IPN Retreat 2013
Abstract Booklet

Contents

Poster Presentation Sessions

Posters will be presented at the following times:

Session A posters:

September 19th: 1:00 pm to 3:00 pm

September 19th: 5:15 pm to 6:15 pm

Session B posters:

September 19th: 6:15 pm to 7:15 pm

September 20th: 12:30 pm to 2:30 pm

The Abstract booklet is only available in this electronic format. However a printed version of the poster titles will appear on a couple of poster boards, along with the floor plans, to help you find any specific posters you are interested in.

Instructions for Poster Presenters

Putting up your posters

Session A posters must be put up between 8:30 and 10 am on September 19th – before 9 am if you are coming to the lab Notebook Workshop.

Session B Posters should be put up before 3:00 pm on September 19th.

Presenting your posters

You will present your posters during two sessions at the Retreat; one lunch time session and for an hour during the evening of the 19th. Of course, it's fine to leave the posters to grab some lunch. If you will be away for some time, you might want to leave a note saying when you're likely to be back.

Taking down your posters

All posters must be removed during the break between 4:00 pm and 4:15 pm on September 20th at the latest. Any posters left up after this time can be considered lost for good.

If, for any reason, you cannot present during the sessions you have been assigned, just leave a note on your poster and show up when you can. No further changes can be made to the schedule at this time.

Poster	Abstract authors	Abstract Title
A1	Jayabal, S.*, Watt., A.J.	Developmental abnormalities in the cerebellum of SCA6 mice
A2	*Robert Nahas, Magali Millecamps, Laura S Stone, Alfredo Ribeiro-da-Silva	Effects of pre-emptive running exercise on pain-related behaviours in a mouse model of neuropathic pain.
A3	Marie-Pier Girouard*, Madeline Pool, Alyson Fournier	Understanding the mechanism and the functions associated with the proteolytic cleavage of RhoA
A4	Juzwik, C.*, de Faria Junior, O., Bar-Or, A., Fournier, A.	Identifying microRNA regulators of neuronal viability and repair in multiple sclerosis.
A5	Ricardo Sanz*, Gino Ferraro and Alyson E. Fournier	Proteolytic cleavage of IgLON adhesion proteins by MMPs is involved in neurite outgrowth
A6	Marc-Andre Dery* and Andrea C. LeBlanc	Role of Mahogunin and USP3 in cytosolic PrP ubiquitination and Bax-mediated cell death
A7	Sarah Peters* & Andrea C. LeBlanc	E3 Ligase Hrd1 mediates Prion protein retrotranslocation in mammalian cells
A8	*Prateep Pakavathkumar, Jan-Eric Ahlfors, Andrea C. LeBlanc	Study of a novel Caspase-6 inhibitor for protection against axonal degeneration
A9	Micaela Das Gupta*, Emily Deane, Alina Ilie, John Orlowski and Anne McKinney	Characterization of Sodium/Proton Exchangers 6 and 9 in the Mouse Hippocampus
A10	Sonia Jego (*), Stephen D. Glasgow, Carolina Gutierrez Herrera, Mats Ekstrand, Jeffrey Friedman, Denis Burdakov3 & Antoine R. Adamantidis	Optogenetic dissection of the MCH system: implications for sleep-state modulation
A11	Daniel Morales*, Chris Law, Artur Kania	Spontaneous neural activity is not required for ephrin-A:EphA signaling in chick motor neuron axon guidance
A12	Ghorayeb, K.*, Brodeur, M.B., & Debruille, J.B.	Affordance and anterior N400
A13	Liam Crapper*, Carolina Gigek, Alpha Diallo, Gilles Maussion, Gustavo Turecki, Carl Ernst	Development of a Neuronal Model of Lesch-Nyhan Disease
A14	Florence Shahabi*, Colleen Manitt*, Alanna Grant, Stephanie Gallant, Harrod Ling and Cecilia Flores.	Autoradiographic quantification of dopamine D1 receptor expression in striatal regions of dcc haploinsufficient mice from early adolescence to adulthood
A15	Kangjoo Lee, Jean-Marc Lina, Jean Gotman, Christophe Grova	Stable brain network organization in sparse GLM reveals functional modular structure in resting-state fMRI

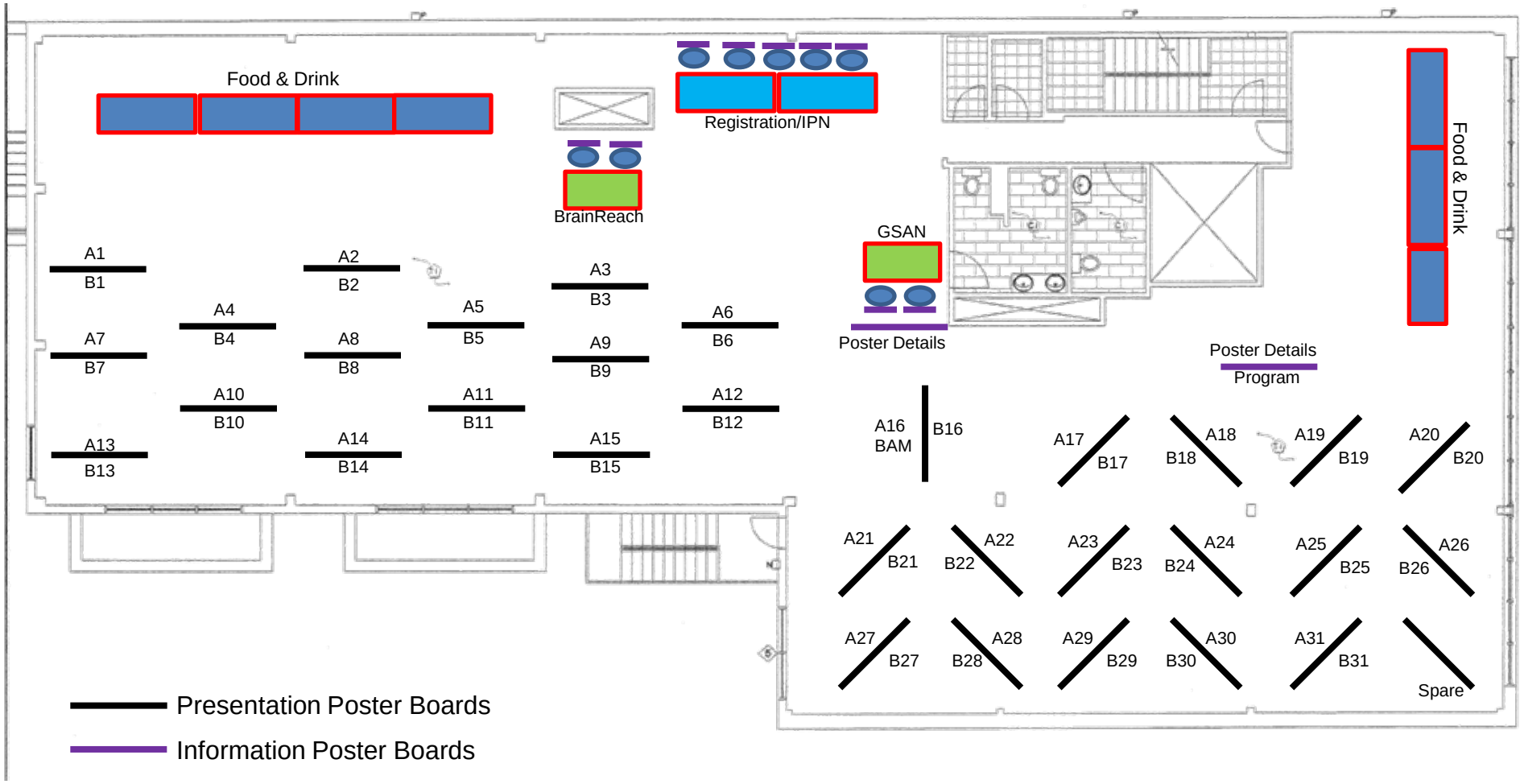
Poster	Abstract authors	Abstract Title
A16	Brain Awareness Montreal	Brain Awareness Montreal
A17	L. D. Liu*, C. C. Pack	Influence of surround suppression in cortical area MT on motion discrimination performance
A18	Adiel Mallik*, Mohammad Qasaimeh and David Juncker	Design and Fabrication of a Transparent Microfluidic Probe for Local Stimulation of Neurons
A19	*Altimimi, Haider F. Bailey, Nicole J. Stellwagen, David	The involvement of Tumor Necrosis Factor alpha in the neuronal response to stress
A20	Patricia Brown*, Mark Aurousseau, Hugo McGuire, Rikard Blunck, Derek Bowie	Heteromerization shapes the pore properties of kainate receptors
A21	Dawe, G. Brent*; Daniels, Bryan A.*; Musgaard, Maria; Andrews, Elizabeth; Biggin, Philip C.; Bowie, Derek	Covalent crosslinking of GluK2 kainate receptor dimer interface prevents full activation
A22	Marc Cuesta*, Nicolas Cermakian, Diane B. Boivin	Glucocorticoids induce phase shifts of peripheral circadian clocks in humans independently of the central clock
A23	Chaychi, Samaneh*; Chorfi, Sarah; Polosa, Anna; Jung, Suna; Dorfman, Allison L.; Yang, Xiaojuan; Chemtob, Sylvain; Lachapelle, Pierre	Oxidative-Retinopathies: Female Neonate Rats Handle Bright Light Better Than Hyperoxia, Males React Strongly To Both
A24	Martin Munz, Delphine Gobert, Jessie Poquérusse and Edward S. Ruthazer	Developing retinal ganglion cell axon arbors exhibit different dynamic branch behaviors and elaboration in response to correlated and asynchronous visual stimulation
A25	Brishna Kamal, Constance Holman and Etienne de Villers-Sidani	Shaping the aging brain: Role of auditory input patterns in the emergence of auditory cortical impairments
A26	Filip Liebsch*, Mark Aurousseau, Derek Bowie, and Gerd Multhaup	LIVE-IMAGING OF BACE1 SECRETION FROM HEK-CELLS AND PRIMARY NEURONS
A27	Marcio L. De Paula*, Qiao-Ling Cui, Shireen Hossain, Jack Antel and Guillermina Almazan	PTEN inhibition enhances myelination by amplifying IGF-1 signaling in rat and human oligodendrocyte progenitors
A28	Kathryn Vaillancourt*; Gang Chen; Gustavo Turecki	Differential DNA Methylation in Cocaine Addiction
A29	Michael Tibshirani*, Katie Mattina, Hongru Zhou, Wencheng Yang, Michael J. Strong, Lawrence Hayward, Heather Durham	Cytoplasmic accumulation of ALS-linked FUS/TLS alters posttranslational modifications of histones regulating gene transcription

