaluate the mu-OR expression in the VP. The multiple sodium depletion induced a salt sensitization in a subpopulation of FURO rats, 40% salt sensitized group (SS) and not in remaining rats, non sensitized group (NO). Salt sensitization was first demonstrated by a significant increase in spontaneous salt intake of SS rats compared to NO rats. Then, an increased in need-induced salt intake was observed in SS, but not in NO rats after 3 Na⁺ depletions. Finally, mu-OR expression in the VP was significantly lower in SS rats than in NO rats. This study suggests that multiple sodium depletions induced a salt sensitization in a subpopulation of Sprague-Dawley rats. This sensitization was correlated with a significant decrease in mu-OR expression in the VP of SS rats.

Funding: CIHR, Heart and Stroke Foundation

Title: Alterations of electrophysiological properties of the rat MnPO neurons induced by multiple sodium deficits

Authors: Aurore VOISIN¹, Guy DROLET², Didier MOUGINOT³

Affiliation: 1-3 Centre de Recherche du CHUQ-CHUL

Abstract: OBJECTIVES: Repeated sodium depletions have been reported to increase salt intake, a motivated behavior resulting from sodium depletion in the extracellular body fluid compartments. In this study, we sought to determine whether multiple sodium depletions might generate plastic changes in the neuronal basic properties of the median preoptic nucleus (MnPO), involved in the regulation of the sodium homeostasis. METHODS: Patch-clamp recordings were performed on rat brain slices containing the MnPO. Neurons were tested for sodium sensitivity, response to transient application of DAMGO and pattern of action potential discharge. Rats were sacrificed after one, two or three consecutive sodium depletions. RESULTS: Multiple sodium depletions reduced the amplitude of the membrane depolarization induced by hypernatruric aCSF, as well as the percentage of sodium-responsive neurons by 15% and 22%, respectively. Interestingly, the percentage of Na⁺-sensor neurons expressing functional mu-opioid receptor increased after repeated sodium challenges. Moreover, a majority of MnPO neurons displayed a light adaptation in their spiking pattern, whereas other neurons displayed a strong spike frequency adaptation. The percentage of the former category was increased by 20%, whereas the percentage of the latter was reduced by 25% after the third sodium depletion. CONCLUSION: Multiple sodium depletions are associated with the alteration of primary intrinsic properties of the neuronal MnPO populations. These plastic changes might strongly alter the signal output of this nucleus, thereby promoting adaptation of the neuroendocrine, autonomic and behavioral responses involved in the hydromineral homeostasis.

Funding: CIHR (MOP#81124) and The Heart and Stroke Foundation of Québec

Title: Stimulation of the dorsal raphe nucleus by corticotropin releasing hormone and behavioural outcomes related to mPFC functioning

Authors: Adam Neufeld¹

Affiliation: 1- Carleton University

Abstract: Variations of serotonin (5-HT), and corticotropin-releasing hormone (CRH) have been implicated in mediating the behavioural effects of uncontrollable stressors. In this regard, it seems that CRH may play a role in the regulation of 5-HT activity. In the present investigation, we demonstrated that CRH (1.0ug) administered directly to the dorsal raphe nucleus (DRN) increased

the utilization of 5-HT, reflected by elevated 5-HIAA accumulation at the prefrontal cortex (PFC) and hippocampus of CD-1 mice. In parallel, the CRH treatment elicited dose-dependent signs of anxiety, reflected by diminished open arm entries in an elevated plus maze test and reluctance to enter the open arms, as well as increased latencies and less time spent in the central core of an open field. The CRH receptor antagonist, α -helical CRH, had the opposite effect. When CRH (1.0ug) was administered in conjunction with a social stressor, plasma corticosterone was increased by both treatments (additively) as was 5-HIAA accumulation within the PFC. Many of the actions of CRH on monoamine activity were attenuated by pretreatment with the CRH₂ antagonist astressin. These results suggest that CRH infusion into the DRN promotes a stress-like response with respect to behavioural, corticoid and central monoamine functioning, although it did not appear that CRH and stressor treatments had synergistic actions in this regard.

Funding:

Title: Stress and activity-dependent lability of endocannabinoid signalling at hypothalamic synapses

Authors: Jaclyn I. Wamsteeker¹, J. Brent Kuzmiski², Jaideep S. Bains³

Affiliation: 1-3 Hotchkiss Brain Institute

Abstract: Endocannabinoids (eCBs) regulate glucocorticoid (CORT) hormone responses following stress through direct actions at GABA and glutamate synapses onto hypothalamic parvocellular neuroendocrine cells (PNCs). Intriguingly, prolonged *in vivo* exposure to CORT (during repetitive stress) compromises eCB signalling in PNCs through functional downregulation of presynaptic CB1 receptors. Since presynaptic g-protein coupled receptors can be upregulated by synaptic recruitment, we hypothesized that patterned afferent activity after stress would re-establish CB1 function and eCB signaling. To assay eCB signalling at GABA synapses in hypothalamic slices, we induced calcium-dependent liberation by depolarizing PNCs (5s, +20mV) and examined the ensuing transient eCB-mediated reductions of evoked inhibitory post-synaptic current (eIPSC) amplitude. We investigated the impact of low-frequency stimulation of synaptic inputs (LFS: 2Hz, 5 min) on eCB-mediated eIPSC suppression in naïve or repetitive restraint stress-exposed rats (5 days, 30 mins). LFS rapidly enhanced eCB signaling in cells from stressed but not naïve rats. Compared to naïve cells (41.8±7.5% decrease from baseline), repetitive stress reduced eCB-mediated eIPSC suppression (13.4±7.4% decrease). After LFS, depolarization induced a robust

reduction of eIPSC amplitude in stressed cells ($43.1 \pm 7.8\%$ decrease) but not naïve cells ($36.5 \pm 5.0\%$ decrease). LFS of inputs to stressed cells also enhanced suppression of eIPSCs by the CB1 receptor agonist WIN55,212-2 ($5 \mu\text{M}$, 25 mins): $51.9 \pm 11.4\%$ decrease from baseline vs $18.2 \pm 7.0\%$ in untetanized cells. These data demonstrate that patterned synaptic activity erases stress induced changes in eCB signalling.

Funding: Canadian Institutes of Health Research, Alberta Innovates- Health Solutions, Hotchkiss Brain Institute (T.Chen Fong)

Title: Chronic treatment with 17beta-estradiol of ovariectomized monkeys increases striatal dopamine transporter and Akt/GSK3 signaling

Authors: Maria Gabriela Sánchez¹, Marc Morissette², Thérèse Di Paolo³

Affiliation: 1-3 Molecular Endocrinology and Genomic Research Center, Centre de recherche du CHUQ (CHUL), Quebec and Faculty of Pharmacy, Laval University, Quebec (QC), Canada

Abstract: The present experiments sought the effect of a chronic treatment with 17beta-estradiol on striatal dopaminergic activity and the Akt/GSK3 signaling pathway in the brain of monkeys. Eight female monkeys (*macacca fascicularis*) were ovariectomized (OVX) and a month later, half received a month treatment with 17beta-estradiol and the other with vehicle. The DA transporter (DAT) was measured by autoradiography with [¹²⁵I]RTI-121 and the vesicular DA transporter (VMAT2) with [³H]TBZ-OH at three rostro-caudal parts of the caudate nucleus and putamen subdivided in their lateral/medial, ventral/dorsal sub-regions. Specific binding to DAT was increased in all sub-regions of the caudate nucleus and the putamen of 17beta-estradiol-treated compared to vehicle-treated monkeys whereas specific binding to VMAT2 remained unchanged. We measured by Western blot the phosphorylated forms at serine 473 and threonine 308 of Akt, serine 9 and tyrosine 216 of GSK3beta and serine 21 of GSK3alpha in anterior, medial and posterior caudate nucleus and putamen. 17beta-estradiol treatment induced an increase in caudate nucleus and putamen of pAkt(Ser473)/betaIII-tubulin, pGSK3beta(Ser9)/betaIII-tubulin and in putamen of Akt/betaIII-tubulin compared to vehicle-treated monkeys. In anterior and medial putamen, pAkt(Thr308)/betaIII-tubulin was also increased in monkeys treated with 17beta-estradiol. pGSK3beta(Tyr216)/betaIII-tubulin and pGSK3alpha(Ser21)/betaIII-tubulin did not change with the 17beta-estradiol treatment. These results suggest that 17beta-estradiol activates striatal DA neurotransmission as reflected with increased DAT specific binding and

down-stream activation of the Akt/GSK3 signaling pathway. This supports a beneficial role of a chronic treatment with 17beta-estradiol by increasing the activity of signaling pathways implicated in cell survival.

Funding: for this study was provided by CIHR Grant.

Title: Fatty acid desaturase variants, gene expression, and activity levels in major depressive disorder

Authors: Erika Freemantle¹, Naguib Mechawar², Gustavo Turecki³

Affiliation: 1-3 Douglas Mental Health University Institute

Abstract: "Background: Peripheral and cerebral alterations in fatty acids (FAs) and related genes, such as fatty acid desaturase 1 (FADS1), have been implicated in major depressive disorder (MDD) and suicide. Recent genomic studies have highlighted genetic variability in the FADS1/2/3 gene cluster as an important contributor to polyunsaturated FAs (PUFA) alterations in serum lipids and measures of FA desaturase activity estimated by ratios of relevant FAs. Methods: Fatty acids in brain tissue (Brodmann area 47; BA47) were analysed by GC-MS, gene expression was measured by Real-time PCR, and DNA sequencing was performed by the Sanger method. Results: Variants in FADS gene cluster were sequenced and analyzed for their influence on both FADS gene expression and FAs in BA47 from 47 subjects (18 controls, 17 suicides, and 12 MDD suicides). Our results suggest an association of the minor allele (C/C vs. C/T and T/T) in the 3'UTR of FADS1 (rs174546) of MDD suicides (chi-square=0.025), accompanied by an alteration in estimated desaturase activity in brain tissue (p=0.0001). Given that this SNP was previously shown to be in linkage with other variants in a 48kb region of FADS1/2 gene cluster ($D' > 0.9$), it is unlikely the effect reported is due to this variant alone. Expanding sequencing coverage and haplotype analysis in a larger sample will be conducted to confirm this hypothesis. Discussion: This study suggests that genetic variability in fatty acid destaurase genes has an effect on both gene expression and fatty acid profiles in brain tissue. "

Funding:

Funding: provided by Fonds de la Recherche en Santé Québec and the Canadian Institutes of Health Research

Title: Gastrin-releasing peptide induces PER1 in the hamster suprachiasmatic nucleus during the late night

Authors: Roxanne Sterniczuk¹, Michael Antle²

Affiliation: 1-2 University of Calgary

Abstract: Located within the anterior hypothalamus, the suprachiasmatic nucleus (SCN) contains the mammalian circadian pacemaker, which regulates the timing of daily physiology and behaviour. The SCN is a very heterogeneous structure that is composed of distinct cell groups, which process and transmit photic information from the retina to rhythmic clock cells. Rhythmic clock cells in the SCN are driven by a transcription-translation feedback loop that restarts daily. Gastrin-releasing peptide is a prominent neuropeptide within the retinorecipient region of the SCN and has been shown to mimic the effects of light by altering the phase of the circadian clock. There is strong evidence to suggest that GRP is a primary output signal that conveys photic information from the arrhythmic retinorecipient region, to the rhythmic non-retinorecipient portion of the SCN. The mechanisms by which GRP exerts its phase altering effect are still unclear. The present study sought to determine whether GRP induces the expression of one of the main proteins involved in the transcription-translation feedback loop. Hamsters received a microinjection of either saline or GRP, 10 hours following the onset of activity, and were sacrificed 6 hours later for immunohistochemical evaluation. GRP was found to significantly induce PER1 expression within the SCN in the late night ($p < .01$). This finding is consistent with previous work demonstrating that GRP induces Per1 and Per2 gene expression in the early night. This provides further evidence that GRP is acting at the individual cellular level to regulate rhythmic clock cells and mediate the phase shifting effects of light.

Funding: Natural Sciences and Engineering Research Council of Canada, Alberta Heritage Foundation for Medical Research, Sir Izaak Walton Killam Pre-Doctoral Scholarship

Title: "TRPV1 Δ 1-283: A candidate osmoreceptor channel."

Authors: Cristian Zaelzer¹, Pierce Hua², Masha Prager-Koutorsky³, Sorana Ciura⁴, Sukhee Lee⁵, Wolfgang Liedtke⁶, Charles W. Bourque⁷

Affiliation: 1-4,7 Research Institute of MGH 5-6 Center for Translational Neuroscience, Duke University Medical Center

Abstract: "Mammalian osmosensory neurons exposed to hypertonicity display a shrinking-

induced increase in non-selective cation conductance causing membrane depolarization and excitation. This response is important for triggering homeostatic responses including thirst and antidiuresis. The molecular identity of the osmoreceptor channel is unknown, but appears to involve a capsaicin-insensitive N-terminal variant of the transient receptor potential vanilloid type 1 (TRPV1) channel (Ciura and Bourque, 2006; Sharif-Naeini et al., 2006). Here, we report that heterologous expression of TRPV1 variant $\Delta 1-283$, which lacks exons 1-5, confers osmosensory characteristics similar to those found in native osmosensory neurons. Whole cell voltage clamp recordings examined the effects of hyperosmolality (+mannitol) on human embryonic kidney (HEK293) cells expressing $\Delta 1-283$ TRPV1, GFP or wild type TRPV1. Dynamic imaging confirmed that all HEK cells exposed to hypertonicity underwent visible shrinking. However in contrast to GFP (n = 10) and TRPV1 (n = 32) transfected cells, which were unresponsive, ~41% (84/206) of cells transfected with $\Delta 1-283$ TRPV1 showed a progressive and sustained increase in non-selective cation current and membrane conductance when exposed to hypertonicity for 2-5 minutes. These effects were dose-dependent (+5 to +80 mosmol/kg), mimicked by suction induced shrinking (n=6), and abolished by Ruthenium Red. In agreement, FURA-2 loaded HEK cells transfected with $\Delta 1-283$ TRPV1 displayed hypertonicity-induced increases in intracellular $[Ca^{2+}]$, whereas those transfected with wild type TRPV1 did not. These results show that channels encoded by $\Delta 1-283$ TRPV1 can operate as functional osmoreceptors."

Funding: CIHR grant MOP-82818

Poster Category F : Techniques in Neuroscience

Title: Interrogation of cultured neurons and synaptic activity using novel patch-clamp chips

Authors: Geoffrey Mealing¹, Marzia Martina², Collin Luk³, Dolores Martinez⁴, Tanya Comas⁵, Robert Monette⁶, Mike Denhoff⁷, Naweed Syed⁸, Christophe Py⁹

Affiliation: 1,2,5,6 Institute for Biological Sciences NRC 3,8- University of Calgary 4,7,9- IMS NRC

Abstract: Planar patch-clamp chips have recently been developed at the National Research Council of Canada suitable for recording ion channel activity from cultured neurons. Using these chips, we report characteristics of synaptic communication between cultured pre- and postsynaptic neurons from *Lymnaea stagnalis*. Both large postsynaptic Left Pedal Dorsal 1 (LPeD1) and smaller presynaptic Visceral Dorsal 4 (VD4) neurons were examined in the whole-cell configuration. Pairing these neurons in close proximity encouraged soma-soma synapse formation, making it possible to record postsynaptic responses from single-site chips in response to presynaptic stimulation via sharp electrodes. Tetanic stimulation transiently potentiated these cholinergic postsynaptic responses which were blocked by tubo-curarine. Subsequently, simultaneous dual-site chip recordings were obtained from synaptic pairs, and dedicated cytoplasmic perfusion of individual neurons via on-chip subterranean microfluidics was possible without disrupting the whole-cell configuration. Packaged single-site chips will soon be available for evaluation to researchers with applications that could benefit from this technology.

Funding:

Title: Common Pathological Mechanisms in Huntington's and Alzheimer's Diseases Uncovered by FLIM-FRET

Authors: Ray Truant¹, Lise Munsie², Nicholas Caron³, Jianrun Xia⁴

Affiliation: 1-4 McMaster University

Abstract: Huntington's disease (HD) is caused by an expanded CAG tract in the IT15 gene encoding the 350 KDa huntingtin protein. Cellular stresses can trigger the release of huntingtin from the endoplasmic reticulum (ER), allowing huntingtin nuclear entry. Endogenous, full-length huntingtin localizes to nuclear cofilin-actin rods during stress and is required for the proper stress response involving actin remodeling. Mutant huntingtin induces a dominant, persistent nuclear rod phenotype similar to that described in Alzheimer's disease for cytoplasmic cofilin-actin rods.

Using live cell temporal studies, we show that this stress response is similarly impaired when mutant huntingtin is present, or when normal huntingtin levels are reduced. In clinical lymphocyte samples from HD patients, we have quantitatively detected cross-linked complexes of actin and cofilin with complex formation varying in correlation with disease progression. By live cell FLIM-FRET studies and western blot assays, we quantitatively observed that stress-activated tissue transglutaminase 2 (TG2) is responsible for the actin-cofilin covalent cross-linking observed in HD. These data support a direct role for huntingtin in nuclear actin re-organization, and describes a new pathogenic mechanism for aberrant TG2 enzymatic activity in neurodegenerative diseases. This also suggests that the huntingtin protein may be involved in some AD pathology, for non-genetically-determined reasons. We will present data on the development of a new live cell FLIM-FRET sensor for TG2 activity, as well as small molecule kinase inhibitors that can affect the conformation of huntingtin in vivo, restoring mutant huntingtin conformation to wild-type with direct implications for HD therapeutic development.

Funding: The CIHR, The Krembil Foundation, CHDI Inc., Huntington Society of Canada, the Canadian Foundation Institute.

Title: Combined fluorescent in situ hybridization and immunofluorescence: limiting factors and a substitution strategy for slide-mounted tissue sections

Authors: Didier Mougnot¹, Méline Henry², Benjamin Nehmé³

Affiliation: 1-3 Centre de recherche du CHUQ (CHUL) et Axe Neurosciences, université Laval

Abstract: The simultaneous localization of several anatomical markers is required to understand and analyze the organization of brain nuclei or to identify neuronal networks recruited during a biological stimulus. Gathering information is usually achieved by the combined detection of both mRNA and proteins. Despite the promise offered by the combination of fluorescent in situ hybridization (FISH) and immunofluorescence (IF) in terms of reduced bench time and easy visualization of multiple labels at once, this technique was performed using free-floating brain sections that were at least 30 μm thick. In the present study, we have adapted a combination of FISH and IF for slices mounted on a microscope slide (thickness $\leq 20 \mu\text{m}$), using mRNA (GAD65 mRNA) and proteins (NeuN, FosB or TH) to validate this method. We demonstrated that the prehybridization protocol widely used for ISH is incompatible with IF staining. This incompatibility results from the proteolytic activity of proteinase K, which digests or denatures

numerous antigenic sites of target proteins. Our modified protocols indicate that either stabilization of the riboprobe-mRNA complex with 2X standard saline citrate solution , or membrane permeabilization using Triton X-100 or Tween 20 are critical steps for producing a high quality FISH and IF labeling in the majority of the brain regions of interest . Our study provides several substitution strategies to PK for efficient FISH and IF staining on thin brain slices.