Poster	Abstract authors	Abstract Title
A30	Sarah F. Rosen*, Jeffrey S. Mogil	Complete reversal of neuropathic and inflammatory mechanical allodynia in pregnant mice.
A31	Roberto A. Gulli*, Sebastien Tremblay, Nour Malek, Sylvain Williams, Antoine R. Adamantidis, Julio C. Martinez-Trujillo	Optogenetic manipulation of cortical and subcortical circuits in non-human primates
B1	Lalanne Txomin*, Oyrer Julia, Chung Andrew, Farrant Mark & Sjostrom Per Jesper	Synapse-specific expression of Cp-AMPARs in neocortical inhibitory neurons
B2	I.D. Blum*, L. Zhu, K.-F. Storch	The Dopaminergic Ultradian Oscillator
B3	Peter S.B. Finnie*, Karine Gamache, Johnna Perdrizet, & Karim Nader	Endogenous beta-amyloid regulates memory stability
B4	Erin Nuro* , Denise Cook, Emma V. Jones, Haider F. Altimimi, Edith Hanna, W. Todd Farmer, David L. Nelson, Joseph Rochford, David Stellwagen, Jean-Claude Béïque, Keith K. Murai	Investigating the role of Fragile X-related Protein 1 (FXR1P) in Brain Plasticity
B5	Falisha Karpati*, Chiara Giacosa, Virginia Penhune, Nicholas E.V. Foster, Krista L. Hyde	Long-term dance training changes gray matter structure
B6	Anastasia Sares*, Nicholas E.V. Foster, Kachina Allen, Krista L. Hyde	Pitch and time processing in speech versus music: effect of musicianship and attention
B7	*Ana Tryfon, Nicholas E.V. Foster, Tia Ouimet, Krissy Doyle-Thomas, Evdokia Anagnostou, Alan C. Evans, Lonnie Zwaigenbaum4, Krista L. Hyde	Brain and behavioral correlates of auditory-motor synchronization in children with autism spectrum disorder
B8	Anne Loeffler*, Marcel Brass, Jelle Demanet, Lize DeCoster, Dorit Wenke	Intentional binding and sense of responsibility: Temporal (un-)binding of actions and effects when inflicting pain on others
B9	NANTES J.C.*, WHATLEY B., YUSUF A., TAYLOR-SUSSEX R., & KOSKI L.	Intracortical Inhibition Alterations In Multiple Sclerosis
B10	Oestereich, Felix*; Bittner, Heiko; Weise, Chris; Hildebrand, Peter; Multhaup, Gerhard; Munter, Lisa	Amyloid beta peptide generation is determined by key residues within the Amyloid Precursor Protein transmembrane sequence

Poster	Abstract authors	Abstract Title
B11	JP. Landry*, S. Wiebe, D. Sheen, M. Pompeiano	Acute moderate hypoxia reduces the number of activated Hypocretin/Orexin neurons in chicken embryos.
B12	Zahra Shiri*, Charles Behr*, Shabnam Hamidi*, Rochelle Herrington, Pariya Salami, Maxime Lévesque,, Massimo Avoli	Neural network synchronization in the limbic system and its contribution to temporal lobe epilepsy
B13	Daina Crafa*	Transcultural Neuropsychiatry: Considerations for Clinical Research and the RDoC
B14	Mary O'Sullivan, Martin Lepage, Mathieu B. Brodeur, Bianca Lapierre*	Bank of Standardized Stimuli (BOSS)- the impact of normative variables and image modality on episodic memory.
B15	Tatiana Ruiz* Melissa Maguire Mathieu B. Brodeur	The effect of context on recognition of objects
B16	Adele Tufford*, Pierre Mattar, Alanna Watt, Michel Cayouette	The characterization of a novel subtype of photoreceptor cell in the mammalian retina
B17	Katarina Stojkovic*, Sanjeev K. Bhardwaj, Lalit K. Srivastava, Nicolas Cermakian	Fragmentation of circadian locomotor activity in the Sandy mouse model of schizophrenia
B18	Silke Kiessling* Nathalie Labrecque Nicolas Cermakian	The circadian clock in B16 melanoma cells controls tumor growth in vitro and in vivo.
B19	Karim Habbal*MSc, Vincent Joseph PhD	Anatomical Localization of Membrane Progesterone Receptors in Brainstem Respiratory Areas
B20	Lorena R. S. Almeida*, Rebecca A. Gruber, Jan Goldstein Elman, Nádja Negreiros, Guilherme Valença, Elen Beatriz-Pinto, Jamary Oliveira-Filho	Impact of freezing of gait on self-perceived balance confidence for functional activities in people with Parkinson’s disease
B21	Paul de Roos*, Krzysztof Nesterowicz, Mohammad Fereshtehnejad	Creative, curiosity driven research question generation and project development around Parkinson’s Disease, at an international Parkinson’s Disease Summer School, report on 4 years process improvement.
B22	Marcello Moccia*, Marina Picillo, Marianna Amboni, Roberto Erro, Katia Longo, Carmine Vitale, Roberto Allocca, Anna De Rosa, Giuseppe De Michele, Lucio Santoro, Giuseppe Orefice, Maria Teresa Pellecchia, Paolo Barone	Gender differences in non-motor symptoms in early, drug naive Parkinson’s disease
B23	Mathieu Gauvin*, M Hébert,, R.K. Koenekoop, J.M. Little, Jean-Marc Lina and Pierre Lachapelle	DWT Analysis of the Photopic Hill Reveals New Diagnostically Relevant Features of the Human ERG.