Funding: This research study was supported by the Canadian Institutes for Health Research Grant MOP-178002

Title: RC::PFtoX, a new genetic tool for turning-off neurotransmission in highly selective cell populations in the living mouse

Authors: Junchul Kim¹, Susan Dymecki²

Affiliation: 1- University of Toronto, Department of Psychology 2- Harvard University

Abstract: "Our goal has been to generate a set of genetic tools for use in mice that allow the following key features of a neuron type to be delineated and linked in vivo: cell origin, cell fate, and cell function. Our starting point has been a dual recombinase-based, highly cell type-selective molecule delivery system with plug-and-play modularity. Building up from this versatile system, we have generated fate mapping and neuronal silencing alleles in order for: (1) neurotransmission to be altered in otherwise undisturbed, freely behaving animals or in developing embryos in utero; (2) considerable cellular specificity and reproducibility in the neuron population targeted for silencing; (3) cellular specificity to be based on a molecular signature so that cellular resolution is not only great but that the revealed neuron function can be related to gene expression occurring either at the time of silencing or earlier during the development of that cell and circuit _ this would allow for the identification of gene products that might function in the development, differentiation, and/or maintenance of a particular kind of circuit; (4) visualization of the silenced neurons; (5) versatility, in that the tool could be applied differentially to silence virtually any type of neuron. Utility of the developed tools, including strengths and weaknesses, has been demonstrated through investigations of two very different types of circuits: a precerebellar-to-cerebellar circuit involved in motor behaviors, and circuits involving serotonin neurotransmission. "

Funding: NSERC

Title: Combining a micro-optrode with optogenetics enables resolving intrinsic membrane properties by extracellular recordings in vivo.

Authors: Suzie Dufour¹, Nicolas Doyon², Martin Deschênes³, Réal Vallée⁴, Yves De Koninck⁵

Affiliation: 1-3,5 Centre de Recherche Université Laval Robert-Giffard⁴- COPL Université Laval

Abstract: Extracellular recordings are extremely popular for in vivo experimentation. Glass and metal microelectrodes can record a neuron activity from the extracellular medium, leaving the cell body membrane completely unaltered and are thus a non-invasive recording technique. But without an access to the membrane or the intracellular medium, extracellular recordings exclude all depolarizing and hyperpolarizing pulse tests that provide fundamental data on the electrophysiological properties of the cell membrane. Here, we show that using optogenetic tools and the micro-optrode recently developed in our laboratory, we can mimic the pulse tests usually achieved with intracellular and patch clamp techniques. In our experiments, we used transgenic mice expressing the light-gated cations channel channelrhodopsin 2 (CHR2). The optrode has the advantage of providing a confined illumination volume and was used in two distinct ways: 1) to identify and record fluorescently labeled cells that express CHR2 and 2) to provide the light stimuli that depolarize the membrane. Cell depolarization and repolarisation were recorded with the optrode. A signal reconstruction allowed us to estimate the amplitude and time course of the subthreshold depolarization occurring in the cell. Our results show that using micro-optrodes and optogenetic tools, voltage-gated ionic currents can be identified and that membrane resistance changes can be quantified. This demonstration extends the use of extracellular recordings, and gives access to ionic current and cell identification as well as membrane electrophysiological properties in vivo.

Funding: Funded by CIHR. S.D. is supported by the CIHR Neurophysics Training Program.

Title: In situ examination of calpain activity in brain tissue

Authors: Kevin Duffy¹

Affiliation: 1- Dalhousie University

Abstract: Proteases are involved in a multitude of neuronal processes that are critical for maintenance of normal cell function, and their aberrant activity has been linked to a large number of brain diseases. Calpain is a calcium-dependent cysteine protease found within cells and distributed throughout various brain regions, and its activity contributes to normal and abnormal

brain function. A limitation with common approaches to studying calpain activity is the requirement for homogenization of tissue samples, which reduces the ability to resolve the spatial location of calpain activity, and which also introduces the possibility that calpain will interact with inhibitors that would have otherwise been kept spatially separated in vivo. We have developed a method for the investigation of calpain activity in thin brain slices that provides far better spatial resolution than alternatives, and that alleviates the concern of protein interactions in homogenate. We examined calpain activity in brain tissue sections by observing fluorescence signal produced by fragmentation of a casein substrate that was embedded in an agarose gel solution overlaying the section. The specificity of this technique is presented within the context of a cell lysate assay and within polyacrylamide zymograms. Evaluation of calpain activity by in situ zymography preserved the anatomical organization of the tissue, and provided spatial resolution sufficient for ready examination of calpain activity in neurons, glia, and blood vessels within a single tissue section.

Funding: NSERC, CIHR

Title: Exploiting retrogradely transported viral vectors encoding fluorescent proteins to label the entire dendritic tree of spinal projection neurons

Authors: Claire Magnussen¹, Guillaume Lavertu², Yves De Koninck³, Alfredo Ribeiro-da-Silva⁴

Affiliation: 1,4- McGill University 2,3- Centre de Recherche Université Laval Robert-Giffard- Université Laval

Abstract: Projection neurons are an important component of a nociceptive circuit where they relay noxious information from the dorsal horn of the spinal cord to higher brain centers. To gain further insight into the morphology of these neurons, and how this morphology might be altered by nerve injury, these projection neurons can be visualized in the spinal cord using a technique called retrograde tracing. Commonly used retrograde tracers include cholera toxin subunit B (CTb), fast blue and fluorogold among many others. While these tools are useful, they are limited by their inability to label the entire dendritic arborisation. As a result, novel tools capable of labelling a neurons' entire dendritic tree are needed. To this end, we have developed new retrograde tracers, namely viral vectors expressing fluorescent proteins (FP). We used adenoviruses (Ad) and serotypes of adeno-associated viruses (AAV) that are retrogradely transported from the neuron terminals in the brain to the cell body in the spinal cord where the

host cell's replication machinery produces the FP. This approach produced Golgi-like fillings, labelling even the more distal dendrites and spines, unlike CTb. In addition, this approach labels many neurons, unlike intracellular injections of biocytin which are laborious and can yield only a few neurons at most. Thanks to the extensive labelling of neurons using the viral vector transduced FP, we were able to reconstruct entire dendritic trees, allowing a much greater insight into the morphology of these neurons in naive animals and in animals with neuropathic pain.

Funding: Funded by CIHR and FRSQ (Quebec Pain Research Network)

Title: Development of STED microscope to monitor at nanoscale the spatial dynamics of neuronal proteins.

Authors: Christian Tardif¹, Hugues Dufour², Daniel Côté³, Paul DeKoninck⁴

Affiliation: 1-4 Centre de Recherche Université Laval Robert-Giffard

Abstract: The ability to follow the spatial dynamics of proteins in the highly compartmentalized neuron can contribute to the understanding of the signaling cascades implicated in neuronal communication and plasticity mechanisms. For example, the recruitment of proteins at synapses plays a key role in synaptic signaling and remodeling, but the underlying mechanisms and nanoscale redistribution of these proteins are not well understood. A major limitation has been the resolution of optical methods, which is diffraction limited, such that nanoscale structures are difficult to resolve. We are therefore developing a super resolution microscope based on stimulated emission depletion (STED). This poster describes our STED microscope setup, which includes a supercontinuum laser to both excite and deplete fluorescence. We show improved resolution of fine intraneuronal structures, such as microtubules, by immunostaining with Atto-594 as label. We also characterize the effectiveness of new genetically-encoded probes to follow protein dynamics in live neurons with STED. The setup is being optimized to minimize photobleaching in order to monitor proteins on the move over time (multiple frames). We aim to follow the dynamic translocation of CaMKII between microtubules, actin and the post synaptic density under various patterns of neuronal activity. Learning more about the rules that govern the rapid recruitment of the kinase to those different compartments should help us understand the cellular and molecular basis of neuronal plasticity.

Funding: This research is supported by FQRNT, CFI, CIHR and NSERC.

Title: Wide-field multiphoton imaging of cellular dynamics in thick tissue by temporal focusing and patterned illumination

Authors: Olivier Dupont-Therrien¹, Benoit Aubé², Stéphane Pagès³, Paul De Koninck⁴, Daniel Côté⁵

Affiliation: 1-5 Université Laval

Abstract: Wide-field temporal focusing is a novel technique that provides optical sectioning for imaging without the need for beam scanning. However, illuminating over large areas greatly reduces the photon density which limits the technique applicability to small regions, precluding functional imaging of cellular networks. Here we present a strategy that combines beam shaping and temporal focusing of amplified pulses (>1 microjoules/pulse) for fast imaging of cells from the central nervous system in acute slices. Multiphoton video-rate imaging over total areas as wide as 4800 micrometers square with an optical sectioning under 10 micrometers at 800 nm is achieved with our setup, leading to imaging of calcium dynamics of multiple cells simultaneously in thick tissue.

Funding: NSERC training Program in Biophotonics, Canadian Foundation for Innovation, Canadian Institute of Health Research (CIHR) training program in Neurophysics.

Title: New tools for the three dimensional optogenetic stimulation of a group of neurons in the brain

Authors: Alexandre Castonguay¹, Christian Casanova², Frédéric Lesage³, Matthieu Vanni⁴

Affiliation: 1,2,4 École d'optométrie de l'Université de Montréal 3- École Polytechnique Montréal

Abstract: The study of functional connectivity in the brain may require stimulating selectively a group of neurons. This is generally achieved by electric or pharmacological stimulation. These approaches are not very specific and do not allow to spatially modulate neuronal activation. Optogenetics is a developing field in which specific neuronal activation is achieved by light excitation of channels/pumps coupled with photosensors. The goal of this study was to develop new tools to spatially excite group of neurons according to their 2D cortical position and layer. Transgenic mice expressing Channelrhodopsin-2 (ChR2) were used in combination with intrinsic optical imaging recordings (IOI) in the cortex. A 473nm laser (150 mW) was used to excite ChR2, controlling the intensity with modulated liquid crystals. Spatial patterns of illumination were achieved using a digital micromirror device (DMD) leading to both temporal and spatial control of activation. In a second set of experiments, specific layer excitation was accomplished using a 0.3

mm polished optical fiber emitting light at an angle of 90 degrees. With the DMD, maximum neuronal responses were observed at 2mW/mm² for spots of 0.1 mm. The insertion of the optical fiber in the visual cortex produced responses in supra- and infragranular layers. In conclusion, DMD was used to produce spatial patterns of optogenetic stimulation with micrometric precision over the whole cortex. The polished optical fiber allowed the selective activation of cortical layers. Further developments will focus on reducing the stimulus diameter and motorize the fiber displacement, allowing the complete mapping of regions of interest.

Funding: NSERC (CC, FL) FRSQ vision network (AC)

Title: Identification of Ultrastructural Features of Artificial Synapses using Cryo-TEM

Authors: Gopakumar Gopalakrishnan¹, Patricia Yam², Isabelle Rouiller³, Carolin Madwar⁴, Mihnea Bostina⁵, David Colman⁶, Bruce Lennox⁷

Affiliation: 1-7 McGill University

Abstract: Synapses are specialized cell-cell junctions formed between neuronal cells or between neurons and muscle cells through which neurons communicate each other. Synapses are generally formed between axons (presynaptic side) and dendrites (postsynaptic side). In chemical synapses, the presynaptic side has an active zone that contains neurotransmitters that are ready to be released upon depolarization of neurons, which will then be received by the postsynaptic side. Although optical microscopy has been the most popular method being used extensively to observe synapse formation and related studies, electron microscopy is indeed required for observing the cellular level structural details of such structures. Different neurological disorders and head injuries sometimes result in nerve degeneration and as a result, neuronal activities along the axons and at the synapses do not occur. Artificial synapse formation has thus been experimented by several laboratories on various planar and spherical substrates in order to successfully create alternative methods to regenerate damaged nerve terminals. Using cryo-TEM, we observed ultrastructural details of presynapses induced and formed between artificial substrates of 500 nm silica beads and hippocampal neurons when interfaced using unique molecular coatings such as poly-D-lysine and supported lipid bilayers. Known molecular features at the presynapses are clearly present at the artificial synapses as visible on the cryo-TEM images. Key synaptic features such as membrane contact area at synaptic junctions, active zone containing presynaptic vesicles, as well as microtubular structures can be visually identified. This is the first direct observation of

ultrastructural details of artificial synapses.

Funding: Grant from the Regenerative Medicine and Nanomedicine Initiative of the Canadian Institutes of Health Research (CIHR)

Poster Category G : Cognition and Behavior

Title: A novel treatment target for post-anesthetic memory deficits

Authors: Agnieszka A Zurek¹, Beverley A Orser²

Affiliation: 1,2 University of Toronto

Abstract: "Post-anesthetic cognitive deficits occur frequently in patients and have been extensively characterized in laboratory animals; however, no treatment strategies exist. We previously showed that administration of L-655,708, a drug that decreases the activity of $\alpha 5$ -subunit containing γ -aminobutyric acid type A receptors ($\alpha 5$ GABAA-R), prior to anesthesia prevents post-anesthetic memory deficits (*Anesthesiology* 113(5): 1061-71, 2010). Unfortunately, preemptive administration of L-655,708 could cause intra-operative awareness. The aim of this study was to determine whether the administration of L-655,708 after exposure to anesthesia could treat learning and memory deficits. We also sought to develop a mouse model of post-anesthetic memory impairment that mimics memory tasks in humans. We used the object recognition paradigm because it is an ethologically-relevant, non-aversive learning paradigm that models human declarative memory. Isoflurane anesthesia (1.3 %, 1 h) impaired object recognition in wild-type mice for at least 24 h. As shown previously, this deficit was attenuated by administering L-655,708 (0.7 mg/kg) prior to anesthesia. Moreover, a low dose of L-655,708 (0.35 mg/kg) administered after exposure to isoflurane treated the memory deficit. To confirm that $\alpha 5$ GABAA-Rs are an appropriate treatment target, we examined object recognition 24 h after anesthesia in $\alpha 5$ GABAA-R null mutant mice (*Gabra5*^{-/-}). In contrast to wild-type mice, the performance of *Gabra5*^{-/-} mice was not altered by isoflurane anesthesia nor L-655,708. These results suggest that $\alpha 5$ GABAA-Rs are necessary for post-anesthetic memory impairment and provide proof of principle that these receptors can be targeted for treatment of post-anesthetic cognitive deficits."

Funding: This work was funded by CIHR grants to B.A.O. (MOP-38028 and MOP-79428) and NSERC CGS to A.A.Z.

Title: Effects of Aging and Selective Attention on the Time Course of Auditory Perceptual Organization

Authors: Graham Raynor¹, Ingrid Johnsrude²

Affiliation: 1-2 Queen's University

Abstract: "A primary complaint of older adults is that they cannot understand speech in situations with competing sounds. The ability to hear speech in background sound depends in part on the ability to perceptually segregate different sound sources. In this study, we compared performance by younger and older adults on a rhythmic-deviant detection task. Accuracy on this task can be used to index how listeners perceptually organize repetitive patterns of two tones (ABA-ABA-) over time (Carlyon et al. 2010). Perceptual integration of two complex tones at different frequencies (A and B) yields accurate performance on this task; perceptual segregation results in very poor performance. We used two task conditions with different attentional demands: the tone patterns were either played continuously over a block of 165 sec or each trial was preceded by 5 seconds of silence. The tones were separated by either 6 or 8 semitones. When such stimuli are preceded by silence, participants initially perceive the tone pattern as a whole and 'build up' a segregated organization of the tones over time (Cusack, Deeks, Aikman, & Carlyon 2004). The present study replicates these findings in both younger and older adults. Although there was no main effect of age group, this factor interacted with both task condition and semitone separation. Higher accuracy rates over time suggest that older adults tend to have lengthier 'build up' periods, which may account for their speech comprehension deficits. Additionally, the interaction with semitone separation indicates an age difference in the acoustic features necessary for segregation."

Funding: Queen's University, CoNCH Lab

Title: A Superadditive Interaction Between Inflammation and Etomidate for Memory Blockade

Authors: William To¹, Dianshi Wang²

Affiliation: 1- University of Toronto

Abstract: "Seriously ill patients typically require lower doses of general anesthetics; however, the underlying reasons for the reduced anesthetic requirements have not been clearly elucidated. A major concern associated with the use of lower doses of general anesthetics is that patients will experience the explicit recall of surgical events and "intraoperative awareness". It is suspected that general anesthetics and systemic inflammation may cause memory deficits in animal models. The goal of the present study was to determine whether systemic inflammation and etomidate interact in a sub-additive, additive or supra-additive manner to modify learning and memory."

Memory performance in 3-4 month old, male 129/Sv_{ev} C57BL/6 mice was assessed with contextual fear conditioning assay. The endotoxin lipopolysaccharide (LPS; 125 microgram/kg, i.p.) was used to trigger systemic inflammation. Three hours prior to contextual conditioning, each mouse received an injection of either vehicle (saline) or LPS. Additionally, 30 min before training, the mice received either vehicle or etomidate (2, 6, or 10 mg/kg, i.p.). Twenty-four hours after the training session, learning and memory performance was assessed by measuring the percent of time spent freezing in response to the conditioned context. Etomidate caused a concentration-dependent impairment of contextual fear memory as evidenced by a decrease in the freezing scores at all three concentrations of etomidate, $F(2,42) = 24$, $p < 0.0001$. Inflammation induced by LPS supra-additively intensified the memory blocking properties of etomidate."

Funding: CIHR

Title: Disrupting AMPA trafficking reverses the spatial memory deficits produced by acute or chronic expression of Familial Alzheimer's Disease (FAD)-linked genes in mice

Authors: Adelaide Yiu¹, Valentina Mercaldo², Asim Rashid³, Derya Sargin⁴, Rachael Neve⁵

Affiliation: 1-4 Hospital for Sick Children 5- MIT

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by memory impairment. The amyloid precursor protein (APP) gene has been implicated in familial Alzheimer's disease (FAD). Transgenic mouse models chronically expressing FAD genes recapitulate AD cognitive deficits. However, the effects of acute expression of APP mutant genes are unknown. Here we used viral vectors to acutely express FAD genes in adult mice. The effects of 5-day expression of genes encoding human APP with the Swedish, Indiana and both mutations on spatial memory were examined. Acute expression of the FAD genes in the CA1 region of the hippocampus disrupted the formation but not retrieval of spatial memory. These memory deficits occurred in the absence of amyloid plaques or cell death. As changes in dendritic spines may contribute to synaptic plasticity and memory, we also examined the effects of FAD expression on spine density. Acute expression of FAD genes had no effect on overall neuronal structure (dendritic length, branching, surface area, etc.) but decreased spine density. Previous electrophysiological data suggest that amyloid beta may disrupt synaptic transmission by interfering with normal trafficking of GluA2 subunit-containing AMPA receptors. To examine whether the memory deficits produced by expressing FADs were mediated by increasing

endocytosis of GluA2 receptors, we co-expressed FAD with a peptide that specifically disrupts regulated endocytosis of GluA2. Co-expression completely reversed (blocked) the memory deficits produced by acute and chronic expression of FAD. These findings indicate the importance of AMPA trafficking in the memory deficits in AD.

Funding: Alzheimer's Society of Canada Restracom CIHR

Title: Propofol Directly Increases Tau Phosphorylation

Authors: Carl Julien¹, François Marcouiller², Marie-Amélie Papon³, Noura B. El Khoury⁴, Françoise Morin⁵, László Virág⁶, Charles W. Emala⁷, Robert A. Whittington⁸, Emmanuel Planel⁹

Affiliation: 1-5,9 Centre Hospitalier de l'Université Laval, Neurosciences, Québec, 6-8 Department of Anesthesiology, College of Physicians and Surgeons, Columbia University

Abstract: In Alzheimer's disease (AD) and other tauopathies, the microtubule-associated protein tau can undergo aberrant hyperphosphorylation leading to the development of neurofibrillary tangle (NFT) pathology. Understanding the factors accelerating NFT formation is extremely important, as the distribution pattern of tau pathology is highly hierarchical and correlates to the degree of dementia. The cause of the majority of AD cases is considered to be multifactorial, with external factors interacting with biological or genetic susceptibility known to accelerate the manifestation of the disease. Anesthesia could be such an external factor since patients with AD are at risk for anesthesia-related neurocognitive side effects but there is also now growing concerns that anesthetics may accelerate the disease, as recent preclinical studies have provided strong evidence that certain anesthetics can exacerbate the neuropathogenesis of AD. Here, we investigated whether a general anesthetic dose of propofol, an intravenous sedative-hypnotic commonly used as an anesthetic for procedures requiring general anesthesia, would increase the phosphorylation of tau. Significant increases in tau phosphorylation at several epitopes were observed in several regions of the brain, thirty min following intra-peritoneal administration of propofol in mice. Tau hyperphosphorylation persisted 2h following propofol, although the sedative effects of the drug were no longer evident at this time point. By 6h following propofol, levels of phosphorylated tau returned to control levels. These findings suggest that propofol increases tau phosphorylation, and that further studies are warranted to determine the impact of this anesthetic on the acceleration of neurofibrillary pathology.

Funding: "Columbia University Department Anesthesiology Research Allocation (RAW); CIHR

(MOP-106423, PCN-102993), NSERC (354722), FRSQ (16205, 20048) (EP); Alzheimer Society Canada (CJ)."

Title: The judgement of humanity of an interlocutor is in the Eye of the Beholder

Authors: Catherine Lortie¹, Matthieu Guitton²

Affiliation: 1-2 Laval University

Abstract: The expanding need for human-like autonomous agents able to lure a protagonist about his nature is not to be yet fulfilled, as agents aren't cognitively convincing so subjects would believe that they interact with an actual human being. Using as a model of linguistic behaviors during computer-mediated communication the Loebner Prize of Artificial intelligence, we investigated here whether the study of linguistic behavior of human subjects who are perceived as non-human by human judges would enable us to identify the core parameters of judgment of humanity. Indeed, through the years, some human participants of the Loebner Prize failed to convince judges of their humanity, and were thus perceived as machine. The use of this model enable us to study semantic aspects of dialogs that can delude a judge on his interlocutor humanness, through examination of faulty events that were responsible for the negative behavioral response directed toward the participant. Using cognitive and emotional dimensions in a global behavioral characterization, we suggested the importance of multimodal, emotional and cognitive parameters when analyzing complex social behaviors. Results have shown that the judgment of an interlocutor' humanity during a social interaction will depends on his behavior, obviously, but will also depend on the judge himself. We here demonstrate that the judgment of humanity is in the eye of the beholder.

Funding: The present work was supported by the Natural Sciences and Engineering Research Council of Canada.

Title: Social organization in virtual settings depends on proximity to human visual aspect

Authors: Catherine Lortie¹, Matthieu Guitton²

Affiliation: 1-2 Laval University

Abstract: Virtual environments are inherently social spaces, in which humans interact through avatars. However, the parameters which favor inter-individual social structuring in those settings are still far to be understood. Particularly, the putative influence of anthropomorphic similarity of

visual aspect on social organization of avatars is a key issue to understand the cognitive processes used to form social interactions in virtual worlds. Using the highly popular massively multiplayer online role-playing game World of Warcraft as a model of socially-active virtual setting, we analyzed the social behavior of 11,649 avatars as a function of their visual aspect. Our results show that social structuring in virtual settings depends on proximity to human visual aspect. Social groups formed by human-like avatars display more homogeneity than what the optimal use of the interface would predict, while this effect is not observed for social groups formed by non-human avatars. Thus, immersion in virtual environments depends more on visually-triggered social dynamics (role-play) than on optimal use of the interface (game-play). Furthermore, social aspect may override the immediate reward of interface optimization, thus representing a major factor of immersion in virtual environments.

Funding: The present work was supported by the Natural Sciences and Engineering Research Council of Canada.

Title: Adult Hippocampal Neurogenesis and Memory Interference

Authors: Shira Rosenzweig¹, Gordon Winocur², Suzanna Becker³, Paul Luu⁴, J.M. Wojtowics⁵

Affiliation: 1,4,5 Department of Physiology, University of Toronto 2- Rotman Research Institute, Baycrest Centre 3- Department of Psychology, Neuroscience & Behaviour, McMaster University

Abstract: The hippocampus is involved in the formation of distinct, contextually-rich memories and when its function is impaired memories become more susceptible to interfering influences. Evidence suggests that discrete neuronal representations within the dentate gyrus allow memories to be readily distinguished from one another, thus minimizing the effect of competing memories during selective recall. Adult neurogenesis is a unique feature of the dentate gyrus and is known to contribute to normal hippocampal function. We therefore hypothesized that neurogenesis plays a central role in the modulation of interfering influences during learning and memory. **Methods:** adult neurogenesis in rats was alternatively suppressed using low-dose irradiation, or enhanced by allowing the rats to engage in running. Some rats were subjected to both treatments. The rats were then trained in a visual discrimination task under conditions of high or low interference. **Results:** neurogenesis in irradiated rats was reduced and the animals exhibited a concordant increase in their susceptibility to memory interference, resulting in loss of memory for the previously learned discrimination. Learning and memory retention of the

discrimination response under low interference conditions were unaffected. Irradiated rats that engaged in running activity exhibited increased neuronal growth and protection from memory impairment under high interference conditions. Conclusion: our results suggest that adult-born hippocampal neurons play a role in differentiating between conflicting, context-dependent memories, and provide further evidence of the importance of neurogenesis in hippocampus-sensitive memory tasks.

Funding: Supported by CIHR.

Title: Systemic inflammation increases anesthetic potency for memory blockade

Authors: William To¹, Dian-shi Wang², Beverley A. Orser³

Affiliation: 1-2 Department of Physiology, University of Toronto 3- Department of Anesthesia, Sunnybrook Health Sciences Centre

Abstract: Background: Severe inflammation typically increases the potency of general anesthetics for adverse side-effects such as hemodynamic instability and respiratory depression. It remains unknown whether inflammation alters the potency of anesthetics for memory blockade. It is important to identify factors that modify anesthetic potency to avoid overdosing, inadequate amnesia and intraoperative awareness. Our study determined whether systemic inflammation alters the potency of the anesthetic etomidate for memory blockade. Methods: Animal ethics board approved all experiments. Adult male mice were used to study fear conditioning to context and auditory tone. To trigger systemic inflammatory response, mice were treated with the endotoxin lipopolysaccharide (LPS) 3 h prior to conditioning. Controls were treated with saline. Various doses of etomidate (2 mg/kg to 10 mg/kg) were administered 30 min prior to fear conditioning. Memory performance was assessed by measuring the time spent freezing. Experimenters were blinded to the treatment groups. Results: Etomidate caused a dose-dependent impairment of contextual and auditory fear memory as evidenced by a decrease in freezing scores ($p < 0.001$). LPS and etomidate interacted synergistically to block memory to context ($p = 0.025$). Notably, LPS failed to influence the potency of etomidate for memory blockade of auditory events. Conclusions: Systemic inflammation increased the amnestic potency of the etomidate for certain forms of explicit memory in vivo. These results may have implications related to the high incidence of auditory recall in critically ill patients who experience

intraoperative awareness.

Funding: Canadian Institutes of Health Research

Title: Time-dependent global reorganization of functional networks supporting contextual fear memory

Authors: Anne Wheeler¹, Afra Wang², Catia M Teixeira³, Xuejian Xiong⁴, Natasa Kovacevic⁵, Anthony McIntosh⁶, John Parkinson⁷, Paul Frankland⁸

Affiliation: 1,2 University of Toronto, Institute of Medical Science 3,4,8 Hospital for Sick Children, Neuroscience and Mental Health 5,6- Rotman Research Institute, Baycrest Centre for Geriatric Care 7- Hospital for Sick Children, Molecular Structure and Function

Abstract: Previous lesion and electrophysiological recording studies provide direct support for the idea that the organization of memories in the brain changes over time, however these studies represent only a narrow window on what is appreciated to be a much more global process of reorganization. In order to track global changes in memory organization, we quantified the expression of the activity-dependent gene c-fos after recall of a recent and remote context fear memory in 84 brain regions in mice covering the majority of the forebrain. Computation of a complete set of inter-regional correlations allowed us to identify collections of brain regions where Fos expression co-varied, and presumably form a functional network that is engaged during memory expression. These analyses indicated that remote memories are supported by a broadly distributed network and provide evidence that network reorganization leads to the strengthening of inter-cortical functional connectivity and that the prefrontal cortex plays an emergent role in memory expression. Graph theoretical measures indicate that the remote memory network is highly segregated and contains a collection of 'hub' regions including the anterior cingulate cortex and the reuniens thalamic nucleus. We are now testing whether lesions of these highly connected hub regions lead to deficits in remote but not recent memory which would further suggest that these hub regions are important for remote fear memory. These results identify and describe functional memory networks underlying recent and remote memory expression and provide strong evidence for reorganization and distribution of functional memory networks over time.

Funding: Canadian Institute of Health Research and Ontario Mental Health Foundation.

Title: Time-dependent global reorganization of functional networks supporting contextual fear memory

Authors: Anne L Wheeler¹, Afra H Wang², Catia M Teixeira³, Natasa Kovacevic⁴, Xuejian Xiong⁵, Anthony McIntosh⁶, John Parkinson⁷, Paul W Frankland⁸

Affiliation: 1,2 University of Toronto, Institute of Medical Science 3,8 Hospital for Sick Children, Neuroscience and Mental Health 4,6- Rotman Research Institute, Baycrest Centre for Geriatric Care 5,7 Hospital for Sick Children, Molecular Structure and Function

Abstract: Previous lesion and electrophysiological recording studies provide direct support for the idea that the organization of memories in the brain changes over time, however these studies represent only a narrow window on what is appreciated to be a much more global process of reorganization. In order to track global changes in memory organization, we quantified the expression of the activity-dependent gene c-fos after recall of a recent and remote context fear memory in 84 brain regions in mice covering the majority of the forebrain. Computation of a complete set of inter-regional correlations allowed us to identify collections of brain regions where Fos expression co-varied, and presumably form a functional network that is engaged during memory expression. These analyses indicated that remote memories are supported by a broadly distributed network and provide evidence that network reorganization leads to the strengthening of inter-cortical functional connectivity and that the prefrontal cortex plays an emergent role in memory expression. Graph theoretical measures indicate that that the remote memory network is highly segregated and contains a collection of hub regions including the anterior cingulate cortex and the reuniens thalamic nucleus. We are now testing whether lesions of these highly connected hub regions lead to deficits in remote but not recent memory which would validate that they are important for remote fear memory. These results identify and describe functional memory networks underlying recent and remote memory expression and provide strong evidence for reorganization and distribution of functional memory networks over time.

Funding: Canadian Institute of Health Research, Ontario Mental Health Foundation

Title: Working memory representations of visual motion direction are encoded in the firing patterns of neurons in dorsolateral prefrontal cortex, but not in area MT

Authors: Diego Mendoza-Halliday¹, Julio Martinez-Trujillo²

Affiliation: 1-2 McGill University

Abstract: It is thought that primate dorsolateral prefrontal cortex (dlPFC) neurons play a role in the maintenance of visual information in working memory. It has been recently suggested that this process also involves the recruitment of neurons in early visual cortex selective for the stimulus features to be remembered. Supporting this hypothesis, recent fMRI studies reported that the contents of visual working memory can be decoded from patterns of BOLD signals in visual areas (Harrison and Tong, 2009). However, because fMRI does not directly measure the neurons' spiking activity, it remains controversial whether this effect is attributable to variations in the firing patterns of neurons, or in the amplitude of other signals such as local field potentials. We investigated this issue by recording the spiking activity of single neurons simultaneously from the dlPFC (n=53) and early visual area MT (n=33) of two rhesus monkeys during a working memory task requiring them to remember the motion direction of a sample random dot pattern and match it to the direction of one of two test patterns serially presented. During the memory period, the activity of most dlPFC neurons remained above or below baseline and approximately 1/6 of these neurons showed sustained tuning to the remembered direction. In all of the recorded MT neurons, activity remained at baseline levels and no sustained direction tuning was present during the memory period. Our results show that working memory representations of motion direction are encoded in the firing patterns of neurons in dlPFC but not in MT.

Funding: Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Institutes of Health Research (CIHR) and EJLB Foundation

Title: Coherent theta synchronization between the medial prefrontal and lateral entorhinal cortex during late acquisition and remote retrieval of associative memory

Authors: Kaori Takehara-Nishiuchi¹

Affiliation: 1- University of Toronto

Abstract: Associative memory depends on a cortical network that includes the medial prefrontal and entorhinal cortices. To examine the nature of their interplay, we investigated synchronization of local field potentials (LFPs) between the prelimbic prefrontal and lateral entorhinal cortices during acquisition and remote retrieval in trace eyeblink conditioning. Over the course of 10 days of the conditioning, rats acquired an association between a neutral tone (CS) and a mild electric shock to the eyelid, which were presented with a 500-msec interval between them. In parallel, LFPs recorded in the prelimbic and lateral entorhinal cortices became rhythmically synchronized

at theta frequencies (7 - 11 Hz) after the CS onset. This theta phase synchrony was absent early in acquisition and began to appear late in acquisition. In addition, theta synchrony was also prominent when memory retention was tested one month after the conditioning. Moreover, theta synchrony was stronger when the rats successfully retrieved the association than when they did not. This stimulus-locked increase in theta synchrony accompanied phase resetting of local theta oscillations at the CS onset: the CS presentation induced shifts in the phase of local theta oscillations in such a way that the increased phase entrainment took place over time that lasted for about two oscillation periods. These results suggest that the prelimbic and lateral entorhinal cortices interact with one another during late acquisition and remote retrieval of associative memory. Prefrontal-entorhinal interplay may be a central network process in the neocortex that supports consolidated memory.

Funding: NSERC Discovery Grants (386686).

Title: Successful cognitive aging: a crucial role of mGluR5 glutamate receptors and Homer 1 proteins

Authors: Caroline Menard¹, Remi Quirion²

Affiliation: 1-2 DMHUI, McGill University

Abstract: Normal aging is associated with impairments in cognition, especially learning and memory. With the classical Morris Water Maze acquisition task, we discriminated a population of Long Evans aged rats in two groups - memory-impaired (AI) and memory-unimpaired (AU) by a comparison of their cognitive performance with 6-months adult animals. AI subjects presented deficits for learning, reverse memory acquisition and remote memory. We discovered biochemical alterations characterizing those behaviours in the AI and AU hippocampal formation. We observed an increase in metabotropic glutamate receptors 5 (mGluR5) level in both homogenates and post-synaptic densities (PSD) for the AU rats. Post-synaptic mGluR are coupled to signalling effectors by Homer proteins. Both Homer 1a and 1b/c levels were up-regulated in the AU PSD. We evaluated regional specificity using immunohistochemistry and detected that mGluR5 as well as Homer 1b/c staining were enhanced in the CA1 region of AU animals. We confirmed mGluR5 and Homer 1 co-localization in the AU dendrites by immunofluorescence. Homer 1b/c and 1 mGluR group coupled the signalling machinery while Homer 1a uncoupled them. The ratio of Homer 1a/Homer 1b/c bound to mGluR5 was four times lower for AU animals with no change for

mGluR1alpha. Consequently, AU animals presented higher PKCgamma, ERK, p70S6K, mTOR and CREB activation. We finally evaluated immediate early gene Arc/Arg3.1 expression since it has been associated with memory consolidation. Arc level was significantly higher for the AU. We propose a model of successful cognitive aging with a critical role of mGluR5 and Homer 1 proteins.

Funding: This research was supported by grants from the Canadian Institutes of Health Research to RQ and a fellowship to CM.

Title: The role of adult neurogenesis in context discrimination

Authors: Yosuke Niibori¹, Tzong-Shiue Yu², Jonathan Epp³, Sheena Josselyn⁴, Paul Frankland⁵

Affiliation: 1-5 Neuroscience and Mental Health, Hospital for Sick Children

Abstract: Neurogenesis persists into adulthood in the dentate gyrus of the hippocampus. While adult-generated neurons likely contribute to hippocampal memory processing, the exact nature of this role is unclear. Previous computational and neurogenetic studies have suggested that the dentate gyrus may play a role in disambiguating similar (but nonetheless discrete) representations (or pattern separation). Here we test whether suppressing adult neurogenesis disrupts this process using a contextual fear paradigm. In one-trial contextual fear conditioning experiments, weak conditioning to context occurs if the shock is delivered immediately following placement of the animal in a novel conditioning apparatus, a phenomenon known as the immediate shock deficit. This immediate shock deficit is alleviated by pre-exposure to the training context. In our experiment, mice were initially pre-exposed to two similar contexts (A and B). The following day they were placed in context A and shocked immediately. One day later, we placed them back in contexts A and B and measured conditioned freezing behavior. In this test, control mice froze more in context A compared to B, indicating that they could discriminate between these two familiar contexts. However, levels of discrimination were significantly reduced in mice treated with the antimetabolic drug Temozolomide (TMZ). These results suggest that the suppression of adult neurogenesis impairs the discrimination of two familiar contexts, and suggest that adult neurogenesis at dentate gyrus in hippocampus has the crucial role for the pattern separation.