Poster	Abstract authors	Abstract Title
B24	Josephy, S.*, Aboulkassim, T., Maira, M., Pirvulescu, I., Menard, C., Quirion, R., Saragovi, U.	Identifying regulatory mechanisms of long term memory
B25	Karen Hei Man Fung* and Stefano Stifani	Role of RUNX1 in Glioblastoma Tumour-Initiating Cells
B26	Soheila Samiee*, Sylvain Baillet	A method for Measuring Cross-Frequency Phase Amplitude Coupling in Neural Oscillations
B27	Hibatulnaseer Nasir*, Stephanie Ding, André Laferrière, Terence Coderre	Inhibition of spinal PKM Zeta reverses persistent referred allodynia induced by injection of acidic saline in the rat thigh
B28	Cornea V.M *., Jong Y-J. I., Ribeiro-da-Silva A., O'Malley K.L. and Coderre T.J.	Involvement of spinal nuclear metabotropic glutamate 5 receptors (mGluR5) in persistent pain: anatomical and biochemical evidence
B29	*Peter Helfer Thomas Shultz Oliver Hardt Karim Nader	A Computational Model of Systems Memory Reconsolidation
B30	Jenea M. Bin*, Sarah-Jane Bull, and Timothy E. Kennedy	Netrin-1 function in myelin maintenance assessed using an oligodendrocyte precursor cell-organotypic slice culture transplant model
B31	S. G. Ricoult*, G. H. Thompson-Steckel, G. Ongo, J. P. Correia, T. E. Kennedy, and D. Juncker	Protein Digital Nanodot Gradients with Adjustable Reference Surfaces to Investigate Axonal Migration in Response to Nanopatterned Cues

3rd Floor: Crowley Arts Centre



A1

Developmental abnormalities in the cerebellum of SCA6 mice

Jayabal, S.*, Watt., A.J.

Spinocerebellar ataxia type 6 (SCA6) is a devastating, progressive neurodegenerative disorder affecting motor coordination. SCA6 is caused by a polyglutamine expansion in P/Q type voltage gated calcium channels (P/Q channels) that are richly expressed in Purkinje cells of the cerebellum. The cellular underpinnings of this disorder are not yet fully understood, and no treatment or cure exists at present. A knock-in mouse model of SCA6 was recently developed (SCA6 KI) that recapitulates several key features of the human disease, including the delayed onset of motor deficits at 7 months of age. P/Q channels play an important role in the developmental refinement of the cerebellar microcircuit and contribute to Purkinje cell functionality in the adult. Although the mutant P/Q channels are expressed early in development in the SCA6 KI mouse, it has yet to be determined how cerebellar development is affected in the SCA6 brain. The development of climbing fiber synapses onto Purkinje cells is a well characterised form of synapse elimination that depends on P/Q channels. While adult Purkinje cells are innervated by a single climbing fiber; early in development, Purkinje cells are innervated by multiple climbing fibers. During the first few postnatal weeks of development, extra climbing fiber inputs are eliminated so that only one climbing fiber remain, a process that is disrupted in the P/Q channel knock-out mouse. Developmental abnormalities that result in multiple-climbing fiber innervation in the adult mouse are often associated with impaired motor performance and ataxia.
Here we determine if climbing fiber synapse elimination is affected in the developing SCA6 KI mice. We make whole-cell patch clamp recordings from Purkinje cells in acute cerebellar slices from SCA6 KI and wildtype (WT) sister-matched controls. We stimulate climbing fibers with an extracellular stimulation electrode and record evoked excitatory post-synaptic currents (EPSCs) in the Purkinje cells. At an age where the majority of WT Purkinje cells receive input from one or two climbing fibers (P10-13; 83% input from 1 or 2 climbing fibers, N = 17), Purkinje cells in the SCA6 KI mouse receive input from significantly more climbing fibers (47% receive input from more than 2 climbing fibers, N = 15; significantly different by Wilcoxon rank sum test, P= 0.025). These results suggest that climbing fiber elimination may be delayed in the SCA6 KI mouse. We will extend this study to other ages to further understand how this developmental process occurs in the SCA6 KI mouse, and whether these abnormalities may contribute to the onset of disease symptoms.

A2

Effects of pre-emptive running exercise on pain-related behaviours in a mouse model of neuropathic pain.

*Robert Nahas, Magali Millecamps, Laura S Stone, Alfredo Ribeiro-da-Silva

Background: The mechanisms that trigger and maintain neuropathic pain are still not fully clear and current therapies are ineffective or insufficient for most patients. Rehabilitative exercise and environmental enrichment attenuate pain behaviours in various animal models of pain. Studying the effects of pre-emptive exercise in an animal peripheral nerve injury model might allow us to better understand the changes associated with chronic neuropathic pain. In particular we aim to characterize the effects of pre-emptive voluntary running exercise on the development of pain-associated behaviours in an animal model of neuropathic pain.
Methods: Male cd-1 mice were housed for two months in cages containing either a freely rotating exercise wheel or a fixed non-rotating control treadmill. Animals then received a unilateral chronic constriction injury of the sciatic nerve and were returned to cages with fixed non-rotating control treadmills. Animals were tested for mechanical and cold pain thresholds (von Frey/acetone) and for anxiety and depression-like behaviours (black and white/open field/tail suspension) prior to the exercise period, post-exercise/pre-injury, and at various time points after injury. Animals were perfused one month after injury and tissue was processed for immunohistochemisty.
Results: Mice with two months of exercise prior to a chronic constriction injury of the sciatic nerve had a significantly lower response to the acetone on the ipsilateral but not contralateral paw as compared with unexercised controls ($p<0.05$) and a trend towards an increased threshold to mechanical stimulus ($p=0.06$) at three weeks. Exercised mice showed significantly less anxiety-like behaviour (black and white) than controls but no difference in depression-like behaviour (tail suspension test).
Conclusions/Future Directions: Pre-emptive exercise attenuated the development of cold pain hypersensitivity and may have resulted in increased thresholds to mechanical stimuli. Exercised animals displayed reduced anxiety-like behaviours. Spinal cord and hind paw tissue will be processed for immunohistochemistry to characterize changes in dorsal horn and peripheral neuroanatomy that might underlie this behaviour effect.

A3

Understanding the mechanism and the functions associated with the proteolytic cleavage of RhoA

Marie-Pier Girouard*, Madeline Pool, Alyson Fournier

Every year, a significant amount of people has to live with severe medical consequences resulting from neurological injuries, such as stroke or traumatic brain injury, and the lack of recovery following those. In the central nervous system, this failure of recovery is due to the neurons that are unable to spontaneously regenerate and recover functions after injury. This is partly due to the presence of inhibitory molecules such as myelin-associated inhibitors (MAIs) and chondroitin sulfate proteoglycans (CSPGs) at the injury site, both of which inhibit neuronal regeneration by activating the small GTPase RhoA. This protein plays a central role in these signaling mechanisms and some clinical trials are currently underway to promote repair by limiting the activity of this protein. Our laboratory has identified that RhoA is proteolytically cleaved to generate a novel 10kDa N-terminal cleavage fragment that has been detected following over-expression of RhoA in COS7 cells and in cortical neurons. From this observation, we hypothesized that we could modulate this mechanism to promote axonal regeneration. In order to identify the protease responsible for the cleavage of RhoA, a screen with several protease inhibitors was conducted. This revealed the involvement of calpain and caspases in this mechanism but did not provide the type of protease directly involved. Further experiments will be required to address this unresolved issue. Through biochemical approaches and mass spectrometry, the cleavage site was found to be located at the amino acid L57 and a cleavage resistant mutant was created to evaluate the relevance of this mechanism. Furthermore, the N- and C-terminal RhoA cleavage fragments were over-expressed in Swiss3T3 cells and their effects on the formation of actin stress fibers were analyzed. Interestingly, some C-terminal fragments localize to the nucleus and appear to form a network at the nucleic membrane. The implications of these findings are yet to be evaluated. In addition, the effects of these RhoA cleavage fragments will be assessed in neurons to evaluate if this mechanism could be modulated to promote neurite outgrowth and eventually to promote neuronal regeneration following injury.

A4

Identifying microRNA regulators of neuronal viability and repair in multiple sclerosis.

Juzwik, C.*, de Faria Junior, O., Bar-Or, A., Fournier, A.

Multiple sclerosis (MS) is an autoimmune disease characterized by the infiltration of peripherally activated immune cells into the central nervous system. Current MS therapies are immunomodulatory rather than neuroregenerative, making neural repair and viability an ideal direction for future work. Previously we have identified neurite outgrowth inhibition by immune cell subtypes and their conditioned media. Our lab is interested in how immune cells and their products affect neuronal viability and repair.