Funding: CIHR

Title: Increasing CRTC (CREB regulated transcription coactivator) levels in the dorsal hippocampus is sufficient to induce contextual fear memory

Authors: Melanie J Sekeres¹, Paul W. Frankland², Sheena A. Josselyn³

Affiliation: 1-3 Hospital for Sick Children

Abstract: "Previous research has established that the CREB (cAMP/Ca²⁺ responsive element binding protein) family of transcription factors is critical for memory in many species. CREB-regulated transcription co-activators (CRTCs; also known as Transducers of Regulated CREB activity, or TORCs) are latent cytoplasmic co-activators that shuttle to the nucleus in response to cAMP and calcium signals to stimulate CREB-mediated transcription in a CREB phosphorylation-independent manner. It has been suggested that, while there may be sufficient cellular levels of other CREB co-activators (CBP and p300) to support transcription, CRTC may be a limiting factor. The CRTC1 isoform is highly expressed in the rodent hippocampus and has been shown to stimulate CREB-dependent transcription and enhance synaptic plasticity. However, the role of CRTCs in memory is unknown. Here we examine whether acutely increasing CRTC1 levels in the dorsal hippocampus is sufficient to enhance the formation of context fear memory in mice. Recently, we found that CREB in the dorsal hippocampus is both necessary and sufficient for the formation of spatial memory. Here we show that increasing wild-type (WT) CRTC1 levels in the dorsal hippocampus is also sufficient to induce a strong contextual fear memory under weak training conditions that do not support contextual memory formation in WT mice. This is the first evidence demonstrating that CREB/CRTCs play a pivotal role in the dorsal hippocampus molecular machinery underlying the formation of contextual memory. "

Funding: CIHR (SAJ & PWF). EJLB Foundation, NSERC (SAJ). Restracom Fellowship (Sick Kids), CIHR Banting & Best CGS Doctoral Award (MJS)

Title: The Road to Faster Recovery: Combinatory Effects of Growth Factor Infusion and Behavioural Rehabilitation

Authors: Matthew Jeffers¹, Meighan Kelly², Amy Hoyles³, Cindi Morshead⁴, Molly Shoichet⁵, Dale Corbett⁶

Affiliation: 1,2 Memorial University of Newfoundland 3-5 University of Toronto 6- University of Ottawa

Abstract: "Behavioural rehabilitation is the only treatment option for chronic stroke deficits. Unfortunately, even lengthy rehabilitation often provides incomplete recovery. This study demonstrates that application of growth factors intended to promote endogenous stem cell

mobilization can significantly reduce treatment time. Rats (N=53) received a stroke via injection of endothelin-1 at two sites in the sensorimotor cortex. This was followed by either a 2-week infusion of epidermal growth factor (EGF) and erythropoietin (EPO) or artificial cerebrospinal fluid (aCSF). Two weeks post-ischemia, animals began either a 6-week enriched rehabilitation program or standard housing treatment: (1) EGF/EPO + rehab; (2) aCSF + rehab; (3) EGF/EPO + no rehab; and (4) aCSF + no rehab. Functional assessments were performed pre- and post-stroke and biweekly thereafter using the Montoya staircase reaching task, beam traversing and cylinder test of forelimb asymmetry. The EGF/EPO + rehab group showed significantly faster recovery on the Montoya staircase reaching task than the aCSF + rehab group ($p < .05$). Further, animals receiving either EGF/EPO + rehab or aCSF + rehab recovered to a significantly greater extent in the staircase test ($p < .05$) than animals that did not receive rehabilitation. Similar results were found with the beam traversing task ($p < .05$). Combining behavioural rehabilitation with growth factors that promote endogenous stem cell mobilization may accelerate recovery beyond that of rehabilitation alone. This has the potential to reduce the rehabilitation time necessary to recover from stroke deficits and may be particularly useful in treating severe stroke. "

Funding: Heart and Stroke Foundation Natural Sciences and Engineering Research Council (NSERC)

Title: Enhanced tonic inhibition by the pro-inflammatory cytokine interleukin 1beta impairs memory and synaptic plasticity

Authors: Dian-Shi Wang¹, Agnieszka A. Zurek², Paul Whissell³, Beverley A. Orser⁴

Affiliation: 1,2,4- Departments of Anesthesia and Physiology, University of Toronto 3- Institute of Medical Science, University of Toronto

Abstract: "Acute inflammatory illnesses cause a "sickness behavior" that is characterized by learning and memory deficits, particularly difficulties in remembering recent events. There are no effective treatments for memory deficits associated with inflammation despite extensive efforts to identify the underlying mechanisms. Here, we show that a key pro-inflammatory cytokine interleukin (IL)-1 β increases the activity of a subtype of $\alpha 5$ subunit-containing GABA_A receptors ($\alpha 5$ GABAARs), which regulates memory and synaptic plasticity. IL-1 β increases a tonic inhibitory conductance generated by $\alpha 5$ GABAARs but not synaptic inhibition via the activation of IL-1 receptor and p38 MAP kinase signaling pathway in cultured hippocampal neurons. This increase

in $\alpha 5$ GABAAR activity blocks synaptic plasticity in vitro and causes memory deficits in mice in vivo. The $\alpha 5$ GABAARs are necessary for IL-1 β -mediated memory blockade as the memory deficits are absent in IL-1 β -treated $\alpha 5$ GABAAR null mutant mice. These results identify a novel downstream effector of IL-1 β -mediated signaling that can be targeted to mitigate learning and memory impairment associated with inflammation. "

Funding: This work was supported by CIHR grants to B.A.O. (MOP-38028 and MOP-79428).

Title: Retinoic acid is required for long-term memory, but not learning, following operant conditioning

Authors: Cailin Rothwell¹, Gaynor Spencer²

Affiliation: 1-2 Brock University

Abstract: "The active metabolite of vitamin A, retinoic acid (RA), is important for vertebrate development and has recently been implicated in both learning and memory in adult vertebrates. No previous studies have determined whether RA is also important for learning and memory in invertebrates. RA classically acts to regulate gene transcription by binding with retinoic acid receptors (RARs) and retinoid X receptors (RXRs), though non-transcriptional effects of RA also occur. We examined the role of RA in learning and memory following the operant conditioning of aerial respiration in the mollusc *Lymnaea stagnalis*. RA signalling is conserved in this mollusc and the central nervous system (CNS) contains endogenous retinoids. Additionally, an RAR, RXR, and the RA-synthesizing enzyme retinaldehyde dehydrogenase (RALDH) have been cloned from the *Lymnaea* CNS. *Lymnaea* can form intermediate-term memory (ITM; 2 hour) and long-term memory (LTM; 24 hour) depending on the training procedure employed. RA synthesis was disrupted in *Lymnaea* using two different RALDH inhibitors, citral and 4-diethylamino-benzaldehyde (DEAB). Incubation in the RALDH inhibitors impaired LTM formation, but not learning, following operant conditioning. Interestingly, exposure to the RALDH inhibitors did not interfere with ITM formation. Thus, we next examined the role of the ligand activated transcription factors RAR and RXR. Incubation in the pan-RAR antagonist LE540 partially impaired LTM while incubation in the pan-RXR antagonist HX531 completely abolished LTM formation. These data suggest that RA classically acts to regulate gene transcription during LTM formation in *Lymnaea* and RA's role in memory may be conserved between vertebrates and

invertebrates. "

Funding: "NSERC Discovery Grant to GES; NSERC PGS D scholarship to CMR"

Title: Increasing adult neurogenesis retroactively disrupts recently-acquired hippocampal memories

Authors: Alonso Martinez-Canabal ¹, Katherine Akers², Anne Wheeler³, Leonardo Restivo ⁴, Sheena Josselyn ⁵

Affiliation: 1,3 Institute of Medical Science, University of Toronto 2,4,5 Department of Neuroscience and Mental Health, The Hospital for Sick Children

Abstract: "New neurons are continuously added to the hippocampus throughout adult life. The functional significance of this process of adult neurogenesis has generally been studied by examining the effects of either increasing or decreasing new neuron production on subsequent memory formation. While there are conflicting data, the prevailing view is that new neurons generally promote the formation of new hippocampal memories. However, little attention has been paid to the effects of similar manipulations on previously acquired memories. Here we studied the effects of running-induced increases in neurogenesis on recently-acquired memories using two contextual memory paradigms (contextual fear conditioning and contextual pre-exposure). In both paradigms, post-training running increased neurogenesis, but impaired subsequent expression of these memories. In contrast, when running occurred prior to training, subsequent memory formation was not affected suggesting that running does not simply interfere with memory expression in a non-specific manner. We speculate that increased new neuron production in the dentate gyrus might persistently increase excitatory input to CA3, leading to the depression of excitatory connections through homeostatic synaptic scaling causing destabilization of hippocampal memory storage. Consistent with this, we found that memory deficits induced by post-training running were strongly correlated with 1) levels of neurogenesis in the dentate gyrus; 2) increased activation of dentate gyrus granule cells; and 3) decreased activation of CA3 pyramidal neurons. Together, these data suggest that adult neurogenesis may represent a mechanism for clearing existing memories from the hippocampus, and, in so doing, maintain capacity for rapid encoding of new information. "

Funding: Funded by: CIHR, OMHF and CONACyT

Title: Social Density and Physical Aggregation of Avatars in Virtual Spaces

Authors: Anna Lomanowska¹, Matthieu Guitton²

Affiliation: 1-2 Laval University

Abstract: The growing popularity of social engagement using the Internet has spurred substantial interest in the dynamics of social interactions in virtual spaces. Many virtual spaces are designed to resemble multiple aspects of the physical world, particularly its three-dimensional (3D) quality. This enables avatars, the graphical representations of users, to navigate through virtual spaces in similar ways that humans move through the real world. In these environments avatars must traverse virtual distances in order to engage in interactions with other avatars. The aim of this study was to examine how virtual distance affects the physical aggregation of avatars. We used models developed in population ecology to investigate the spatial distribution of avatars within the virtual world of Second Life. Analysis of the distribution of over 400 avatars using the measure of nearest neighbour distance unveiled population density-dependent effects on physical aggregation. For very low populated spaces, avatars were either randomly distributed or aggregated. However, when more than 3 avatars were simultaneously located in a given space, we observed a strong tendency of the avatars to physically aggregate. Thus, our results indicate that the social density of avatars in a 3D virtual space affects the dynamics of social interactions.

Funding: provided by NSERC

Title: The Effect of Muscarinic Receptor Blockade (Scopolamine) on Visual Discrimination Performance in Rats

Authors: Claudia Tsui¹, Hans Dringenberg²

Affiliation: 1-2 Queen's University

Abstract: "The effects of muscarinic receptor antagonists (e.g., scopolamine) on the acquisition of a variety of behavioral tasks have been well documented. However, less is known regarding the role of muscarinic receptors in sustaining performance after the initial task acquisition is completed. Here, rats were trained in a visual discrimination task using a Y-shaped water maze apparatus. To successfully navigate to a hidden escape platform located in one of the two goal arms, rats learned to discriminate between two different visual cues, indicating the platform's presence (CS+) or absence (CS-), respectively. Following task acquisition, testing continued using both regular trials (both CS+ and CS- present) and probe trials (only one of the cues present).

Performance on probe trials (one cue present) was impaired, but rats rapidly learned to rely on a single cue for accurate platform localization. Interestingly, this learning was not apparent under conditions of scopolamine treatment (1.0 mg/kg, i.p.), even though performance on regular trials (both cues present) was completely unaffected. In a second experiment, probe trials were administered with the platform removed from the maze to avoid reinforcement and subsequent learning. Scopolamine treatment resulted in a trend toward a dose-dependent (saline, 0.125-1.0 mg/kg) impairment on probe trials (higher dose, lower performance), without affecting regular trial performance. These experiments demonstrate the importance of the cholinergic system in (a) adapting a novel strategy (based on a single cue) to perform visually guided navigation; and (b) performance under conditions of reduced information availability (one instead of two visual cues)."

Funding: NSERC

Title: The effects of physical vs. cognitive activity on adult neurogenesis: unraveling their mechanisms of action

Authors: Catherine-Alexandra Grégoire¹, Anne Aumont², Karl Fernandes³

Affiliation: 1-3 Université de Montréal

Abstract: In the adult brain, neural stem cells continue to produce new neurons within the dentate gyrus of the hippocampus, where these neurons are implicated in the processes of learning and memory. Remarkably, this process of adult neurogenesis is highly stimulated by environmental enrichment (EE), such as access to a voluntary running wheel. Our recent work has led us to hypothesize that physical activity primarily regulates the post-mitotic survival and maturation of newly generated neurons, while EE-induced cognitive stimulation may be responsible for the activation of quiescent hippocampal stem cells (Bednarczyk et al 2010). In the present study, we are attempting to dissect i) the differential contributions and ii) the mechanisms of action of physical activity versus cognitive stimulation during adult neurogenesis. First, using adult mice, we are establishing new housing paradigms to evaluate the effects of moderate and high physical activity, social housing, isolation and environmental complexity on the adult neurogenesis pathway. Furthermore, we are identifying the strain and gender conditions that will give us the highest resolution for detecting the different effects of physical and cognitive stimulation. Second, we are using genetic and biochemical approaches to identify intracellular

regulatory pathways responsible for stage-specific effects of different EE on the hippocampal stem cell niche. Together, these studies should provide novel insights regarding the mechanisms and molecules through which EE can act on adult hippocampal neurogenesis.

Funding: Canadian Institutes of Health Research (CIHR) and Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Frequency of Spontaneous Entorhinal-Prefrontal Theta Coupling Predicts Associative Memory Acquisition

Authors: Mark Morrissey¹, Kaori Takehara-Nischiuchi²

Affiliation: 1-2 University of Toronto

Abstract: The medial prefrontal (mPFC) and lateral entorhinal cortices (LEC) are necessary for the acquisition of associative memory, suggesting that functional connectivity between these two brain regions may strengthen in association with learning. Consistent with this prediction, we recently found that theta oscillations in the mPFC and LEC synchronized after a neutral tone (CS) was paired with an aversive stimulus. To further examine learning-related changes in functional connectivity between the mPFC and LEC, the current study sought to investigate whether theta oscillations in these two regions become more frequently synchronized without any external triggering stimuli in association with learning. To this end, we counted instances of significant theta phase synchrony during inter-trial intervals, during which rats waited for the upcoming CS. Theta phase synchrony was quantified with the stability of phase differences between the two regions within a series of 200-msec windows (SI). Significance was determined by calculating the percentage of SI values above the 95th percentile of the distribution of a set of surrogate data from mismatched trials. As the rats acquired conditioned responses, frequencies of spontaneous theta synchrony between the mPFC and LEC gradually increased. Moreover, they were positively correlated with increased expression of the conditioned response across sessions ($r = .486$, $p = .007$). These results provide further support for close interaction between the LEC and mPFC during memory acquisition. Spontaneous entorhinal-prefrontal oscillatory coupling may reflect internal replay of paired stimuli or anticipation of the stimulus to come, thereby possibly promoting learning.

Funding: NSERC Discovery Grant

Title: Enhanced tonic inhibition by the pro-inflammatory cytokine interleukin-1beta impairs memory and synaptic plasticity

Authors: Dian-Shi Wang¹, Agnieszka A. Zurek², Paul Whissell³, Beverley A. Orser⁴

Affiliation: 1-2 Departments of Anesthesia and Physiology, University of Toronto 3- Institute of Medical Science, University of Toronto 4- Department of Anesthesia, Sunnybrook Health Sciences Centre

Abstract: "Acute inflammatory illness causes a "sickness behavior"" that is characterized by learning and memory deficits. Despite extensive investigation, the underlying mechanisms of memory dysfunction associated with inflammation remains poorly understood. Here, we report that a key pro-inflammatory cytokine interleukin (IL)-1beta increases the activity of alpha5 subunit containing GABA-A receptors (alpha5GABA-ARs) that are known to regulate memory and synaptic plasticity. IL-1beta increases the tonic inhibitory conductance generated by alpha5GABA-ARs via the activation of IL-1 receptor and p38 MAP kinase signaling pathways in cultured hippocampal neurons. This increase in alpha5GABA-AR activity blocks synaptic plasticity in vitro and causes memory deficits in mice in vivo. Thus, alpha5GABA-ARs are downstream effectors of IL-1beta-mediated signaling and novel therapeutic targets for drugs aimed at attenuating learning and memory deficits associated with inflammation."

Funding: This work was supported by CIHR grants to B.A.O. (MOP-38028 and MOP-79428).

Title: Effect of social context on neurogenesis in the adult zebra finch (*Taeniopygia guttata*)

Authors: Leslie Phillmore¹, Emily McGuire², Caitlin Wolfe³, Hannah Kinoshita⁴, Harold Robertson⁵,

Affiliation: 1-5 Dalhousie University

Abstract: "One model system in which adult neurogenesis has been studied is the songbird, such as the zebra finch (*Taeniopygia guttata*). Zebra finch males produce two types of song directed and undirected. Social context mediates which of these he performs: directed song is sung only during courtship of a female. The dopamine pathway may modulate these behaviours by providing motivation and reward for participation in courtship activities. A number of recent studies show that dopamine may also promote adult neurogenesis. In this study, we examined the relationship between social context, behaviour, and adult neurogenesis in the zebra finch by assigning birds to same-sex or opposite-sex pairs and administering the cell birth marker bromodeoxyuridine (BrdU), then counting the number of labeled cells in the subventricular zone (SVZ), a zone of cell

birth in the avian brain. Two different sacrifice timelines were used to investigate which stage of neurogenesis may be affected by social context (proliferation and survival). We also analyzed video recordings of pairs to quantify song and other behaviours. Males tended to have more BrdU+ cells than females, and there were more cells in the SVZ after 1 day than after 10 days; this may be attributable to cell migration. However, social context did not seem to have an effect on proliferation or survival in the SVZ. Considerations for future studies include increased sampling of behaviours, a more controlled auditory environment, longer time in social pairings to increase likelihood of an effect, and double-labeling methods for positive identification of neurons."

Funding: NSERC

Title: "Behavioral characterization of CamKIIcre-GSK3 β Flox mice"

Authors: Camille Latapy¹, Veronique Rioux², Jean-Martin Beaulieu³

Affiliation: 1-3 Centre de Recherche Universite Laval Robert-Giffard

Abstract: "Psychotropic drugs acting on monoamine neurotransmission are major pharmacological treatments for neuropsychiatric disorders such as schizophrenia, depression and bipolar disorders. Clinical studies, as well as biochemical and behavioral approaches in normal or genetically modified mice, leads to hypothesize a central involvement of glycogen synthase kinase 3 (GSK3) in behavior regulation by dopamine and serotonin. This constitutively active kinase is represented by two isoforms sharing several activation processes and targets but their specific roles in the behavior remains unclear. Contrary to GSK3 α , a total GSK3 β knock-out causes an embryonic death. Nevertheless, the investigation of behavioral consequences induced by a neuronal total lack of GSK3 β has been made possible thanks to the tissue-specific mice line CamKIIcre-GSK3 β Flox. Immunohistochemistry characterization of positives mices shows a drastic diminution of GSK3 β positive neurons in the cortex and hippocampus without affecting other structures such as the striatum. This study will focus on the consequences of GSK3 β tissue-specific lack in various behavioral paradigms like anxiety, depressive-like response and sociability, all implied in neuropsychiatric disorders and dependant of the brain structures pre-cited."

Funding: This work was supported by CIHR operating Grant (NSA 93798)

Title: STRENGTH OF RESPONSE SUPPRESSION TO DISTRACTER STIMULI DETERMINES ATTENTIONAL-FILTERING PERFORMANCE BY PRIMATE PREFRONTAL NEURONS

Authors: Therese Lennert¹

Affiliation: 1- McGill University

Abstract: "Neurons in the dorsolateral prefrontal cortex (dlPFC) filter attended targets from distracters through their response rate. The extent to which this ability correlates with the organism's performance, as well as the neural processes underlying it remain unclear. We recorded the responses of dlPFC neurons of two monkeys while the animals were performing a target selection task. They were presented with two white moving random dot patterns (RDPs) to the left and right of a fixation cross. After a variable interval both RDPs simultaneously changed to a different color. Prior to training, we chose a set of colors and built an ordinal scale by arbitrarily assigning a fixed ordinal position (rank) to each color (grey < pink < green < blue < red < cyan). Animals were rewarded for keeping gaze on the cross and releasing a lever after the higher-ranked RDP (the target) changed motion direction. The animals' performance improved as ordinal distance between the stimuli increased. 122/222 units reliably signaled the position of the target. Importantly, dlPFC neurons improved their filtering performance with increasing ordinal target-distracter distance; units encoding target representations increased their firing rate by similar amounts while units encoding distracter representations gradually suppressed their rates as the inter-stimulus ordinal distance increased. These results suggest that attentional-filtering performance in primates relies upon the ability of dlPFC neurons to suppress distracter representations."

Funding: JMT: CIHR, NSERC, EJLB Foundation, Canada Research Chair program. TL: CIHR Canada Graduate Vanier Scholarship

Title: Amphetamine Suppresses Lever Press Expression At Doses That Do Not Suppress Acquisition

Authors: Tyson Baker¹, Richard Beninger²

Affiliation: 1-2 Queen's University

Abstract: The neurotransmitter dopamine has been implicated in the acquisition and expression of operant behavior. Amphetamine is an indirect dopamine agonist with psychogenic properties that suppresses lever press expression. Low-dose amphetamine augments the natural dopamine signal, high-dose amphetamine occludes the natural dopamine signal. We tested the hypothesis that low doses of amphetamine will enhance and high doses will suppress lever press acquisition.

We tested the effects of 0 - 1.0 mg/kg amphetamine on lever press acquisition and expression in 15-min daily sessions on continuous reinforcement for the first 20 trials, then random ratio 2 for subsequent trials each session. The doses tested did not suppress lever press acquisition but suppressed lever press expression. We found no evidence of a low-dose augmentation of lever pressing. Our previous research has shown that some antipsychotic drugs suppress lever pressing more potently during acquisition than expression: the opposite pattern of amphetamine. This suggests that dopamine augmentation disrupts lever press acquisition less than expression while dopamine suppression may disrupt lever press acquisition more than expression.

Funding: This work was funded by an NSERC grant to RJB.

Title: Exercise and neurogenesis in a mouse model of Alzheimer's disease.

Authors: Ewelina Maliszewska-Cyna¹, Isabelle Aubert², JoAnne McLaurin³

Affiliation: 1-2 Sunnybrook Research Institute 3- University of Toronto

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder resulting from a progressive loss of neurons leading to cognitive impairment and disruption of adult neurogenesis. Several non-invasive treatments are being developed to reduce the rate of neuronal degeneration. For example, it has been shown that exercise influences adult neurogenesis, synaptic plasticity, and cognitive functions in healthy adults and recent epidemiological studies suggest that exercise potentially benefits AD patients as well. However, we only begin to understand the effects exercise has on pathology, plasticity, and cognition in AD mouse models. Here we evaluated the effects of moderate voluntary exercise on cognitive function by giving AD mice access to running wheels for 30 and 60 days and assessing their functional recovery with a battery of behavioural tests. Our results to date from a Y-maze behavioural task show an improvement in maximum alternation, an index of spatial working memory, in transgenic running animals suggesting that running may improve memory function in those mice after 60 days of exercise ($p=0.04$, $n=5$). In summary, we were able to show that exercise can improve spatial working memory in an AD mouse model. Subsequent research in those animals will test whether moderate exercise has a positive effect on adult neurogenesis by assessing the number of cells labelled at the onset of exercise (cell differentiation) and after several weeks of exercise (cell proliferation). Exercise has the potential to improve cognition even when cognitive impairment results from several different

neuropathological events.

Funding: CIHR

Title: Improved working memory following novel combinations of physical and cognitive activity

Authors: Kristopher Langdon¹, Dale Corbett²

Affiliation: 1- Memorial University of Newfoundland 2- University of Ottawa

Abstract: "Background: In humans, retrospective studies suggest that habitual physical or cognitive activity can help maintain or improve cognitive function. Similar findings have been reported using physical exercise in animal studies, however the exercise paradigms differ markedly in duration and frequency making extrapolation difficult. Here, we present a novel, physical and cognitive activity paradigm that combines voluntary wheel running with Hebb-Williams and radial arm maze training. Methods: Fifty-seven Sprague-Dawley rats were divided into four treatment groups: physical activity; cognitive activity; combined physical and cognitive activity; and sedentary controls. Physical (voluntary wheel running) and cognitive (Hebb-Williams mazes) activity consisted of a moderate two hours/day five days/week treatment paradigm. Results: Animals exposed to a combination of physical and cognitive activity made significantly fewer working memory errors and exhibited superior choice accuracy than either physical or cognitive activity-alone animals in the 8-arm baited configuration of the radial arm maze. Additional analyses revealed that the cognitive improvements were independent of exercise intensity/duration. Assessment of brain-derived neurotrophic factor (BDNF) levels revealed a significant increase in hippocampal BDNF only in physical activity-alone animals. Conclusion: A novel combination of physical and cognitive activity improves learning and memory abilities independent of activity intensity, BDNF or pCREB levels. This is the first report of significant changes in cognitive ability using a paradigm involving moderate levels of physical activity plus cognitive stimulation. An adaptation of this paradigm may be particularly beneficial in slowing the development of mild cognitive impairment and subsequent dementia."

Funding: This work was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada

Title: Consolidation, robustness, and decay of visual working memory in the macaque monkey

Authors: Mariann Oemisch¹, Evelien Heyselaar², Kevin Johnston³, Martin Pare⁴

Affiliation: 1-4 Queen's University

Abstract: Visual working memory (VWM) is an executive function that allows us to temporarily retain visual information that can be used to execute an appropriate response. A fundamental characteristic of VWM is its limited storage capacity, which is reflected by a drop of performance with increasing memory load. We recently implemented a sequential-comparison task in the macaque monkey to investigate the neural mechanisms of VWM. In this task, a memory array of 2 to 5 highly discriminable colored stimuli is first presented. Following a retention interval, a test array is presented in which one of the original stimuli has changed color. The animal is required to report the changed stimulus with a single foveating saccade. Here we assessed other potential limiting aspects of VWM in four monkeys. First, we examined the time course of VWM consolidation by manipulating the duration of the memory array (50-500ms). We found that performance reached a plateau within 200 ms across monkeys. Second, we examined the decay time of VWM by manipulating the retention interval duration (0-1000ms). Performance declined exponentially with increasing interval. The decay time constant averaged 350ms across monkeys, but significantly decreased (from 512 to 240ms) with increasing memory loads (set size 2 to 5). Third, we examined the susceptibility of VWM to distraction by presenting task-irrelevant distracters (white stimuli) during the retention interval. Such distracters had no consistent effect across monkeys, suggesting that they possess robust VWM in this sequential-comparison task. These results help characterize further our monkey model of VWM.

Funding: NSERC

Title: The involvement of the anterior cingulate cortex in the acquisition, consolidation and reconsolidation of contextual fear memory

Authors: Einar Einarsson¹, Jennifer Pors², Karim Nader³

Affiliation: 1-3 McGill University

Abstract: The standard view of systems-consolidation posits that cortical structures including the anterior cingulate cortex (ACC) play a specific role in remote memory, such as that of contextual fear conditioning. Here we report that intra-ACC infusions of the protein-synthesis inhibitor anisomycin immediately following conditioning, or retrieval of a recent (3d) or remote (30d) old memory, disrupted later memory expression. Furthermore, we found that pre-test ACC AMPA-receptor inactivation impairs remote memory retrieval. However, if retrieved 6 hours earlier,

subsequent memory expression is only impaired if both the ACC and dorsal hippocampus (DH) are inactivated, while at 24 hours only ACC inactivation impaired expression again. Furthermore, following the retrieval of a 30 d old memory, context discrimination is restored 24 hr later, but not at 6 or 48 hr. Moreover, we find that AMPA-receptor inactivation of the ACC 6 hr after the reminder selectively impairs responding to the novel context, but not the original training context, thus restoring context discrimination. These findings suggest that the circuits supporting contextual fear memory involve the ACC from memory formation for cellular consolidation and reconsolidation at recent and remote time-points, and that the DH is re-engaged transiently with the ACC following the reactivation of remote memory. Our results also suggest that the ACC mediates generalized memory expression, whereas the dorsal hippocampus supports more discriminative memory expression.

Funding: NSERC, CIHR, Steacie Foundation

Title: Age-related changes in visuo-spatial learning and memory in mouse models of Alzheimer Disease

Authors: Richard Brown¹, Rhian Gunn², Timothy O'Leary³

Affiliation: 1-3 Dalhousie University

Abstract: The Morris Water maze (MWM) is the most commonly used test for visuo-spatial learning and memory in rodents and is frequently used to test cognitive deficits in mouse models of Alzheimer Disease (AD) (D'Hooge & De Deyn, 2001, Brain Res Rev 36, 60-90). We have tested males and females of five strains of AD model mice (APPInd, APPSwInd, AppSwe/PS1, AppSwe/PS1dE9, 5XFAD) and their wildtype (WT) control strains in the MWM between 3 & 24 months of age. Both longitudinal and cross-sectional studies were completed. This paper summarizes the effects of genotype, age and sex on measures of learning (latency, search strategy) and memory (percent time in correct quadrant, annulus crossings) in the MWM and discusses background gene effects which influence performance independently of transgene effects. The most significant genotype effects were found in the APPInd and 5XFAD mice. There were few significant sex differences, although females appear to use a different search strategy than males. All strains showed reduced performance with age. Background genes for retinal degeneration and albinism affect performance independently of AD transgenes. The results demonstrate the use of the MWM as an analytical tool in dissociating the factors underlying visuo-spatial learning and

memory in AD model mice.

Funding: NSERC & Alzheimer's Association

Title: NR2B-subunit dependent facilitation of LTP in V1 following visual discrimination training of adult rats.

Authors: Peter Gagolewicz¹, Hans Dringenberg²

Affiliation: 1-2 Queen's University

Abstract: "Long-term potentiation (LTP) is an important mechanism mediating changes in synaptic connectivity following various types of experiences. We examined the effects of visual discrimination training on LTP in the mature, rodent thalamocortical visual system. Adult rats underwent visual discrimination training in a modified Morris Water Maze containing a Y-maze insert, requiring rats to associate visual cues with the location of a hidden escape platform located in one of the two goal arms of the Y-maze insert. On the day following task completion (average of 9 training days), rats were anesthetized (urethane) and LTP in the thalamocortical system was characterized. In task-naïve rats, theta-burst stimulation of the lateral geniculate nucleus resulted in modest (39%) potentiation of field postsynaptic potentials recorded in V1. Rats trained on the visual discrimination task showed significantly greater levels of LTP (62%), while rats that swam in the maze without learning to associate visual cues with the platform location failed to show this LTP facilitation. An antagonist of the NMDA receptor NR2B subunit (Ro 25-6981; 2 mM, applied locally at the recording site in V1) of the NMDA receptor reversed the training-induced LTP enhancement without affecting LTP in task-naive rats. An antagonist of metabotropic glutamate receptors (LY 341495; 2 mM) was ineffective in reversing the training-induced LTP enhancement. These data suggest that behavioral training can result in changes in NMDA receptor composition and/or functioning, leading to an expansion of the plasticity range exhibited by the mature, thalamocortical visual system."

Funding: Supported by the Natural Sciences and Engineering Research Council of Canada.

Title: Effects of acute and chronic buspirone and escitalopram treatments on anxiety-like behavior and plasma corticosterone response of rats

Authors: Dietmar Hestermann¹, Ingeborg van Kroonenburgh², Harry Steinbusch³, Arjan Blokland⁴, Yasin Temel⁵, Lee Wei Lim⁶

Affiliation: 1-6 Maastricht University

Abstract: "Selective serotonin reuptake inhibitors (SSRIs) are widely used in clinical management of mood and anxiety disorders. However, initiating SSRI treatment may result in an increase in anxiety, jitteriness, insomnia and irritability (jitteriness / anxiety syndrome). The hypothalamic-pituitary-adrenal (HPA) axis has been shown to play an important role in the regulation of anxiety-like behaviour. To examine the effect of SSRIs on behaviour and HPA interplay, eighteen male albino Wistar rats were given acute and chronic treatment of escitalopram (ESCIT; 10mg /kg) and buspirone (BUSP; 3mg /kg), and tested in the open-field (OF) for anxiety-related behaviors (mobility, immobility, head weaving, rearing and self-grooming). Levels of corticosterone were measured 30 min after the OF test. Acute treatment of BUSP and ESCIT decreased time-spent in OF center and frequency of rearing. Acute treatment of BUSP but not ESCIT increased time-spent in OF corners, immobility and head weaving. Levels of plasma corticosterone were significantly elevated after acute and chronic BUSP treatment and chronic ESCIT treatment but there was no significant correlation between baseline levels of corticosterone and anxiety-related behaviors. These results are consistent with previous findings showing anxiogenic-like behavior induced by acute treatment of BUSP. However, the lack of a significant correlation between corticosterone levels and anxiety-related behaviour suggests that elevated corticosterone levels following SSRI treatment might not be the cause of anxiety-like behaviour. Both behavioural and hormonal changes could be the result of drug activity on serotonergic and/or noradrenergic pathways. "

Funding:

Title: Food anticipatory activity: different neuronal response to food with different hedonic value

Authors: Arojit Mitra¹, Jessica Martin², Christophe Lenglos³, Elena Timofeeva⁴

Affiliation: 1-4 IUCPQ, University Laval

Abstract: Introduction: Search of Food entrainable oscillators (FEO) and its behavioural output as Food-anticipatory activity (FAA) has long, rigorous but still an ill understood realm in behavioural and physiological biology. Ablation, silencing, and lesion of various brain structures were never ample enough to withdraw the capability of an organism to anticipate the food window of its niche. In this study, powerful tool of c-fos mapping was used to investigate the neural substrates that could play a pivotal role in FAA and could be a promising FEO. Method: Rats were maintained

for 3 weeks on ad libitum feeding of regular chow, or were submitted to the restricted feeding with 2-hour daily access to chow or chow and 10% sucrose. The neuronal activation, estimated by detection of expression of immediate early gene c-fos, was assessed during FAA and following feeding. Horizontal activity and oxygen consumption were also measured during FAA. Result: Data clearly shows that restricted feeding schedules for regular chow and daily limited access to palatable food (along with chow) in free-feeding rats, activated different brain regions during FAA. The paraventricular and dorsomedial hypothalamic nuclei and posterior lateral hypothalamus (LH) expressed c-fos mRNA in rats maintained on restricted chow regime, whereas adding sucrose along with restricted chow displaced the magnitude of activation to prefrontal cortex, lateral septum, nucleus accumbens and anterior LH. Recorded horizontal activity and oxygen consumption demonstrated no difference between the restricted groups. Conclusion: The obtained data provide evidence towards possibility of having palatability-dependent different oscillatory epicenters in the brain.

Funding: CIHR and NSERC

Title: Activity in the lateral septum governs response selection in a modified shock-probe burying test.

Authors: Meaghan M. Wilkin¹, Janet L. Menard²

Affiliation: 1-2 Queens University

Abstract: "In the shock-probe burying test (SPBT), rats shocked from an electrified probe attempt to "bury" it with bedding material (wood chips) spread about the test chamber's floor. We recently developed a modified version of this test in which rats are provided with the additional option of "hiding" in a small hide-box placed inside of the test chamber. We determined that burying is the predominant response when bedding material is available. Specifically, under these conditions, rats tested with an electrified probe displayed significant increases in shock-probe burying but did not spend more time concealed within the hide-box. By contrast, when burying was precluded, by testing rats in a chamber without bedding, we observed reliable shock-induced increases in hiding. The purpose of the current experiment was to determine whether the lateral septum, which is known to regulate burying, also contributes to hiding behaviour. Rats were infused with either muscimol or saline into the lateral septum and then tested in either the 'bedding' or 'no-bedding' condition of the modified SPBT. As expected, intra-lateral septal

muscimol decreased shock-probe burying. Remarkably, these muscimol-induced decreases in burying were accompanied by dramatic increases in hiding behaviour. By contrast, intra-lateral septal muscimol had virtually no effect on hiding behaviour in the 'no-bedding' condition. Together, the results suggest that activity in the lateral septum elicits the burying response while at the same time inhibiting structures that regulate competitive behaviours (e.g., hiding). Such a mechanism could ensure the selection of contextually appropriate defensive behaviour."

Funding: This research is funded by NSERC

Title: Effect of breeding condition on volume of song control nuclei and FoxP2 expression in Female Black-capped Chickadees (*Poecile atricapillus*)

Authors: Ryan Wilson¹, Stephanie Martin², Leslie Phillmore³

Affiliation: 1-3 Dalhousie University

Abstract: Changing photoperiod affects both behaviour and brain of songbirds. In black-capped chickadees, production of male fee-bee song increases in spring, when days are long (Phillmore et al 2006), and production of chick-a-dee calls is greater in fall and winter (Avey et al 2008). Song control nuclei, (HVC, RA) are larger in breeding than non-breeding male chickadees but the difference small compared to that seen in other species (e.g. Tramontin et al 2000), and there is no difference in Area X. Unlike their male counterparts, female chickadees do not produce fee bee songs, but they do produce the chick-a-dee call. However, there is little research examining the seasonal plasticity (Breeding vs. Non-breeding) of the song-control system in females. This study investigated two measures of seasonal plasticity in the vocal-control nuclei of female black-capped chickadees: volumetric measures (raw and proportional) of both HVC and Area X, and levels of the "language" gene FoxP2 in Area X relative to a control area in striatum. There was no difference in nuclei volume between females in breeding and non-breeding condition. However, the ratio of FoxP2 expressed in Area X relative to a striatal control area was significantly larger for females in breeding condition (mean ratio = 5.38) compared to those in non-breeding condition (mean ratio = 2.55). These results suggest that changes in FoxP2 expression, a gene related to plasticity in vocal learning (Fisher et al 2009) may be related to song "reception" as well as song production.

Funding:

Title: Modeling covert stroke: effects of chronic hypoperfusion and medial thalamic stroke on attention set shifting ability in rats

Authors: Christopher Cordova¹, Krista Hewlett², Kristopher Langdon³, Matthew Jeffers⁴, Shirley Granter-Button⁵, Dale Corbett⁶

Affiliation: 1-5 Memorial University of Newfoundland 6- University of Ottawa

Abstract: Covert strokes arising from small vessel disease often lead to cognitive impairments affecting executive function, attention and memory. Ischemic covert stroke produces white matter damage and small (lacunar) infarcts frequently arising in midline thalamic nuclei that may interrupt connections of the prefrontal cortex and contribute to the development of executive cognitive dysfunction. A rodent model of covert stroke was developed that incorporates pathological features of the disease by using a bilateral occlusion of the common carotid arteries to induce mild hypoperfusion (2-VO) and unilateral injections of the vasoconstrictor endothelin-1 to create lacunar infarcts in the mediodorsal nucleus of the thalamus (MD). Additionally, common clinical co-morbidities including age, hypertension and diet were modeled using middle-aged rats (8-12 mo) fed a diet high in fat, refined sugar and salt. Impairments in executive function were assessed using a rodent attention set-shifting test that requires animals to flexibly allocate attention to stimuli in different stimulus dimensions. Rats with an MD stroke + 2-VO and rats with an MD stroke alone exhibited a selective impairment in the ability to perform an extradimensional attention shift, but this effect was not observed in rats with 2-VO or sham surgery alone. Further, there were no differences in either intradimensional set-shift or reversal tests among groups, indicating a selective extradimensional set-shifting deficit. This effect is consistent with attention set shifting impairments in humans following covert stroke, and demonstrates for the first time the feasibility of an animal model that accurately reflects the neurobiological and clinical features of covert stroke.