MicroRNAs (miRNA) are RNA sequences ~22 nt in length. A single miRNA is able to target several different mRNA, providing significant information about pathological processes. Altered miRNA expression has been identified in blood cells of MS patients, as well as active and inactive MS lesions. MS-related miRNA dysregulation has not been investigated in neurons, though there are several examples of miRNA gene expression control in neuronal development and differentiation. An investigation of neuronal miRNA expression can provide further insight into immune-neural interactions in MS.

We developed a list of candidate miRNAs to investigate during neurite outgrowth inhibition in mouse cortical neurons following treatment with peripheral blood mononuclear cell conditioned media (PBMC-CM). Specifically, levels of mmu-miR-27a-3p increase in response to stimulation with PBMC-CM raising the possibility that it may be involved in neurite outgrowth inhibition. Preliminary data suggests an involvement of miR-27a in outgrowth. Probing for different targets of miR-27a can unravel its function in neuronal viability and repair.

A5

Proteolytic cleavage of IgLON adhesion proteins by MMPs is involved in neurite outgrowth

Ricardo Sanz*, Gino Ferraro and Alyson E. Fournier

Matrix metalloproteinases (MMPs) are a family of endopeptidases capable of cleaving extracellular matrix and cell surface proteins resulting in degradation or release of biologically active fragments. Processing of surface proteins through MMPs affects developmental processes including axon guidance, survival and synaptogenesis. Moreover, MMPs process ligands and receptors that regulate neuronal plasticity and neurite growth following injury in the Central Nervous System. In the present study, we evaluated the role of MMPs in regulating neurite growth. We find that pan-MMP inhibitors inhibit outgrowth of cortical neurons and dorsal root ganglion neurons and that this effect is dependent on the stage of neuronal maturity. Through tandem mass spectrometry we identified the IgLON family of glycosyl-phosphatidyl inositol (GPI)-anchored neural cell adhesion molecules as proteins that are shed in an MMP-dependent manner. IgLONs are the earliest and most abundant GPI-anchored proteins expressed in the nervous system and are known to regulate neuronal outgrowth and cell adhesion. Different members of this family have been identified, including Neurotrimin (NTM/CEPU), Opioid-binding cell adhesion molecule (OBCAM), Limbic system associated membrane protein (LSAMP), and Neuronal growth regulator 1 (NEGR1/ Neurotractin/ Kilon). Our findings suggest that surface expression of IgLON family members represses neuronal extension. We are focussing on elucidating the role of MMP-dependent processing of IgLON family members in regulating neurite outgrowth in cortical and DRG neurons.

A6

Role of Mahogunin and USP3 in cytosolic PrP ubiquitination and Bax-mediated cell death

Marc-Andre Dery* and Andrea C. LeBlanc

Over the last decade, a growing body of evidence points toward a protective role of cytosolic prion protein (CyPrP) against Bax-mediated cell death in neurons and the breast carcinoma MCF-7 cell line. CyPrP was reported to interact with the ubiquitin ligase Mahogunin (Mgrn1) and a yeast two-hybrid screen ordered by our laboratory identified the deubiquitinase USP3 as an interacting partner of CyPrP. Interestingly, these two CyPrP-interacting proteins are involved in ubiquitination, a post-translational modification characterized by the covalent conjugation of ubiquitin molecules to targeted proteins. This conjugation directs the target protein for proteasomal degradation and can be reversed by the activity of deubiquitinases. We hypothesize that CyPrP deubiquitination may increase its protective nature by limiting its proteasomal degradation, whereas Bax ubiquitination would favor cell survival by enhancing its degradation. The objective of this study is to investigate the role of the interaction between CyPrP and Mgrn1 or USP3 in Bax-mediated cell death. We first postulated that Mgrn1 or USP3 could simply influence the ubiquitination of CyPrP. This hypothesis was first investigated by assessing the impact of Mgrn1 or USP3 overexpression on levels of ubiquitinated CyPrP (Ub-CyPrP) in mouse neuroblastoma N2a cells. Ub-CyPrP was detected in the insoluble fraction of N2a cells overexpressing human CyPrP in the presence of proteasome-inhibiting epoxomicin. Surprisingly, both the ligase Mgrn1 and the deubiquitinase USP3 reduced levels of Ub-CyPrP in these conditions. Concurrently, we investigated the putative role of Mgrn1 and USP3 as regulators of Bax ubiquitination. In this model, PrP could interact with Mgrn1 and USP3 and affect their ability to ubiquitinate or deubiquitinate Bax. This would modulate its degradation by the proteasome and influence the levels of Bax available to conduct apoptosis. To determine if Mgrn1 and USP3 influenced Bax levels, each protein was co-expressed with eGFP or eGFPBax using a bicistronic vector. The expression of Mgrn1 or USP3 did not impact Bax levels. However, immunoprecipitation of the conformationally active form of Bax revealed that USP3, but not Mgrn1, overexpression could reduce Bax activation levels. Current work aims at detecting ubiquitinated Bax in order to directly address if it's ubiquitination is modulated by Mgrn1 or USP3 overexpression or silencing. Lastly, we wanted to determine if Mgrn1 or USP3 influenced cell death. Preliminary results indicate that Mgrn1, but not USP3, co-expression reduced Bax-mediated cell death, assessed by quantifying condensed chromatin. Overall, this work suggests an involvement of USP3 or Mgrn1 in CyPrP deubiquitination and in the regulation of Bax-mediated cell death.

A7

E3 Ligase Hrd1 mediates Prion protein retrotranslocation in mammalian cells

Sarah Peters* & Andrea C. LeBlanc

Background: Prion diseases are fatal neurodegenerative disorders. Approximately 15% of human cases can be attributed to mutations of the prion gene (PRNP), which encodes the normal cellular isoform of the prion protein (PrP). While the majority of PrP is expressed at the cell surface, 10% is found in the cytosol (CyPrP) arising from retrotranslocation of PrP from the endoplasmic reticulum (ER) through the ER-associated degradation (ERAD) pathway. CyPrP protects against Bax-mediated apoptosis in primary human neurons and MCF-7 cells. In addition, most familial PrP mutants completely or partially lose their ability to prevent Bax-mediated apoptosis through defective retrotranslocation of PrP. The E3 ubiquitin ligase Hrd1, encoded by the gene synoviolin or SYVN1, is an ER-resident component of the ERAD pathway known to ubiquitinate and retrotranslocate primarily glycoproteins, such as PrP, destined for proteasomal degradation. In fact, in yeast, Hrd1p was found to mediate the retrotranslocation of human PrP. We propose Hrd1 could be responsible for generating neuroprotective CyPrP in humans and provide a mechanism by which familial mutants disrupt this process in disease
Objective: Determine if Hrd1 is the E3 ligase responsible for mediating the retrotranslocation of prion protein in the human CNS.
Methods: Experiments were carried out in human glioblastoma cell line CR7 due to their high PrP expression. PRNP and SYVN1 expression and response to ER stress were determined in cells treated with ER stress inducing tunicamycin and analyzed by either RT-PCR or western blotting. To examine PrP retrotranslocation, control GFP or eYFP-Hrd1-transfected cells were treated with proteosome inhibitor epoxomicin and Golgi disaggregating brefeldin A, followed by subcellular fractionation to yield homogenate, membrane and cytosolic fractions, which were analyzed by western blotting. Lastly, Hrd1 was selectively knocked down using SYVN1-targeting siRNA, followed by subcellular fractions and western blotting.
Results: CR7 endogenously expresses both PrP and Hrd1 and both are upregulated during ER-stress. Subcellular fractionation of CR7 cells treated with epoxomicin and brefeldin A, revealed a small amount of PrP in the cytosol. CyPrP migrated as two distinct bands at 25 and 30 kDa, consistent with unglycosylated and immature glycosylated PrP, lacking both the N- and C-terminal SP. CR7 cells transfected with eYFP-Hrd1 and treated with epoxomicin/BFA showed in increase in CyPrP as compared to pMaxGFP-transfected cells, indicative of an increase in retrotranslocation. Lastly, targeted knockdown of Hrd1 using siRNA, revealed a substantial decrease in CyPrP generation, indicating that endogenous PrP retrotranslocation was disrupted in cells lacking the Hrd1 protein.
Conclusions: Results indicate that PrP is retrotranslocated in CR7 cells under the regulation of E3 ligase HRD1. Identifying the mechanism of CyPrP generation could provide insight into the pathogenesis of familial prion disease as well as provide a potential pharmacological target as upregulation of Hrd1 could increase the amount of neuroprotective CyPrP, thereby protecting against Bax-mediated apoptosis in familial PrP disease.
Future Experiments: Despite providing convincing evidence for the role of Hrd1 in PrP retrotransloation, a number of issues remain to be addressed. Firstly we will confirm that the M232R and V210I PrP mutants block the retrotranslocation of endogenous PrPC in CR7 cells. Next we will determine if PrPC degradation is dependent on Hrd1-mediated ubiquitination and compare that to PrP mutants using an in vitro ubiquitination assay. We will then confirm a direct interaction between both PrPC and mutant PrP using immunoprecipitation and compare relative binding strength to Hrd1 using a binding affinity assay. This will allow us to test the hypothesis that PrP mutants bind tightly to Hrd1, thereby blocking retrotranslocation in a dominant negative fashion. Lastly, we will determine if mutant PrP also blocks the Hrd1-mediated retrotranslocation of modal ERAD substrate D18D transthyretin (TTR) mutant. If so, this will provide preliminary evidence suggesting familial prion disease pathology is not simply the result of a loss of CyPrP protection but is further compounded by a buildup of Hrd1-substates, leading to ER-stress and subsequent apoptosis.

A8

Study of a novel Caspase-6 inhibitor for protection against axonal degeneration

*Prateep Pakavathkumar, Jan-Eric Ahlfors, Andrea C. LeBlanc

Background: Caspase-6 activity is found abundantly in the neuropil threads, neuritic plaques and neurofibrillary tangles of familial and sporadic forms of Alzheimer disease. Active Caspase-6 induces the accumulation of ubiquitin, Tau, and green fluorescent protein (GFP) within the intact axonal membrane of neurons leading to axonal degeneration. Therefore, inhibiting Caspase-6 may be neuroprotective and represent a potential treatment for Alzheimer disease. Unfortunately, there are no known natural inhibitors of Caspase-6. In this study, we investigated a newly developed irreversible Caspase-6 inhibitor, NWL-117, developed by New World Laboratories.

Objective: Determine if the NWL-117 compound can be used as a potent, specific, and non-toxic inhibitor of active Caspase-6.

Methods: The toxicity of the Caspase-6 (Casp6) inhibitor, NWL-117, was verified on the HCT116 cell line and human primary neurons (HPNs) by MTT. Dose-dependent inhibition of Casp6 was assessed by in vitro fluorogenic assays with purified recombinant active Casp6, in HCT116 cells transfected with a self-activating form of Casp6 and in serum-deprived HPNs. Dose and time course studies measuring the activity and abundance of Casp6 in the presence of NWL-117 were performed in Casp6 transfected HCT116 cells. NWL-117 inhibition of Casp6-dependent neuritic beading in amyloid precursor protein (APPwt) or GFP transfected HPNs was monitored by live fluorescence imaging.

Results: NWL-117 mitochondrial reductive potential in HCT116 cells or to HPNs at 20 to 100 µM concentrations. In vitro, a dose-dependent inhibition of recombinant active Casp6 was observed between 50 nM (50%) and 5 µM (100%). The IC50 in HCT116 cells is 1 µM, that is, 50% of the activity of Caspase-6 is inhibited and there is a dose-dependent inhibition between 5 µM and 100 µM concentrations. Time course studies in HCT116 showed complete inhibition of Casp6 activity within 2 hours of treatment and a reduction in Casp6 protein at 18 hours. Preliminary experiments suggest that the depletion of Casp6 protein is potentially proteasome dependent as it can be partly prevented using the proteasome inhibitor epoxomicin. Western blot analyses showed that Casp6 was processed into its active form in the absence or presence of the inhibitor in transfected HCT116 cells. Further, NWL-117 remained covalently bound to the active p20 subunit of Casp6. In serum-deprived HPNs, NWL-117 shows a slight inhibition of VEIDase activity. Finally, NWL-117 treatment of HPNs transfected with eGFP shows a trend towards a decrease in the number of beading axons.

Conclusions: These results indicate that NWL-117 is cell permeable and non-toxic even at high concentrations. NWL-117 is a potent inhibitor of the processed active form of Casp6 and can enhance Casp6 degradation partly through proteasomal activity. Therefore, NWL-117 can be used to assess if Casp6-mediated axonal degeneration can be inhibited and possibly reversed in HPNs and mouse brains. Further improving the specificity, potency, and bioavailability of NWL-117 may introduce Casp6 inhibitors as a novel therapeutic strategy for Alzheimer disease.

A9

Characterization of Sodium/Proton Exchangers 6 and 9 in the Mouse Hippocampus

Micaela Das Gupta*(1), Emily Deane(1), Alina Ilie(2), John Orlowski(2) and Anne McKinney(1,3). (1) McGill Dept. of Neurology & Neurosurgery, Montreal, Canada; (2) McGill Dept. of Physiology, Montreal, Canada; (3) McGill Dept. of Pharmacology & Therapeutics, Montreal, Canada

Dendritic spines, the post-synaptic compartments of the majority of excitatory neurons in the brain, are dynamic structures which can be modulated by neuronal activity. Abnormal spine morphology and learning deficits have been associated with certain neurological disorders, such as Autism Spectrum Disorders. Recently, mutations in sodium/proton exchangers (NHEs) 6 and 9 have been linked to Autism Spectrum Disorders and Attention Deficit Hyperactivity Disorder. These transmembrane proteins allow electroneutral exchange of alkali cations and protons across biological membranes. In addition to other homeostatic processes, NHEs help regulate pH levels, which is important for protein trafficking and degradation. Previous studies have shown that NHE6 and 9 are expressed in the brain and localize to the endosomal pathway in heterologous cells. However, their precise localization and function in the brain remains unknown. We have initially characterized NHE6 and 9 localization in the mouse hippocampus during postnatal development using in-house antibodies. Cryostat-cut brain sections, organotypic hippocampal slices, and primary hippocampal cultures were prepared from transgenic mice expressing membrane-targeted eGFP in a subset of principle neurons. Sequential confocal imaging of eGFP-positive neurons enabled analysis of fine neuronal structures and precise protein localization using immunocytochemistry. We show that both exchangers are expressed at early timepoints in the hippocampus and intensify throughout development. NHE6 localizes to both pre- and post-synaptic compartments in neurons. Additionally, NHE6 and 9 colocalize ~60% of the time with established markers for recycling endosomes, including transferrin and syntaxin13. This suggests that, through pH regulation, these exchangers may contribute to endosomal trafficking in neurons, which is known to be important for spine plasticity. We envisage that this study will help reveal how NHE6 and 9 are involved in neuronal activity and how mutations in these exchangers may be linked to deficits in cognitive function.

A10

Optogenetic dissection of the MCH system: implications for sleep-state modulation

Sonia Jego (*), Stephen D. Glasgow, Carolina Gutierrez Herrera,
Mats Ekstrand, Jeffrey Friedman, Denis Burdakov3 & Antoine R. Adamantidis

The hypothalamus consists of intermingled inhibitory and excitatory neural circuits. The activity of these circuits correlates with vigilance states, including wakefulness, non-Rapid Eye Movement (REM) sleep and REM sleep. Recent evidence suggests that neurons expressing Melanin-Concentrating Hormone (MCH) are potentially sleep-promoting [1-3]; however, the extent of their ability to selectively modulate sleep states remains unclear. To investigate the specific role of MCH neurons in the modulation of sleep states, we first genetically targeted the expression of excitatory (ChETA, SSFO) or inhibitory (eNpHR3.0) opsins to MCH neurons using an engineered mouse model. We then showed that we could optically activate (ChETA, SSFO) or inhibit (eNpHR3.0) MCH neurons with high reliability. Using real-time detection of vigilance state changes with EEG/EMG recordings, we next found that bilateral optogenetic activation of MCH neurons during NREM sleep increased the probability of NREM-to-REM sleep transitions, while MCH neuron activation during REM sleep extended REM sleep duration. These results were confirmed through the use of a step function opsin (SSFO) which increases excitability of targeted cells through sustained depolarization [4]. In contrast, we showed that optogenetic silencing of MCH neurons during REM sleep reduced the amplitude of the cortical theta rhythm concomitant to an increase of oscillation strength in the slow theta range (3 to 5 Hz). Using an unbiased automatic detection of the slow theta events, we found that their occurrence was significantly increased in NpHR3.0 transfected animals compared to EYFP-expressing controls suggesting that these events are physiological, but rare, in natural sleep. Finally, we demonstrated that optical activation of MCH terminals induced fast GABAA-mediated inhibitory currents in local wake-promoting histaminergic (HA) neurons. This inhibitory tone was enhanced by optogenetically-induced MCH peptide release.
Collectively, these results support a causal role for MCH neurons in the onset and maintenance of cortical REM sleep in the mammalian brain.