Funding: Canadian Stroke Network

Title: Optogenetic Probing of Hypothalamic Modulation of the Sleep-Wake Cycle

Authors: Antoine Adamantidis¹, Sonia Jégo², Luis de Lecea³, Karl Deisseroth⁴, Denis Burdakov⁵

Affiliation: 1-2 McGill University 3-5 Stanford University

Abstract: While the functions of sleep are still a matter of debate and may include memory consolidation and plasticity, the neural substrates of sleep and wakefulness are the subject of

intense study. Successive sleep and wakefulness cycles rely on an appropriate balance between sleep-promoting nuclei of the brain and arousal-promoting nuclei. The posterior hypothalamus contains two distinct neuron populations that have been recently identified as critical modulators of the sleep-wake cycle: the Hypocretins/Orexins (Hcrt,/Ox)- and Melanin-Concentrating Hormone (MCH)-expressing neurons. Whereas the Hcrt system is involved in boundary state control, the precise physiological function and mechanisms of action of the MCH system on the sleep-wake cycle remain unclear. Here we have selectively manipulated the activity of Hcrt and MCH neuronal population using optogenetics to study their modulation of the sleep-wake cycle. We found that activation of the Hcrt facilitates wakefulness. In contrast, activation of the MCH system has the opposite effect and promotes sleep. Based on these results, we have built a conductance-based in silico model of Hcrt and MCH neurons as a first step to understand plasticity mechanisms at the network level. These results suggest a bimodal switch within the lateral hypothalamus that may modulate behavioral states with different circuit dynamics.

Funding: CFI, NARSAD, NIH.

Title: Upregulation of AMPA receptors in the anterior cingulate cortex mediate trace fear learning in adult mice

Authors: Giannina Descalzi¹, Xiang-yao Li², Tao Chen³, Valentina Mercaldo⁴, Kohei Koga⁵, Min Zhuo⁶

Affiliation: 1-6 Department of Physiology, University of Toronto

Abstract: Although the cortex has been extensively studied in long-term memory storage, less emphasis has been placed on immediate cortical contributions to fear memory formation. Here we show that the anterior cingulate cortex (ACC) is actively engaged in trace fear learning and that i.p. and intra-ACC microinjections NR2B antagonists before training disrupts trace fear memory 48hr later. Furthermore, trace fear conditioning induced an immediate 20% increase of membrane AMPA GluR1 receptors within the ACC, which was blocked by the NR2B antagonist pre-treatment. Moreover, in mice exposed to trace fear learning, we found that the calcium permeable AMPA channel antagonist, 1-naphthylacetyl spermine (NASPM), inhibited the EPSC amplitudes of cFos positive neurons within the ACC to 67% off the baseline, and that this effect is eliminated after extinction training. These results suggest that rapid, NR2B dependent upregulation of membrane AMPA GluR1 within the ACC is a critical cellular mechanism for trace fear memory.

Funding: Supported by CIHR 84256, 66975, 81086 and CRC chair to MZ. GD is funded through OGS.

Title: Spike rate correlations between primate dorsolateral prefrontal cortex neurons during a spatial working memory task

Authors: Matthew Leavitt¹, Megan Schneiderman², Julio Martinez-Trujillo³

Affiliation: 1-3 McGill University

Abstract: It has been shown that neurons in the primate prefrontal cortex encode location information during the delay period of spatial working memory tasks, however it remains unclear whether and how these units interact with each other. We investigated this by recording single cell responses using microelectrode arrays (96 microelectrodes/array, Blackrock Inc., UT) implanted in dorsolateral prefrontal cortex (area 8v) of two macaca fascicularis during a working memory task, and measuring spike count correlations between the recorded units. During trials the animals fixated on a central spot for 494 to 800ms, then a circular sine wave grating was presented at one of 16 randomly selected locations for 507ms. The offset of the grating was followed by a delay period that lasted between 494 and 2000ms and ended with the offset of the central fixation point. This latter event instructed the animals to saccade to the remembered location of the previously presented grating. The spiking activity of neurons was recorded simultaneously in blocks of 32 channels and sorted using Plexon software (Plexon Inc, TX). We found that spike rate correlations between the pairs computed across all target locations were significantly higher during the delay period ($r = 0.13$) than during the stimulus presentation period ($r = 0.04$) ($p < 0.01$, paired t-test). Our results suggest that interactions between neurons in the dlPFC increase during working memory maintenance in the absence of visual inputs. Such interactions may be critical for working memory encoding by neuronal populations within the area.

Funding: Canadian Institutes of Health Research, EJLB Foundation, Natural Sciences and Engineering Research Council

Title: Acute stress disrupts two forms of long-term potentiation in the CA1-subiculum pathway in rats via glucocorticoid receptor activation

Authors: Matthew MacDougall¹, John Howland²

Affiliation: 1-2 University of Saskatchewan

Abstract: "The subiculum is the major output structure of the hippocampal formation. Exploring synaptic plasticity within this region is of great importance for understanding the dynamics of hippocampal circuitry and hippocampal-cortical interactions. Exposure to acute stress alters synaptic plasticity within the hippocampus proper by inhibiting long-term potentiation (LTP) or facilitating long-term depression (LTD). Using in vivo electrophysiological recordings in adult male Sprague-Dawley rats, the present experiments demonstrate that two forms of LTP co-exist within the CA1-subiculum pathway following low frequency stimulation (LFS; $23.85\% \pm 5.0$) and high frequency stimulation (HFS; $13.0\% \pm 4.6$). Both forms of LTP are inhibited after exposing rats to 30 min of restraint stress. We further demonstrate that the disruptive effects of acute stress on these forms of LTP are mediated by glucocorticoid receptor (GR) activation using the selective GR antagonist RU38486 (10 mg/kg). To better elucidate the flow of plasticity within hippocampal circuits, simultaneous recordings of CA1 and subiculum were performed while stimulating the CA3/Schaffer collaterals. We demonstrate that subicular plasticity is driven by plasticity in CA1. LFS of the CA3/Schaffer collaterals produced no significant alterations in CA1 ($-6.0\% \pm 7.8$) while potentiation was observed within the subiculum ($23.3\% \pm 10.6$) similar to that following CA1 stimulation. Conversely, HFS produced significant potentiation in CA1 ($42.6\% \pm 2.0$) and significantly enhanced subicular potentiation ($49.3\% \pm 3.4$) compared to potentiation following CA1 stimulation. The present results demonstrate the susceptibility of CA1-subicular potentiation to acute stress while on-going experiments will assess the interplay between CA1 plasticity and the subiculum."

Funding: NSERC and CIHR.

Title: Inhibition of mTOR kinase via rapamycin causes a long-lasting impairment of predator stress-induced hyperarousal.

Authors: Jacqueline Blundell¹, Kathleen Fifield², Mark Hebert³, Greg Gill⁴, Robert Adamec⁵, Victoria Linehan⁶

Affiliation: 1-6 Memorial University of Newfoundland

Abstract: "Traumatic, stressful life events are thought to trigger acquired anxiety disorders such as post-traumatic stress disorder (PTSD). PTSD is characterised by several symptoms including both associative and non-associative fear memories. It has been previously established that the

mTOR pathway plays a key role in associative fear memories (Bekinschtein et al, 2007; Blundell et al., 2008; Parsons et al, 2006); however, it is unknown whether this pathway attenuates non-associative fear memories. We hypothesized that mTOR is not only important for consolidation of associative fear memories, but is also critical for consolidation of non-associative fear memories such as hyperarousal and anxiety-like behaviour. To test this hypothesis, we injected the mTOR antagonist rapamycin which selectively inhibits the activity of mTOR kinase, following predator stress. Predator stress involves an acute, unprotected exposure of a rat to a cat which causes long-lasting non-associative fear memories such as hyperarousal (manifested as increased startle response) and elevated anxiety. Here, we show that rapamycin attenuated predator stress-induced hyperarousal. However, rapamycin had no effect on predator stress-induced anxiety. Consistent with previous data, rapamycin significantly reduced weight gain lasting at least 4 weeks. Taken together with past research, mTOR regulation of protein translation is required for normal consolidation of both associative and non-associative fear memories. Current studies are investigating the role of rapamycin following retrieval of predator stress-induced non-associative fear memories. Overall, these data suggest that rapamycin, a drug already in clinical trials, could be a novel treatment for patients suffering from acquired anxiety disorders such as PTSD."

Funding: NSERC, NARSAD, CIHR, ADAA

Title: Visuo-spatial learning and memory of 13 inbred strains with different visual abilities on a mouse version of the Barnes maze

Authors: Timothy O'Leary¹, Richard Brown²

Affiliation: 1-2 Dalhousie University

Abstract: Visuo-spatial learning and memory were assessed in 13 inbred strains on a small diameter mouse version of the Barnes maze surrounded by a wall and intra-maze visual cues. Mice completed acquisition and reversal training to assess learning, and a probe test to assess memory for the location of the escape hole. C57BL/6J and CAST/EiJ strains showed better learning performance than the other strains, while A/J and 129/SvImJ strains showed poor learning performance due to low levels of exploration. No differences in memory were found between strains in the probe test. However, mice may not have used visuo-spatial cues to locate the escape hole, as (1) all strains primarily used the non-spatial serial/thigmotaxic search strategy, (2) strains did not show a reversal effect when the escape hole location was moved, and (3) learning

and memory performance were not correlated with measures of visual ability. Multivariate and univariate analyses of variance indicated that mice with good visual ability performed better than mice with poor visual ability, but the effect sizes were small. The small diameter and the presence of a wall around the edge of the maze may promote thigmotaxis in mice, increasing the use of a non-visual search strategy, thereby decreasing the sensitivity of this maze design to detect strain differences in visuo-spatial learning and memory. These results indicate that the design of the Barnes maze has a significant effect on learning and memory processes.

Funding: NSERC

Title: Inhibition of mTOR kinase via rapamycin causes a long-lasting impairment of predator stress-induced hyperarousal.

Authors: Kathleen Fifield¹, Mark Hebert², Victoria Linehan³, Greg Gill⁴, Robert Adamec⁵, Jacqueline Blundell⁶

Affiliation: 1-6 Memorial University of Newfoundland

Abstract: "Traumatic, stressful life events are thought to trigger acquired anxiety disorders such as post-traumatic stress disorder (PTSD). PTSD is characterised by several symptoms including both associative and non-associative fear memories. It has been previously established that the mTOR pathway plays a key role in associative fear memories (Bekinschtein et al, 2007; Blundell et al., 2008; Parsons et al, 2006); however, it is unknown whether this pathway attenuates non-associative fear memories. We hypothesized that mTOR is not only important for consolidation of associative fear memories, but is also critical for consolidation of non-associative fear memories such as hyperarousal and anxiety-like behaviour. To test this hypothesis, we injected the mTOR antagonist rapamycin which selectively inhibits the activity of mTOR kinase, following predator stress. Predator stress involves an acute, unprotected exposure of a rat to a cat which causes long-lasting non-associative fear memories such as hyperarousal (manifested as increased startle response) and elevated anxiety. Here, we show that rapamycin attenuated predator stress-induced hyperarousal. However, rapamycin had no effect on predator stress-induced anxiety. Consistent with previous data, rapamycin significantly reduced weight gain lasting at least 4 weeks. Taken together with past research, mTOR regulation of protein translation is required for normal consolidation of both associative and non-associative fear memories. Current studies are investigating the role of rapamycin following retrieval of predator stress-induced non-associative

fear memories. Overall, these data suggest that rapamycin, a drug already in clinical trials, could be a novel treatment for patients suffering from acquired anxiety disorders such as PTSD."

Funding: NSERC, NARSAD, ADAA, CIHR

Title: "Nothing like the first time? The ""shocking"" similarity of contextual events."

Authors: Peter Finnie¹, Szu-Han Wang², Karim Nader³

Affiliation: 1,3 McGill University 2- Edinburgh University

Abstract: The brain has clearly evolved to store biologically significant information about the world in order to predict subsequent similar events. Due to the presence of such predictive memory, familiar events could be encoded differently than unfamiliar events which require storage of entirely novel information. Indeed, it has been reported that pre-training dorsal hippocampal infusion of a N-methyl-D-aspartic acid-receptor (NMDAR) antagonist, DL-2-amino-5-phosphonopentanoate (AP5), disrupts a first contextual fear conditioning task, but not a second in a distinct context. This could suggest that the brain builds upon the memories of past events when encoding related information. This switch in plasticity mechanisms could reflect a process by which the brain integrates new information with old memories, so it is important to identify the kinds of events that can subsequently permit AP5-insensitive learning. Here we show that only when the content and arrangement of two events is very similar can the second be acquired in an AP5-insensitive manner (even if the same conditioned response is acquired during both). We also report that this switch in mechanisms is temporary, but can be re-engaged when a reminder of the first training is given the day before second training. Together these findings indicate that the hippocampus can use different plasticity mechanisms for learning when a similar experience has recently occurred, though this does not seem to rely on having recently acquired a similar conditioned response. This is consistent with a role for the hippocampus in storing representations of contextual stimuli, though not in encoding conditioned behaviour.

Funding: NSERC CIHR EWR Steacie Research Foundation

Title: Neural FOXP2 Expression in the Brown Ghost Knifefish (*Apteronotus leptorhynchus*)

Authors: Sean Roach¹, John Batt², Leslie Phillmore³

Affiliation: 1,3 Psychology Department, Dalhousie University 2- Oceanography Department, Dalhousie University

Abstract: The FOXP2 gene is highly conserved among vertebrates and functions primarily in motor control. It is also involved in the communication systems of disparate vertebrate taxa (e.g., humans, songbirds). Because only one study has examined expression in the fish brain (in zebrafish, Shah et al., 2006), we used immunohistochemistry to detect neural FOXP2 protein expression in a species that uses electric signals for communication. The brown ghost knifefish (*Apteronotus leptorhynchus*) produces a sexually dimorphic wave-type electric signal (electric organ discharge, or EOD) that is used both for location and for intraspecific social communication in a context-dependent manner. The distribution of FOXP2 expression was similar to that found in the zebrafish (e.g., optic tectum, torus semicircularis), which was expected given the highly conserved distribution of FOXP2 expression among vertebrates. Prompted by FOXP2 expression in areas linked to EOD production and modulation, and given FOXP2 expression changes associated with context-dependent communication in other species (e.g., zebra finch), we also report the results of a comparison of FOXP2 expression in individuals housed alone versus with conspecifics.

Funding:

Title: Role of the Frontal Eye Field in Choosing Mixed Strategy Saccades

Authors: Abdullahi Abunafeesa¹, Michael Dorris²

Affiliation: 1-2 Queen's University

Abstract: "In a multi-agent environment, animals must often adopt a stochastic mixed-strategy approach to maximize reward and minimize costs; otherwise, competitive opponents can exploit predictable choice patterns. Here, we tested whether the frontal eye field (FEF) is involved in choosing mixed-strategy saccades. The FEF was selected because of the substantive literature linking it with voluntary saccade selection. Moreover, it provides strong input to the superior colliculus (SC), which we have recently demonstrated is involved in choosing mixed-strategy saccades. Monkeys played a saccade eye movement version of the classic mixed-strategy game 'matching-pennies' against an adaptive computer opponent; while the preparatory activity of saccade related neurons were recorded from the FEF. Signal detection theory was used to analyze how accurately FEF preparatory activity predicted upcoming saccade choices. Our data demonstrates that the FEF became increasingly predictive as the time of saccade execution approached. The pattern of activity is consistent with an accumulation of evidence for each

potential option towards a decision threshold. Lastly, a particular advantage of our experiment is that we had the same monkeys perform this task while successively recording from the both SC and FEF. This allowed us to compare the timing and magnitude of neuronal selectivity across these two structures during strategic decision-making. Our results indicate that the selection of mixed-strategy saccades occurred earlier and was greater in magnitude in the FEF compared to the SC, indicative of a decision process that occurs earlier in the frontal cortex before being relayed on to premotor regions in the midbrain."

Funding: Department of Physiology, Center for Neuroscience Studies, CIHR Group in Sensory-Motor Neuroscience, Queen's University

Title: Parsing out memory structure with reconsolidation

Authors: Michael Honsberger¹, Karim Nader²

Affiliation: 1-2 McGill University

Abstract: Neural populations in the basolateral amygdala have been shown to be an essential substrate for associative fear memories. It remains unclear whether distinct associative memories are mediated by independent or overlapping populations of BLA neurons. To investigate this issue, we used the fact that initiation of reconsolidation is dependant on the reactivation of a particular memory. Therefore, if two memories are stored by the same overlapping population within the BLA, then interference though blockade of reconsolidation of one should lead to impairments in both. Conversely, if the two memories are stored independently of each other within the BLA, then blockade of reconsolidation of one memory should leave the second intact. We have begun to investigate this with two protocols that each result in two distinct fear memories. We have previously shown that one day following a single tone-shock pairing, blocking reconsolidation of fear of the training context led to an impairment in freezing to the tone, even though the tone was not reactivated. Conversely, blocking reconsolidation of auditory fear conditioning had no effect on freezing induced by the training context. We have also developed a two day Pavlovian fear conditioning protocol which results in two pure tones eliciting freezing. This does not result in a generalized freezing to all tones as extinguishing freezing to tone 1 leaves freezing in tone 2 intact. Results from this protocol suggest a new boundary condition constraining reconsolidation. These experiments provide a unique view of how fear memory is

organized in the amygdala.

Funding: NSERC, CIHR, EWR Steacie Foundation

Title: Redefining the role of acetylcholine in the striatum.

Authors: Vania F Prado¹, Xavier DeJaeger², Monica Guzman³, Marco A M Prado⁴

Affiliation: 1,3,4 Robarts Researn Institute/University of Western Ontario 2- University of Western Ontario/ Universidade Federal de Minas Gerais

Abstract: Acetylcholine is thought to play important roles in distinct brain regions, however the contribution of striatal cholinergic transmission for the regulation of physiological functions and behaviours remains poorly understood. Importantly, it has been recently recognized that cholinergic neurons can release acetylcholine and glutamate and the specific contributions of these two neurotransmitters released from cholinergic neurons is unknown. Here we used a striatal-selective vesicular acetylcholine transporter (VACHT) mouse knockout line to address the specific roles of acetylcholine for behaviour. These novel mice do not express VACHT and therefore, do not release acetylcholine in the striatum, whereas in the hippocampus both VACHT expression and ACh release were not affected. We found that selective elimination of VACHT in the striatum does not cause overt disruptions or alterations in several behavioural tasks previously thought to depend on striatal ACh release, such as motor learning and locomotor activity. However, these mice showed increased responses to both D1 and D2 dopamine agonists due to increased expression of D1 and D2 dopamine receptors. Our results indicate that behavioural consequences of selective elimination of VACHT in the striatum are remarkably minimal. Because in Parkinson's Disease an increased activation of cholinergic neurons is observed with the concomitant dopamine deficiency in the striatum, it is possible that VACHT could be used as a molecular target to improve hypercholinergia-related symptoms in PD.