A11

Spontaneous neural activity is not required for ephrin-A:EphA signaling in chick motor neuron axon guidance

Daniel Morales*, Chris Law, Artur Kania

In the developing nervous system, axons are directed to their synaptic targets by the signaling of guidance molecules and receptors. Many neuronal populations exhibit spontaneous electrical activity throughout this process, and the role that it plays in the correct wiring of circuits has been the topic of a long-standing debate. Axons of limb-innervating spinal motor neurons exhibit such activity as they face a choice in their trajectory between the dorsal and ventral limb mesenchyme, a decision that is mediated in part by axonal expression of EphA4 and expression of its ligand, ephrin-A, in the limb. We have demonstrated, in an in vivo setting, that the silencing of spontaneous neural activity in chick limb-innervating spinal motor neurons by expression of the inward-rectifying potassium channel Kir2.1 does not affect their number, molecular identity, EphA4 expression levels, or axon outgrowth properties. Moreover, by injecting the retrograde tracer HRP into the limb, we have shown that ephrin-A:EphA signaling is not affected in axons silenced by Kir2.1, since their trajectories are not disturbed. These results argue strongly for the functional uncoupling of spontaneous neural activity and ephrin-A:EphA signaling in the development of neural circuits and, together our collaborator's mirror experiments in mouse retinocollicular axons, provide important insight into a long-standing debate.

A12

Affordance and anterior N400

Ghorayeb, K.*, Brodeur, M.B., & Debruille, J.B.

Introduction: It has long been demonstrated that concrete stimuli (e.g., pen) automatically initiate plans of actions (e.g., writing), a phenomenon known as motor affordance. However, because these actions are inappropriate or useless in most contexts, plans are rarely executed. The present project used the event-related potentials (ERPs) to probe the brain processes involved in the inhibition of motor affordances. It was hypothesized that these processes would be reflected by changes of voltage amplitude of the N400, one component of the ERPs known for indexing inhibitory processes in other experimental conditions.
Methods: To test this hypothesis, ERPs elicited by names of objects and animals and by pictures of objects and animals were recorded in 18 participants while manipulating the need for the inhibition of motor affordances across 3 different tasks: 1) reporting whether the stimulus fell under the category of animal or object (control condition), 2) reporting the first action that comes to mind which could be done on or with the animal or object (i.e., inhibition of alternative responses required), and 3), reporting all the possible such actions (i.e., no inhibition required).
Results: Amplitudes of the N400s was larger in the task requiring the report of only one action than in the two other tasks. Moreover, the distribution on the scalp of the N400s evoked by animal stimuli was different from that of the N400s elicited by objects.
Conclusion: The present results brought evidence that the inhibitory processes indexed by the N400 apply to motor affordances and that their scalp distributions depend on the nature of the action inhibited.

A13

Development of a Neuronal Model of Lesch-Nyhan Disease

Liam Crapper*, Carolina Gigek, Alpha Diallo, Gilles Maussion, Gustavo Turecki, Carl Ernst

Lesch-Nyhan disease (LND) is a rare genetic disorder caused by the disruption of the gene HRPT1. LND has a variety of metabolic and neurological symptoms including crystals in the urine, gout, dystonia, intellectual disability, and chronic self-injury. While challenging behaviors are seen in many developmental disorders, the prevalence and severity of self-injury in LND is unique. The causes of the neurological symptoms in LND remain unknown and to date no treatments have been effective. Human post-mortem studies and animal models of LND have both implicated dysfunction of the dopaminergic system, presenting the strongest link between HPRT1 dysfunction and the neurological symptoms of LND. However other alterations to the dopaminergic system are not sufficient to cause these symptoms, suggesting a more complex pathogenesis. To address these we have developed novel complimentary models of LND. We have generated induced pluripotent stem cells (iPSCs) from patients with LND, and are differentiating them into neurons. Concurrently we have created RNAi knockdowns of HPRT in immortalized fetal neural progenitors from the ventral midbrain, and differentiated these progenitors into mature neurons. We are using genome wide transcription profiling to examine global alterations in gene expression in the ventral midbrain. This study is the first to use high-throughput transcriptional analysis in a neuronal model of LND, and will provide information vital to the understanding of LND pathogenesis and challenging behaviors in other developmental disorders.

A14

Autoradiographic quantification of dopamine D1 receptor expression in striatal regions of dcc haploinsufficient mice from early adolescence to adulthood

Florence Shahabi*, Colleen Manitt*, Alanna Grant, Stephanie Gallant,
Harrod Ling and Cecilia Flores.

The netrin-1 receptor, deleted in colorectal cancer (DCC), is selectively implicated in the development of dopamine (DA) projections to the medial prefrontal cortex (mPFC) during adolescence. Adult dcc haploinsufficient mice exhibit increased synaptic DA input and DA release in the mPFC, as well as alterations in the dendritic spine density and basilar dendritic arbor morphology of Layer V pyramidal neurons, a major target of mPFC DA inputs. Their nucleus accumbens (NAcc) and dorsal striatum, also innervated by projections from the ventral tegmental area, do not show similar neurochemical and neuroanatomical changes. However, behaviours that require the coordinated function of the mesocortical and mesolimbic DA systems are affected in adult dcc haploinsufficient mice, suggesting that the alterations observed in the mPFC impact mesolimbic DA transmission. The maturation of synaptic DA connectivity in mesocorticolimbic targets coincides with developmentally regulated changes in dopamine receptor expression in these regions: early adolescence is marked by the overproduction of dopamine receptors, followed by a period of progressive pruning. Using quantitative receptor autoradiography, we have begun to investigate D1 DA receptor expression in mesocorticolimbic targets of dcc haploinsufficient mice and control littermates in early adolescence (PND21), mid-adolescence (PND35), and in adulthood (PND60). As expected, wild-type mice exhibited a peak in D1 DA receptor expression at mid-adolescence in the NAcc and dorsal striatum. However, dcc haploinsufficient mice do not display this transient increase in D1 DA receptor density in these regions. These results indicate that in dcc haploinsufficient mice, the developmental trajectory of D1 DA receptors is disrupted in striatal regions that are innervated by cortical inputs.

A15

Stable brain network organization in sparse GLM reveals functional modular structure in resting-state fMRI

Kangjoo Lee1,2, Jean-Marc Lina1,3,4, Jean Gotman2, Christophe Grova1,2
1) Multimodal Functional Imaging Lab., Biomedical Engineering Department, McGill University
2) Montreal Neurological Institute, Department of Neurology and Neurosurgery, McGill University
3) Electrical Engineering Department, École de Technologie Supérieure
4) Centre de Recherche Mathématique, University de Montreal

We developed a powerful macro-scale functional connectivity analysis for functional magnetic resonance imaging (fMRI) based on stability of sparse brain networks by employing a bootstrap approach for sparse dictionary learning (SDL). Recently, a popular SDL algorithm called K-SVD (M. Aharon et al., IEEE Trans. Sig. Proc., 2006) has proven effective for sparse general linear model (GLM) to explore brain functional organization by detecting resting-state networks in healthy subjects and in patients with neurological diseases such as Alzheimer's (K. Lee et al., IEEE Trans. Med. Imag., 2011). The assessment of stability of the networks was proposed to identify the consistent resting-state networks (CRSNs) across runs and subjects by applying a bootstrap analysis of stable clusters (BASC) (P. Bellec et al., Neuroimage, 2010). Motivated by BASC, we propose a stable sparse brain network model to obtain CRSNs and their stable sparse representations, whose stability can be guaranteed by the consistency among the resampled data using circular block bootstrap. In this framework, an optimal sparsity level (k) for each region of interest is estimated using minimum description length criterion and can be visualized as a k-map, indicating that each ROI involves k CRSNs selected from N total CRSNs. The proposed method is applied on resting-state fMRI data from 24 healthy subjects. The average of z-transformed k-maps shows high functional connectivity primarily within regions of default mode network (DMN) in the brain: posterior cingulate cortex (PCC), lateral perietal cortex, and lateral prefrontal cortex. However, sensory and motor areas have very low connectivity. This result agrees with the cortical hubs revealed in R. L. Buckner et al. (J. Neurosci., 2009), which analyzed their 24 healthy subjects dataset using correlation analysis. Assuming that the PCC area is one of the functional hubs, we analyze the network organization in the PCC. DMN have shown to be most common in the PCC, and other networks such as motor network, visual network, and thalamic networks are also estimated to be consistently involved in the hemodynamic activity in the PCC area. In conclusion, we propose a k-map that provides brain network information, such as functional connectivity levels in different brain regions, in some way related to degree-of-node-map (d-map) based on standard correlation. The proposed method directly provides information about what networks are associated to the identified hub regions. We observed that the PCC involves several networks, notably the motor and visual networks, suggesting that PCC works as a connector hub that is connected to regions involved in the estimated networks supporting communication among them along with task-negative activation. The d-map, in contrast, reflects how much a voxel is connected to the rest of the brain, but it provides no information about which networks are involved. The strength of our method is that we detect reproducible CRSNs and their k stable combinations. Our method is applicable to quantification of neurobiologically meaningful network features based on graph theory, suggesting significant potential to draw modular architecture of human brain functional network in a close relation to functional rich-club organization.

A17

Influence of surround suppression in cortical area MT on motion discrimination performance

L. D. Liu*, C. C. Pack

The responses of single neurons in MT have been shown to correlate with perceptual decisions made during motion discrimination tasks. Previous studies have suggested that such decisions are made by decoding a population of neurons, with special weight given to the most informative neurons (Liu & Newsome 05; Purushothaman & Bradley 05). Based on their relative responses to small and large stimuli, MT neurons can be classified as surround-suppressed (SS) or non-surround suppressed (NS), and these two types of neurons are thought to serve different behavioral and perceptual functions. We therefore examined the relationship between MT activity and perception for SS and NS neurons.
Monkeys performed a 2AFC motion discrimination task with Gabor patches of varies sizes at a fixed duration (typically 50 ms), for which the motion direction of larger patches is more difficult to discriminate than that of smaller patches (Tadin et al. 03). Using ROC analysis, we found that the outputs of SS neurons generally correlated more strongly with perceptual reports, as monkeys found the motion of larger stimuli harder to discriminate. Surprisingly, the outputs of individual NS neurons consistently outperformed the monkey on motion discrimination for large stimuli, suggesting that that the monkey tended to rely on SS neurons to perform the task.
One interpretation of these results is that SS neurons are more common in the output layers of MT, and hence obligatorily more influential on perceptual decisions. We tested this idea by carrying out recordings using linear array electrodes with multiple contacts; the results showed that SS and NS neurons were clustered into columns, with no apparent differences across layers assigned by CSD analysis. Thus we did not find evidence for a larger proportion of SS neurons in the corticocortical projecting layers. Another possibility is that the additional inhibition associated with surround suppression renders SS neurons more selective and thus more informative generally about motion. We directly tested this hypothesis by injecting GABAergic agents to bidirectionally manipulate inhibitory efficacy while monitoring the neuronal response with an injectrode. We found that the GABA antagonist bicuculline increased both baseline and peak response, along with an apparent increase in tuning bandwidth. However, local blockade of GABAA and GABAB receptors did not diminish surround suppression, as previously observed in V1 (Ozeki et al. 04). It thus appears that the disproportionate influence of SS neurons on perception is unlikely to be due either to their laminar locations or to a particularly strong contribution of inhibition.

A18

Design and Fabrication of a Transparent Microfluidic Probe for Local Stimulation of Neurons

Adiel Mallik*, Mohammad Qasaimeh and David Juncker

Microfluidic probes (MFPs) are open, contact-free and channel-free microfluidic systems that combine the concepts of microfluidics and scanning probes [1]. MFPs are positioned above biological samples and deliver microfluidic streams in local regions of interest. Current MFPs are only compatible with inverted microscopes due to bulky designs and material fabrication constraints, which limit widespread application of MFPs. Here we introduce upright microscope compatible MFPs in Off-Stoichiometry Thiol-ene (OSTE) [2] using a new fabrication process and demonstrate the flow confinement of the MFP.The MFP is fabricated by laminating three layers; the fluidic layer, sealing layer and the interface layer. For fabricating the fluidic layer, a two layer SU-8 master was fabricated using traditional photolithography procedures, and was then replicated into PDMS molds. The PDMS molds were used as the master for the OSTE casting. Fluidic access ports were then drilled before bonding the sealing layer. The sealing layer was fabricated using the same steps as mentioned above with a flat PDMS as the master. The interface layer was fabricated by casting OSTE on glass capillaries followed by a UV pre-cure. This layer was then bonded with the fluidic layer and the device was fully-cured in the UV chamber. A proof of concept of the MFP was demonstrated by localized flow of fluorescein over an in vitro culture of neurons. In brief, we designed, fabricated and tested new MFPs that are compatible with upright microscopes, using a new fabrication process in OSTE. These new MFPs open the door for new neurological studies.

References

1. M. Qasaimeh, S.G. Ricoult and D. Juncker. Lab on a Chip 13, 40 (2013).
2. C. Carlborg, T. Haraldsson, K. Oberg, M. Malkoch and W. van der Wijingaart. Lab on a Chip 11, 3136 (2011).

A19

The involvement of Tumor Necrosis Factor alpha in the neuronal response to stress

*Altimimi, Haider F.
Bailey, Nicole J.
Stellwagen, David

Stress is known to influence the nervous and immune systems. Acute stress modulates rodent performance on tasks involving working memory, through altering the strength of neuronal synaptic transmission, mediated in part, by circulating corticosterone. We have previously demonstrated that the immune signalling molecule tumor necrosis factor -alpha (TNF-alpha) regulates synaptic transmission through AMPAR trafficking. In light of evidence for the interaction between stress and immune signalling, we set out to investigate the role of TNF-alpha in the neuronal response to stress. To this end, we subjected animals to a forced swim stress test - a paradigm that has been shown to potentiate synaptic transmission in hippocampal neurons. We found that animals genetically deficient in TNF-alpha failed to potentiate synaptic transmission in response to stress. Corticosterone in serum of wild-type and TNF-alpha deficient animals was not different at baseline, and increased in both groups to the same extent immediately following stress. Moreover, in vitro corticosterone application to hippocampal slices from wild-type and TNF-alpha knockout animals showed comparable potentiation of synaptic strength, indicating no deficiency in the molecular response to corticosterone in TNF-alpha knockout neurons. Future experiments are aimed at comparing the physiological and behavioural responses to stress between wild-type and TNF-alpha deficient animals.

A20

Heteromerization shapes the pore properties of kainate receptors

Patricia Brown*, Mark Aurousseau, Hugo McGuire, Rikard Blunck, Derek Bowie

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

A21

Covalent crosslinking of GluK2 kainate receptor dimer interface prevents full activation

Dawe, G. Brent*; Daniels, Bryan A.*; Musgaard, Maria; Andrews, Elizabeth; Biggin, Philip C.; Bowie, Derek

Excitatory activity in the mammalian central nervous system (CNS) is mediated largely through ionotropic glutamate receptors (iGluRs). These membrane-bound receptors open an ion-conducting pore in response to glutamate binding, thereby contributing to neuronal depolarization. Among iGluRs, -alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate-selective receptors (AMPARs and KARs) exhibit rapid desensitization in the sustained presence of glutamate. Accordingly, iGluR desensitization plays an important role in shaping the profile of synaptic currents throughout the CNS. Recent structural evidence has attributed iGluR desensitization to the separation of subunits assembled as dimers at the ligand-binding domain (LBD). It has therefore been argued that crosslinking between subunits (to prevent their separation) can block desensitization. Using electrophysiological recordings of single-channel activity, we show that disulfide crosslinking of the LBD dimer interface is not enough to sustain full activation of the KAR subunit GluK2. Moreover, the crosslinked mutant receptor displayed frequent, prolonged channel closures, while permitting entry to predominantly lower conductance levels. We also used molecular dynamics (MD) simulations to compare the LBD orientation of the cysteine-crosslinked receptor to a range of iGluR structures, and found that its subunits were positioned closer to a desensitized state than an activated state. Consequently, we tested additional functional properties of the crosslinked KAR, and observed an increased apparent affinity for glutamate, versus the wildtype GluK2 peak response, also suggestive of rearrangements around the ligand-binding site. In addition, the mutant current response was potentiated by a lectin modulator that acts selectively in the presence of desensitization. Taking together our results, it appears that covalent crosslinking can restrict KAR access to the activated state by imposing constraints that restrain the receptor in intermediate conformations.