Funding: "Canadian Institutes of Health Research (CIHR); Canadian Foundation for Innovation (CFI), Ontario Research Fund (ORF) and University of Western Ontario"

Title: Differential roles of striatal NR2A and NR2B-containing NMDA receptor on motor skill learning in mice

Authors: Julie Lemay-Clermont¹, Geneviève Bureau², Michel Cyr³

Affiliation: 1-3 UQTR

Abstract: "The striatum is known to play a role in learning and memory. Indeed, this structure has been implicated in various complex motor skill learning tasks, such as the accelerating rotarod task. However, whether individual NMDAR subunits contribute to memory in the striatum is not known. The aim of the present study was to investigate the role of striatal NMDA receptor, specifically NR2A and NR2B subunits, during the learning of the accelerating rotarod task in mice. To this end, the non-selective NMDAR channel blocker MK-801 as well as the selective antagonists NVP-AAM077 to NR2A and Ro 25-6981 to NR2B subunits were injected directly into the dorsal striatum of mice previous to rotarod training sessions, on day 1, 2, 3 and 8. Intra-striatal injections (1 μ L/side) were performed via chronically implanted cannula. Two general phases of learning were distinguished by this experimental approach; a fast acquisition phase (day 1) and a slower consolidation phase (day 2,3 and 8), characterized by more moderate gains in performance. There was no difference in the performances of mice injected with vehicle (saline) or Ro 25-6981 (5 and 10 μ g/side). However, injections of MK-801 (2.5 and 5 μ g/side) as well as NVP-AAM077 (0.5 and 1 μ g/side) impaired motor performances at day 2, 3 and 8. The injected mice displayed normal motor capacity as revealed by a stepping, wire suspension and pole tests. These findings suggested that activation of the NR2A subunits, but not NR2B, in the dorsal striatum is important in the consolidation of motor skill memory. "

Funding: This work was supported by the Natural Sciences and Engineering Research Council of Canada.

Title: The role of delta-GABAARs in pattern separation

Authors: Paul Whissell¹, Irene Lecker², Beverley Orser³

Affiliation: 1-3 University of Toronto

Abstract: Pattern separation (PS) might underlie our ability to distinguish subtly different stimuli by segregating similar neural inputs into non-overlapping representations. PS is thought to occur in the dentate gyrus and be shaped by network inhibition via gamma-aminobutyric acid (GABA). Extrasynaptic GABA subtype A receptors containing the delta subunit (dGABAARs) mediate tonic inhibition in the dentate gyrus but their contribution to PS is unknown. We tested the hypothesis that dGABAARs were required for PS by comparing the performance of WT and dGABAAR knockout (Gabrd^{-/-}) mice on the object-place recognition (OPR) test. Before and after OPR, we also compared these mice on the novel object recognition test (NOR). When we administered the

OPR and NOR tasks separately, we observed that WT mice, but not Gabrd^{-/-} mice, were able to distinguish similar contexts in the OPR test ($t = 1.84$, $df = 31$, $p < .05$). The genotypes did not differ in NOR test. Surprisingly, when we combined the two tests into a serial assay (OPR followed by NOR), we observed an impairment of NOR in WT but not Gabrd^{-/-} mice ($t = 1.89$, $df = 29$, $p < .05$). We argue that this impairment in NOR arises from interference by the preceding OPR task. Genetic deletion of the dGABAAR impairs OPR, but eliminates this interference. Our findings suggest that dGABAARs play an important role in PS. Activation of dGABAARs during PS tasks, such as OPR, may also lead to a form of working memory interference which impairs the acquisition of other subsequent behaviours, such as NOR.

Funding: This work was supported by CIHR grants to BAO, an NSERC grant to PDW and CIHR/CAS grants to IL.

Title: Infusions of neuropeptide Y into the lateral septum are anxiolytic in some but not all animal models of anxiety.

Authors: Natalie Trent¹, Janet Menard²

Affiliation: 1-2 Queen's University

Abstract: "Neuropeptide Y (NPY) is one of the most abundant peptides in mammalian brain. NPY-like-immunoreactivity is highly expressed in the lateral septum, an area extensively implicated in anxiety regulation. Similarly, intracerebroventricular (i.c.v.) infusions of NPY are known to produce anxiolytic-like effects. Less is known about the contributions of lateral septal NPY to anxiety. In Experiment 1, the effects of NPY (1.5µg/side) infusions into the lateral septum were investigated in three animal models of anxiety: the elevated plus-maze, novelty-induced suppression of feeding, and shock-probe burying tests. We also examined the role of the NPY Y1 receptor by co-infusing NPY with the Y1 antagonist BIBO 3304 (0.15µg/side, 0.30µg/side). In the elevated plus-maze test, lateral septal NPY did not affect rats' open arm exploration, the index of anxiety in this test. In the novelty-induced suppression of feeding test, lateral septal NPY decreased the latency to consume a palatable snack in a novel (but not familiar) environment, suggesting NPY-induced reductions in anxiety independent of increases in appetite. This anxiolysis was blocked by co-infusions of BIBO 3304 (0.30µg/side). Lastly, although lateral septal NPY produced anxiolytic-like decreases in shock-probe burying, this effect was not blocked by co-infusions of BIBO. In summary NPY in the lateral septum produces anxiolytic-like actions in the

novelty-induced suppression of feeding and shock-probe burying tests but not in the elevated plus-maze. Further, it appears that distinct NPY receptors differentially contribute to NPY-mediated anxiolysis in a test specific manner."

Funding: Funded by NSERC

Title: Monkey frontal cortex reflects the time course of changing evidence for reach decisions

Authors: David Thura¹, Paul Cisek²

Affiliation: 1-2 University of Montreal

Abstract: "Recent neurophysiological studies have shown neural correlates of decision-variables in the same regions that are known to be involved in the sensorimotor control of movement. Do these variables simply ""spill into"" the sensorimotor system or is the sensorimotor system directly involved in making the choice? In the present study, we hypothesize that the sensorimotor system determines the commitment to a choice. A monkey performed the "tokens" task, beginning each trial by moving a handle into a central circle in which 15 small tokens were randomly arranged. Next, the tokens began to jump, one-by-one every 200 ms, from the central circle to one or the other of two peripheral target circles. The monkey's task was to move the cursor, as soon as he felt sufficiently confident, to the target that he believed would ultimately receive the majority of the tokens. Among a population of 148 dorsal premotor and prefrontal cortex cells, 31 showed a build-up of activity and reached a fixed threshold at the time, estimated from behavior, at which the monkey committed to his choice. Prior to this, the activity reflected the profile of evidence presented to the monkey. Consistent with behavioral data, preliminary analyses show that neural activity at a given moment is not significantly influenced by the information presented earlier in the trial. Our results suggest that neural activity in frontal cortex combines current sensory information provided by the environment with a growing urgency signal, and decisions are made when this quantity reaches a threshold."

Funding: CIHR, CFI, EJLB Foundation, FYSSSEN Fellowship to DT

Title: Prefrontal-dependent memory is altered in the offspring of rats exposed to a viral mimetic on gestational day 15.

Authors: John Howland¹, Brittany Cazakoff², Ying Zhang³, Quentin Greba⁴

Affiliation: 1-4 University of Saskatchewan

Abstract: "Infection during pregnancy (i.e., prenatal infection) increases the risk of psychiatric illnesses such as schizophrenia and autism in the adult offspring. Recent studies using rats revealed behavioral and neuropathological alterations consistent with impaired functioning of prefrontal cortex. The present experiments examined the effects of prenatal infection on two forms of prefrontal-dependent learning and memory in rats: object-in-place recognition memory and the extinction of classically conditioned fear. Pregnant Long Evans rats were exposed to the viral mimetic polyinosinic:polycytidic acid (PolyI:C; 4 mg/kg; i.v.; gestational day 15). Offspring were tested for recognition memory using three different spontaneous tests (object, object-place, and object-in-place recognition). Fear conditioning and extinction were assessed using a standard protocol that included five pairings of an auditory conditioned stimulus with a footshock on day one. On three subsequent days, 20 conditioned stimuli were presented in the absence of footshock to assess extinction of condition fear. Regardless of treatment, young adult offspring showed intact object and object-place recognition memory (discrimination ratios > 0.15). Interestingly, PolyI:C offspring showed significantly impaired object-in-place memory (saline treated = 0.19; PolyI:C = 0.00). No effect of PolyI:C treatment was observed during fear conditioning or initial fear expression. However, PolyI:C-treated male rats showed evidence of impaired extinction learning (particularly to the context) on subsequent test days. The present results suggest prenatal infection during mid/late gestation selectively impairs types of learning and memory that depend on the prefrontal cortex. Therapeutics targeted at reversing these changes may be useful in the treatment of patients with neurodevelopmental psychiatric disorders."

Funding: CIHR, NARSAD, SHRF.

Title: Disentangling ventral striatal processing through oscillations, spike timing, and behaviour in the rat

Authors: Matthijs (Matt) van der Meer¹

Affiliation: 1- University of Waterloo

Abstract: "The ventral striatum (VS) is a central node in brain networks that mediate the impact of behaviourally relevant events and cues. This includes (1) a role in learning from feedback, commonly thought to occur through prediction error signals from midbrain dopamine neurons, and (2) a role in the performance of certain actions, particularly those involving overcoming effort, attribution of incentive salience to cues, or flexible approach. Consequently, dysfunctions of

ventral striatal processing have been implicated in a range of disorders such as addiction, obsessive-compulsive disorder, and depression; however, the wide range of functions attributed to VS, as well as its complex and heterogeneous organization, make it difficult to disentangle its specific contributions in these and other instances. Recording from the rat ventral striatum, van der Meer, Redish and colleagues found several properties that shed new light on the organization of VS processing. In particular, low and high gamma oscillations within VS had different relationships to behaviour, task events, and spiking activity in putative fast-spiking interneurons. Furthermore, putative medium spiny neurons with anticipatory "ramp" activity showed theta phase precession as animals approached reward sites. Here, I argue that these findings provide a valuable toolkit for dissecting VS processing. For instance, in dual-structure recordings, LFP and spike timing phenomena allow on-line identification of VS ensembles that receive known inputs, such as from the hippocampus (HC). Comparison of HC afferent activity with VS activity can then reveal the processing applied to these inputs, at fine timescales during behaviour. "

Title: Target profitability is represented in the monkey superior colliculus during visuosaccadic foraging

Authors: Michael Dorris¹, Janis Kan²

Affiliation: 1-2 Queen's University

Abstract: "Behavioural choices of animals as they acquire resources in the wild are well characterized by foraging theory; however, the neural mechanisms underlying these behaviours are not well understood. Rhesus monkeys performed a novel visuosaccadic foraging task while we recorded the activity of single neurons in the intermediate layers of the superior colliculus (SCi) which have a well characterized role in saccade planning and preparation. We hypothesized that target profitability - the measure of value in foraging theory - is represented in the SCi. Visual Foraging Task: Monkeys harvested coloured dots representing prey items by fixating them for a pre-specified handling time. On each trial, multiple prey are presented, sharing identical physical attributes except that each was one of three colours. All prey of the same colour shared the same profitability [Profitability = reward magnitude (ml)/handling time (s)]. With limited foraging time, monkeys could maximize reward intake by selecting prey in descending order of profitability. We computed a behavioural index of relative profitability, which compared the order with which monkeys chose prey of each colour, and compared it to concomitant SC activity attributed to the

prey in the neuron's response field. We found that relative profitability of the response field prey was reflected in the level of SCi activity. Profitability information was present in SCi activity throughout the extended fixation period whereas the goal of the next saccade was represented just prior (300-100ms) to the saccade itself. Together, our results highlight the prominent role of profitability in shaping peri-saccadic SCi activity."

Funding: CIHR

Title: Effects of Aging and Selective Attention on the Time Course of Auditory Perceptual Organization

Authors: Graham Raynor¹, Ingrid Johnsrude²

Affiliation: 1-2 Queen's University

Abstract: A primary complaint of older adults is that they cannot understand speech in situations with competing sounds such as voices or music, a task which depends on the ability to perceptually segregate different sound sources. In this study, we compared younger and older adults using a rhythmic-deviant detection task. Accurate performance on this task has been correlated with perceiving the tone pattern as a whole as opposed to perceptually segregating the A and B tones, and therefore provided a measure of how listeners perceptually organized repetitive patterns of two tones (ABA-ABA-) over time (Carlyon et al. 2010). The tones were separated by either 6 or 8 semitones, which differentially biased perceptual organization. We used two task conditions with different attentional demands: the tone patterns were either played continuously over a block or each trial was preceded by 5 seconds of silence. Previous research has shown that when silence precedes the stimuli, participants initially perceive the tone pattern as a whole and 'build up' a segregated organization of the tones over time (Cusack, Deeks, Aikman, & Carlyon 2004). The present study replicates these previous findings in both younger and older adults. While there is no main effect of age group on accuracy, there is an interaction with task condition and semitone separation, indicating that the groups have different patterns of perceptual organization depending on the attentional demands. Higher accuracy rates over time suggest that older adults tend to have lengthier 'build up' periods, which could account for their speech comprehension deficits.

Funding: Queen's University CoNCH Lab

Title: Target profitability is represented in the monkey superior colliculus during visuosaccadic foraging

Authors: Michael C. Dorris¹, Janis Y. Kan²

Affiliation: 1-2 Queen's University

Abstract: "Behavioural choices of animals as they acquire resources in the wild are well characterized by foraging theory; however, the neural mechanisms underlying these behaviours are not well understood. Rhesus monkeys performed a novel visuosaccadic foraging task while we recorded the activity of single neurons in the intermediate layers of the superior colliculus (SCi) which have a well characterized role in saccade planning and preparation. We hypothesized that target profitability - the measure of value in foraging theory - is represented in the SCi. Visual Foraging Task: Monkeys harvested coloured dots representing prey items by fixating them for a pre-specified handling time. On each trial, multiple prey are presented, sharing identical physical attributes except that each was one of three colours. All prey of the same colour shared the same profitability [Profitability = reward magnitude (ml)/handling time (s)]. With limited foraging time, monkeys could maximize reward intake by selecting prey in descending order of profitability. We computed a behavioural index of relative profitability, which compared the order with which monkeys chose prey of each colour, and compared it to concomitant SC activity attributed to the prey in the neuron's response field. We found that relative profitability of the response field prey was reflected in the level of SCi activity. Profitability information was present in SCi activity throughout the extended fixation period whereas the goal of the next saccade was represented just prior (300-100ms) to the saccade itself. Together, our results highlight the prominent role of profitability in shaping peri-saccadic SCi activity."

Funding: CIHR

Title: Luman/CREB3 recruitment factor (LRF) deficiency affects the hypothalamic-pituitary-adrenal axis and prolactin-mediated maternal responsiveness

Authors: Ray Lu¹, Amanda Martyn², Elena Choleris³, Daniel Gillis⁴, John Armstrong⁵, Patricia Turner⁶, Genqing Liang⁷, Kimberly Cai⁸

Affiliation: 1-8 University of Guelph

Abstract: "The hypothalamic-pituitary-adrenal (HPA) axis is attenuated at parturition to prevent detrimental effects of glucocorticoid secretion on lactation and maternal responsiveness. In both

processes, prolactin plays a pivotal role, but the underlying mechanism is unclear. Here we report the first knockout mouse line of Luman/CREB3 recruitment factor (LRF) that has a severe maternal behavioral defect. LRF^{-/-} females lacked the instinct to tend pups; 80% of their litters died within 24-hrs, while most pups survived if cross-fostered. Prolactin levels were significantly repressed in lactating LRF^{-/-} dams, with glucocorticoid receptor (GR) signaling markedly augmented. In cell culture, LRF colocalizes with the known GR repressor, NRIP1/RIP140, and inhibits transcriptional activation by GR. Furthermore, administration of prolactin or GR antagonist RU486 restored maternal responses in the mutant females. We thus postulate that LRF plays a critical role in the attenuation of the HPA axis through repression of glucocorticoid stress signaling during parturition and the postpartum period. These knockout mice may serve as a unique model to study the connection between neuroendocrine stress responses and postpartum depression."

Funding: Canadian Institutes of Health Research

Poster Category H : Ethics, Society and Policy

Title: Evidence-based ethics in imaging genetics: Is the image clear?

Authors: Nicole Palmour¹

Affiliation: 1- Neuroethics Research Unit, IRCM

Abstract: Modern neuroimaging innovations have spurred discussion about current and future ethical uses of translational neuroscience within healthcare and beyond. Imaging genetics as currently practiced is an experimental strategy using neuroimaging technology to interrogate biological processes in individuals with distinct molecular genetic variation, which in turn might mediate individual differences in behavior and associated risk for neuropsychiatric disorders. The data from imaging and genetics diagnostics can potentially be combined to improve pre-symptomatic assessment of individuals having a large impact in the treatment and management of these patients and the rationed healthcare system. This poster presents a review of the literature in both neuroimaging and genetics, along with imaging genetics to identify the salient ethical issues at play in this emerging field. Ethical issues in neuroimaging and genetics include stigma, privacy, autonomy, beliefs and attitudes, resource allocation, translation and public understanding. We address whether the ethical issues have been sufficiently addressed within each domain and whether the combination of technologies changes the ethical topography. Literature searches conducted in 2008 in imaging genetics and ethics yielded no matching articles (Tairyan, 2009) however the lack of synthesized data does not mean that ethical issues are unattended. Accordingly we will address the contours of the issues and highlight the gaps in ethical coverage. Reference: Tairyan K, Illes J: Imaging genetics and the power of combined technologies: a perspective from neuroethics. *Neuroscience* 2009, 164(1):7-15.

Funding: Canadian Institutes of Health Research, States of Mind: Emerging Issues in Neuroethics.

Poster Category: IBRO Student

Title: The effects of eating *Dennettia tripetala* fruit on locomotor and anxiety-related behaviours in CD1 mice

Authors: Daniel Ikpi¹, Richard Brown²

Affiliation: 1-2 Dalhousie University

Abstract: "*Dennettia tripetala* fruit induces a mild stimulating effect when ingested; it is used to spice foods in Southern Nigeria. It is believed to reduce social anxiety and increase sociability. The present study investigated the stimulatory effects of *D. tripetala* on locomotor and anxiety-like behaviours in CD1 mice in the open field, elevated plus maze and light-dark transition box. Thirty singly housed male CD1 mice were divided into 3 groups of 10. The Control group were fed normal rodent pellets. The Low-dose group were fed a 10% *D. tripetala* diet (10g of *D. tripetala* mixed with 90g of rodent pellets). The High-dose group were fed 15% *D. tripetala* diet (15g of *D. tripetala* mixed with 85g of rodent pellets) daily for 4 weeks before testing. Both doses of *D. tripetala* reduced the number of line crosses and the frequency of centre square entries in the open field while the high dose reduced the number of line crosses in the light-dark box and the frequency of rearing in the open field. The low dose of *D. tripetala* increased the time in the open arms in the elevated plus maze, while the high dose decreased the number of stretch attend postures in the light-dark box. The results indicate that chronic ingestion of *D. tripetala* reduced locomotor and exploratory behaviour in CD1 mice at both doses while the low dose may have an anxiolytic effect. Future studies will examine the effect of *D. tripetala* on motor learning, motor control and sociability."

Title: Role of Calcium-stimulated Adenylate cyclase 1 (AC1) in the targeting of the Corticospinal tract and in regeneration after a lesion.

Authors: Hanane Nait Taleb Ali¹, Sophie ., Scotto-Lomassese ², Isabelle Dusart ³, Mohamed Bennis⁴, Patricia Gaspar⁵

Affiliation: 1,4 Faculté des Sciences Semlalia de Marrakech 2,5- INSERM U839 3- UPMC

Abstract: The mammalian CST, arising from layer V neurons in the Somatosensory and motor cortex, is the only direct cortical pathway to the spinal cord. Guidance molecules such as Ephrins and Slits are involved at various decision points for guiding the CST axons. However, previous

analyses of the CST guidance defects in mutant mice lacking these molecules have suggested that there are other molecules involved in CST axon guidance that are yet to be identified. The role of the calcium-stimulated AC1 has been revealed in the fine patterning of the retinal maps. Because the AC1 gene is highly expressed in layer V cortical neurons during the development of the CST, we questioned whether AC1 is involved in the targeting of the CST and in regeneration after a lesion. We used the barrelless (brl) mouse strain which carry a spontaneous mutation of the AC1 gene and investigated the projections of the CST in the cervical spinal cord using anterograde tracers. To investigate the effects of AC1 on axon regeneration in vivo, the brl mice were tested in a model of spinal cord injury. Our study shows an increase in the number of contralateral and ipsilateral projections in the cervical spinal cord in brl mice. Moreover, the density of labelled neurons in the motor cortex is significantly higher in the brl mice. However no major abnormalities of the CST were detected. The brl mice showed greater functional improvement compared to control mice. Increase in the number of corticospinal projections may explain the enhanced functional recovery after a spinal cord injury.

Title: Generating a Proteomic Profile of Neurogenesis

Authors: Shaun Garnett¹, Putuma Gqamana², Susan Kidson³, Jonathan Blackburn⁴

Affiliation: 1-4 University of Cape Town

Abstract: Neurogenesis is the production of new neurons from neural stem cells (NSC). NSC can form neurons, astrocytes and oligodendrocytes, their fate being determined by their environment. Adult neurogenesis discovered in 1965 by Altman is ongoing in two adult neurogenic niches, whose environments are controlled by astrocytes. Neurogenesis can be induced after brain injury in a very limited manner through the release of growth factors by astrocytes in the area surrounding the injury. Traditional suspension cultures of NSCs from foetal brain tissue produce neurospheres, spherical agglomerations of proliferating and differentiating NSCs. A novel human foetal NSC (fNSC) line (CB660) produced by Sun et al 2008 is cultured as an adherent homogenous NSCs, allowing detailed quantitative study of isolated NSCs. In this study we intend performing a quantitative proteomic comparison of fNSC and NSC derived from embryonic and induced pluripotent stem cells to generate a proteomic profile of neural stem cells. The cells lines are then to be differentiated and the signaling pathways responsible for differentiation are to be mapped to signaling pathways in the public databases. The effects of a variety of neurogenic substances can

then be tested against this proteomic pathway model. Regenerative neural stem cell therapy endeavours to either activate neurogenesis in endogenous NSC or transplant in vitro cultured NSC into damaged areas of the brain. The aim of this study is to produce a neurogenic model which can be used to assess in vitro NSCs and neurogenic drugs intended for use in regenerative brain medicine

Funding: National Research Foundation (NRF), Council of Scientific and Industrial Research (CSIR), International Brain Research Organisation (IBRO)

Title: Intracerebral administration effects of MK-801 and (-) nicotine on Glu and GABA extracellular concentrations in PPN from hemiparkinsonian rats

Authors: Lisette Blanco Lezcano¹, Lourdes Lorigados Pedre², Ma. Elena González Fraguera³, Teresa Serrano Sánchez⁴, Nancy Pavón Fuentes⁵

Affiliation: 1-5 International Center of Neurological Restoration

Abstract: "Although the pharmacological manipulation of the glutamatergic and cholinergic system has been studied in animal models of Parkinson's disease (PD), only some results published take into consideration the aminoacid neurotransmitter concentration in pedunculopontine nucleus (PPN) under effects of the intracerebral administration cholinergic and glutamatergic drugs. The present work studied the changes in the glutamate (Glu) and γ -aminobutyric acid (GABA) extracellular concentration (EC) in the PPN from hemiparkinsonian rats. The rats were locally infused by MK-801 (10 μ mol/L) or (-) nicotine (10mM) solution by means of microdialysis cerebral technique. The biochemical studies were carried out through high performance liquid chromatography coupled to fluorescence detection. The MK-801 infusion induced a significant decrease of Glu ($p < 0.01$) and GABA ($p < 0.01$) EC in PPN. On the other hand the (-) nicotine infusion induced a significant increase of Glu ($p < 0.001$) and GABA ($p < 0.001$) EC in PPN from hemiparkinsonian rats. The local blockade of the NMDA receptors by MK-801 infusion facilitates the interaction between Glu and their metabotropic receptors that take part of presynaptic inhibition mechanisms and interfere with the neurotransmitters release process. Meanwhile the (-) nicotine infusion sums the effects of nicotinic receptors activation with the glutamatergic and gabaergic neurotransmission changes produced in the PPN in parkinsonian condition. The cholinergic and glutamatergic drugs infusion in PPN impose a new adjust to the neurotransmission at this level that is aggregated to the neurochemical changes associated to the

dopaminergic denervation."

Funding: Research Project sponsored by International Center of Neurological Restoration, Havana, Cuba

Title: A Descriptive Study of Cerebro-Vascular Accidents in Cameroon

Authors: Marius Trésor CHIASSEU MBEUMI¹

Affiliation: 1- University of Alexandria - Agence Universitaire de la Francophonie

Abstract: A Cerebrovascular accident or stroke is a rapidly installing cerebral deficit of vascular origin lasting more than 24 hours. It represents the second cause of death in the world, third world countries being heavily affected. Our aim in this study was to describe the cerebrovascular lesions (type, localization, size) in Cameroon as well as the age and the sex of the patients. The study was retrospective using brain CT-scan and MRI results of 50 stroke patients admitted in two health centers in Douala. The results indicate that 74% of patients were aged above 50, the 51-60 years being the most affected age group. Men were the most affected with 64% of cases reported. Ischemic stroke represents 60% of cases against 40% for hemorrhagic stroke. With 43.3% of cases the sylvian territory is the most affected site in ischemic stroke, whereas the temporal lobe is the most affected by hemorrhagic stroke with 35% of cases. The median of ischemic and hemorrhagic lesions was respectively 0.97 cm³, and 26.98 cm³. Both hemorrhagic strokes and lacunar infarcts seem to be more important in Cameroon. In addition, the chances of recovery are smaller in case of hemorrhagic stroke since the lesions are bigger than the ones of ischemic stroke. We observed some differences in the results of the two hospitals which may be due to the use of different imaging techniques, the MRI being known to be more sensitive than CT-scan in the detection of acute stroke lesions.

Title: Galectin-3 participates in the Cuprizone-induced demyelination

Authors: Hernan Hoyos¹, Laura Pasquini², Gabriel Rabinovich³

Affiliation: 1-2 Dept. Biological Chemistry. School of Pharmacy and Biochemistry. University of Buenos Aires 3- IBYME-CONICET

Abstract: Galectin-3 is widely spread among different types of cells and tissues. Through specific interactions with a variety of intra- and extracellular proteins galectin-3 is involved in different physiological and pathophysiological processes. We showed that galectin-3 is up-regulated during

oligodendroglial (OLG) differentiation. Moreover, recombinant galectin-3 treatment promotes OLG maturation evidenced by an increased number of MBP+cells and a decrease in A2B5+cells. Accordingly, conditioned media from galectin-3-expressing, but not galectin-3-deficient(Lgals3-/-) microglia, successfully promoted OLG differentiation. Morphometric analysis showed a significant decrease of myelinated axons, myelin turns and g-ratio in the striatum of Lgals3-/- vs WT mice. Cuprizone(CPZ) induces reproducible demyelination in mouse brain. The extent of the CPZ-induced demyelination is characterized by the loss of OLG, myelin sheath degeneration and recruitment of microglia and astrocytes to the lesioned area. We evaluated the susceptibility to CPZ-induced demyelination of 8-week-old-Lgals3-/- vs WT mice. Lgals3-/- mice displayed a decreased myelination evaluated by the number of APC+cells and MBP immunostaining in striatum, corpus callosum(CC), cingulum(CG) and cortex(CX) accompanied by an increase in astroglial activation in CG and microglial response in CC and CG. An increased susceptibility to CPZ-induced demyelination was obtained in Lgals3-/- mice determined by the evaluation of thick horz (average MBP immunostaining) in CX, astroglial response in CC, and APC+ cells in CG at 6 weeks and in eryochrome staining at 4 weeks of treatment. Strikingly, a decreased microglial response to CPZ diet was observed in Lgals3-/- vs WT mice. Our results indicate that galectin-3 participates protecting to the OLG cells against CPZ-induced demyelination.

Funding: IBRO

Title: CG6115, a novel gene involved in neurodegeneration in *Drosophila melanogaster*

Authors: Guillermo Bernabó¹, Carolina Rezával², L. Amaranta Avendaño³, D. Lorena Franco⁴, Santiago Werbajh⁵, M. Fernanda Ceriani⁶

Affiliation: 1-6 Fundación Instituto Leloir

Abstract: *Drosophila melanogaster* has orthologs of about 64% of all unique genes known to be involved in neurodegeneration in humans. To identify novel genes related to this process a misexpression screen has been carried out in our laboratory. It consisted in examining locomotor behavior in young and aged flies. Flies that showed a progressive loss of rhythmic activity could reveal novel genes involved in neurodegenerative mechanisms. One of the mutants, 100B, which, as a result of the P element insertion, shows a striking downregulation of CG6115 expression. CG6115 encodes a gene of unknown function. BLAST analysis of the putative protein encoded by this gene suggests that it belongs to the Complex 1 LYR protein superfamily. Proteins in this family

have been identified as a component of the higher eukaryotic NADH complex. Homozygous 100B mutants cannot progress beyond second instar larvae and have an abnormal feeding behavior. To address CG6115 relevance in different tissues we employed different GAL4 lines to express an RNAi directed towards CG6115. Muscle related GAL4s were found to mimic homozygous mutant behavior in larvae. Interestingly, adult flies expressing the RNAi specific to CG6115 in pigment dispersor factor positive neurons (a key circuit in the control of rest-activity cycles), when screened in locomotor behavior, showed progressive arrhythmicity and period lengthening. In addition, flies expressing CG6115 RNAi under the glass Multimer Reporter promoter show severe eye defects in approximately 23% of the individuals. Taken together these results we propose CG6115 could play a role in neurodegeneration.

Funding: Consejo Nacional de Investigaciones Científicas y Técnicas. Agencia Nacional de Promoción Científica y Tecnológica. National Institutes of Health.

Title: Identification of mutations in the microRNA pathway and their participation in the etiology of severe neuropsychiatric disorders

Authors: Patricia Bolaños¹, Henriette Raventos²

Affiliation: 1-2 University of Costa Rica

Abstract: Schizophrenia is a severe neuropsychiatric disease that has a clear genetic component in its etiology. Nevertheless, the genes involved have not yet been unequivocally identified and only explain a small percentage of the cases. Therefore, we propose that there is another genetic mechanism participating in the development of this disease. During neurodevelopment, protein regulation becomes crucial for the formation of the correct neuronal projections and synapses, and is also involved in the plasticity of mature connections. The precise regulation of the amount of protein in a cell establishes its potential for interaction with other neurons and with the local environment. Within the complex scenario of gene expression regulation, microRNAs have been implicated in the post-transcriptional regulation of messenger RNA (mRNA). This mechanism of protein synthesis regulation has been linked to the correct formation of synapses, which translates into the ability to learn and other higher cognitive functions. Anomalies in the establishment of the correct proteome have also been linked to diseases. This suggests a possible mechanism for disease development based on changes in patterns of gene expression, either by variations in the amount of microRNAs or through interactions with their target mRNA. This

research project focuses on identifying mutations in candidate microRNA genes and their target mRNAs. The candidate genes will be established by an in silico approach, and their sequence will be analyzed in a collection of subjects already recruited in the Central Valley of Costa Rica in a NIMH funded study to localize schizophrenia susceptibility genes.

Funding: University of Costa Rica

Title: PSA-NCAM dependent synaptic remodelling in an experimental model of depression and its treatment with fluoxetine

Authors: Maríía Fernanda Podestá¹, Martín Gabriel Codagnone², Juan Ramiro Lorenzo Lopez³, Margarita López⁴, Alicia Brusco⁵, Silvia Wikinski⁶, Analía Reinés⁷

Affiliation: 1-3,6, 7 Instituto de Investigaciones Farmacológicas (ININFA, CONICET-UBA) 4,5 Instituto de Biología Celular y Neurociencias “Prof. E. De Robertis”

Abstract: Dysfunction of hippocampal plasticity and dendritic atrophy of hippocampal CA3 neurons have been involved in the pathophysiology of depression and the therapeutic action of antidepressants, probably related to excessive glutamate release. Based on our previous results showing decreased hippocampal synaptophysin (SYN) and PSD-95 expression in animals exposed to the learned helplessness (LH) paradigm, an experimental model of depression, we studied the characteristics of the structural remodelling of hippocampal CA3 synapses in the LH paradigm and its pharmacological treatment. Electron microscopy studies in CA3 synapses of LH animals revealed increased synaptic cleft width and number of vesicles morphologically altered. LH animals also showed decreased CA3 immunostaining for NCAM and PSA-NCAM, cell adhesion molecules implicated in plasticity. Interestingly, fluoxetine chronic treatment in LH animals reversed synaptic morphology alterations, strongly reduced NCAM and selectively increased PSA-NCAM levels in CA3. In vitro we studied synaptic changes induced by glutamatergic hyperstimulation in hippocampal neurons in culture in a condition that causes atrophy without neuronal death. Neurons exposed to glutamate presented reduced MAP-2, NCAM and PSA-NCAM immunostaining. Whereas PSD-95 and SYN puncta number diminished, individual puncta size was not modified for PSD-95 and was increased for SYN. Therefore, excessive neuronal exposure to glutamate induced synaptic changes in vitro that resemble those observed in LH group. Our results support the hypothesis that glutamatergic hyperactivity in LH animals could reduce cell adhesion molecule expression compatible with plastic and synaptic connectivity alterations, and

fluoxetine action could involve PSA-NCAM dependent synaptic remodelling that might lead to neuronal connectivity normalization.

Funding: "Grants UBACYT 2008-2010 and 2010-2010; PIP 11220090100937."

Title: Non-classical estrogen apoptotic action in the anterior pituitary gland

Authors: Sandra Zárate¹, Gabriela Jaita², Jimena Ferraris³, Guadalupe Eijo⁴, Daniela Radl⁵, Veronica Zaldivar⁶, María Laura Magri⁷, Daniel Pisera⁸, Adriana Seilicovich⁹

Affiliation: 1-4, 6-9 Instituto de Investigaciones en Reproducción, University of Buenos Aires School of Medicine 5- University of California Irvine, Department of Microbiology and Molecular Genetics

Abstract: In the adult female rat, estrogens are thought to be key players in the cyclic processes of proliferation and death that anterior pituitary cells undergo along the estrous cycle. Although estrogens are known to exert a trophic stimulus on anterior pituitary mitotic activity, the rate of anterior pituitary cell apoptosis has a peak at proestrous, when estrogen levels are the highest. Estrogen actions in this gland are exerted through both classical and non-classical mechanisms of action, the latter involving the activation of estrogen receptors (ERs) associated to the plasma membrane. We have shown that a membrane-impermeant 17beta-estradiol conjugate induces a rapid apoptotic action in anterior pituitary cells, lactotropes and somatotropes from ovariectomized (OVX) female Wistar rats. This effect is completely abrogated by ICI 162,780, the pure antagonist of ERs, suggesting the involvement of classical ERs associated to the plasma membrane. Also, an estrogen-dendrimer conjugate, whose action is restricted to sites near the cell surface, has a rapid apoptotic effect in anterior pituitary cells. The identity of membrane ERs is still a matter of controversy. In cycling rats, we have detected a higher number of lactotropes and a lower number of somatotropes expressing a membrane form of the classical ERalpha (mERalpha) at proestrous than at diestrus. In OVX rats, the acute treatment with E2 increased the number of mERalpha-expressing lactotropes whereas progesterone treatment abrogated this estrogenic effect. We are currently working on the characterization of membrane-associated estrogen receptor/s and intracellular signaling pathways involved in estradiol proapoptotic action in anterior pituitary cells

Funding: Work supported by grants from Agencia Nacional de Investigaciones Cientificas y Tecnologicas, CONICET and the University of Buenos Aires, Argentina.

Title: "The NEUROINFLAMMATORY RESPONSE IN THE BRAIN AND EFFECT OF MEF2 ON MEDIUM SPINY NEURONS; The Implications in Parkinsons Disease. "

Authors: Nwigwe Chikaodonaka¹, Gordon W. Arbuthnott²

Affiliation: 1- Abia State Univeristy 2- Okinawa Institute of Science and Technology Promotion Corporation

Abstract: The microglial has been recognized as the brains intrinsic immune system. The term neuroinflammation refers to more chronic sustained injury when the responses of microglial cells contribute to and expand the neuro destructive effect thereby worsening disease processes. In parkinsons disease, the resulting lack of straital dopamine increases the activity of indirect straitofugal neurons and decreases the straital output along the direct route. More recently, it has been shown that sustained pertubation in straital dopamine levels alter the density of spines and synapses. In this research, the effect of reduction of MEF-2 expression on medium spiny neurons (MSN) was explored. Chronic elevation of straital dopamine level with MEF2 increased MSN spine density whereas dopamine depleting lesions mimicking parkinson's disease tirgged loss of MSN spines and asymmetric synapses.

Title: CRTAM transcriptional regulation in cerebellum and its possible role in Bergman glial cells

Authors: Karla María Pérez Toledo¹, Crystelle Alicia Rojas Marquez², Arturo Ortega Soto³, Vianney Ortiz Navarrete⁴, Esther López-Bayghen Patino⁵

Affiliation: 1- Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional 2-5 CINVESTAV, MEXICO

Abstract: Class-I MHC-restricted T-cell associated molecule (CRTAM), from Nectin-like adhesion family, is highly expressed in activated CD8-T and NKT cells. CRTAM is also involved in early cell-cell and epithelial cell-cell adhesion and its mRNA is highly expressed by Purkinje cells of the cerebellum. Immunocytochemistry revealed that CRTAM is mainly localized in Purkinje and granule cells and Necl-2, known to bind CRTAM, is also expressed in the cerebellum. Bergmann glial cells (BGC) enwrap the Purkinje cell synapses; in development Bergmann fibers associate with migrating granule cells and with Purkinje cells in the adult cerebellum. We propose that

CRTAM/Necl-2 binding may contribute to neuron-neuron or neuron-glia interactions in normal or pathological conditions in cerebellum. This why, we try to understand the possible role and transcriptional regulation of CRTAM in cerebellum, particularly in BGC. Using primary cultures obtained from chick cerebella, we detected the CRTAM messenger in brain and cerebellum (qRT-PCR). In terms of cellular placement, CRTAM co-localized with KBP, the main BGC plasma membrane marker (confirmed by immuno-detection in membrane extracts). qRT-PCR assays were performed on brain and cerebellum from 10, 14 and 18 days old chicks, a differential expression of CRTAM during chicken embryonic development was recorded with higher expression in early stages, diminishing as embryonic development proceeds. In BGC stimulated with glutamate, CRTAM expression decreases, probably reflecting changes in the CRTAM promoter activity. In conclusion, CRTAM expression in BGC may be regulated along development and because the glutamate exposure resulting from neuron-glia interactions.