A22

Glucocorticoids induce phase shifts of peripheral circadian clocks in humans independently of the central clock

Marc Cuesta*, Nicolas Cermakian, Diane B. Boivin

In mammals, the circadian system is composed of a central clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus and peripheral clocks found in various cell types, which are subordinate to the SCN. In humans, we previously showed that the central clock is quickly reset during a simulated shift work with bright light exposure at night, while resetting of peripheral clocks is slower, leading to temporary misalignment between various clocks. Glucocorticoids (GCs) express circadian rhythms controlled by the SCN, and are thought to act as synchronizers of rodent peripheral clocks. In contrast to most of the brain and periphery, the SCN contain no GC receptors. Here, we show for the first time in humans that oral doses of synthetic hydrocortisone (Cortef) administered in the late afternoon (corresponding to the cortisol peak in adapted night-shift workers) can shift peripheral clocks independently of the central clock. We first confirmed the rhythmicity of circadian markers in peripheral (i.e. clock gene expression in peripheral blood mononuclear cells or PBMCs) and central clocks (i.e. core body temperature, plasma cortisol and melatonin). Second, we showed that administration of Cortef acutely increased PER1 expression in PBMCs. Third, while the phases of central markers remained unchanged following 6 days of Cortef administration, the phases of PER2-3 and BMAL1 expression in PBMCs were shifted by ~10-11 h. These results demonstrate, for the first time, the potential of GCs to shift human peripheral clocks. Most importantly, they suggest possible innovative interventions for shift workers or jet-lag travelers combining synchronizing agents for the central clock (e.g. phototherapy, exogenous melatonin) and the peripheral clocks (e.g. GCs or other pharmacological compounds; restricted feeding schedule).

A23

Oxidative-Retinopathies: Female Neonate Rats Handle Bright Light Better Than Hyperoxia, Males React Strongly To Both

Chaychi, Samaneh*; Chorfi, Sarah; Polosa, Anna; Jung, Suna; Dorfman, Allison L.; Yang, Xiaojuan; Chemtob, Sylvain; Lachapelle, Pierre

Purpose: Exposure of neonatal rats to bright light or hyperoxia produce typical retinopathies [Light-Induced (LIR) and Oxygen-Induced (OIR)]. Given that Estrogen (E) was shown to act as a neuroprotective agent and that estrogen receptors were evidenced in the retina, the purpose of this study was to investigate if male and female retinas reacted differently in response to oxidative stress.

Methods: Newborn Sprague Dawley (SD) rats (N =15) were exposed to 80 ± 5% O2 + bright luminous environment of 750 lux (L + H group) between P4-P14. At P14, O2 was interrupted while light exposure continued until P28. A second group of rats were kept at room air with same light intensity from P4 to P28 (Light only group: L group). At P100, retinal function was investigated with the ERG. In parallel, we injected 17 -beta-Estradiol (4 and 12 µg, IP, daily from P6-P14) in SD pups exposed to postnatal hyperoxia from P8-P14. Experimental rats were subdivided as follows: Group 1 (G1: O2 + 4µg E; N = 8); Group 2 (G2: O2 + 12µg E; N = 8); Group 3 (G3: 4µg E; N = 8); Group 4 (G4: 12µg E; N = 8); Group 5 (G5: O2 only; N = 8); Group 6 (G6: control; N = 8). ERG were done at P30.

Results: ERG showed that female rats of the L group had scotopic a-and b waves and photopic b-wave amplitudes that were significantly higher than male [109 ± 41µV vs 52 ± 22 µV(P= 0.01); 398 ± 164 µV vs. 214 ± 85 µV (P=0.039) and 163 ± 53 µV vs. 72 ± 32 µV (P=0.006), respectively].No significant male-female differences noted in the L+H group. Females of the L+H group had significantly smaller scotopic (P=.01) and photopic (P=.002) b-waves compared to females of the L group. In males, only the photopic b-wave showed a similar effect (P=0.01). The latter results suggested that estrogen is detrimental to retinal function, a finding that was further confirm in the second experiment where we showed a significant (P<.01) reduction in ERG amplitudes (scotopic and photopic) in experimental rats receiving the highest dose of estrogen.

Conclusions: Our results reveal that in condition where the primary target of the oxidative stress is the outer retina (i.e. the photoreceptors) the female rats appear to be relatively better preserved than males. However, this sex advantage seems to be lost in situations where the inner retina (or retinal vasculature) as it is the case in OIR, presumably due to a detrimental effect of estrogen on retinal function.

A24

Developing retinal ganglion cell axon arbors exhibit different dynamic branch behaviors and elaboration in response to correlated and asynchronous visual stimulation

Martin Munz, Delphine Gobert, Jessie Poquérusse and Edward S. Ruthazer

Development of precise neural maps relies on molecular guidance signals and activity-dependent cues working in parallel. The mechanisms by which neural activity instructs structural plasticity in the developing brain has been an important subject for theoretical modeling and speculation, however remarkably little experimental evidence exists to directly test these ideas.

Hebbian plasticity, an appealing model for activity-dependent refinement of neuronal connectivity posits that temporal correlation in the firing of pre- and postsynaptic cells strengthens synapses. Many inputs firing synchronously can cooperatively excite the postsynaptic neuron to fire. Thus, Hebbian plasticity would tend to aggregate inputs with common receptive fields, contributing to map refinement. To directly test for Hebbian structural plasticity, we performed two-photon live imaging of retinotectal axon remodeling in transparent albino Xenopus tadpoles while presenting visual stimuli.

Xenopus retinal ganglion cell (RGC) axons normally extend to the contralateral optic tectum. We exploited the existence of infrequent (~20% of animals) axon targeting errors at the chiasm, which result in single retinal axons projecting to the ipsilateral tectum, to assess the Hebbian prediction that the degree of correlation in firing between an axon and its neighboring inputs will influence its growth and branching. Ipsilateral RGC axons were labeled and imaged at 10 min intervals in vivo as they elaborated complex terminal arbors by a process of dynamic branch additions and retractions. Concurrently, a visual stimulus (10ms flash at 0.5Hz) was presented to both eyes, either in or out of phase, respectively to synchronize or desynchronize the activity between the ipsilateral axon and its neighbors. Importantly, the visual stimulus seen by each eye did not change; only the synchrony (phase lag) between the two eyes was altered. We found that the rate of new branch addition was significantly elevated under conditions of asynchronous stimulation compared with synchronous stimulation (or darkness). Furthermore, newly formed branches were more stable, with longer lifetimes, when both eyes were stimulated synchronously, as predicted by the Hebbian model. These effects were cumulative, with asynchronous stimulation over four days resulting in much larger RGC arbors with many more branch tips.

These data indicate that patterned activity instructs axon refinement by a modified Hebbian process in which presynaptic activity promotes exploratory addition of new branch tips, and correlated firing both suppresses new branching and stabilizes existing branches.

A25

Shaping the aging brain: Role of auditory input patterns in the emergence of auditory cortical impairments

Brishna Kamal, Constance Holman and Etienne de Villers-Sidani

Age-related impairments in the primary auditory cortex (A1) include poor tuning selectivity, neural desynchronization and degraded responses to low-probability sounds. These changes have been largely attributed to reduced inhibition in the aged brain, and are thought to contribute to substantial hearing impairment in both humans and animals. Since many of these changes can be partially reversed with auditory training, it has been speculated that they might not be purely degenerative, but might rather represent negative plastic adjustments to noisy or distorted auditory signals reaching the brain. To test this hypothesis, we examined the impact of exposing young adult rats to 8 weeks of low-grade broadband noise on several aspects of A1 function and structure. We then characterized the same A1 elements in aging rats for comparison. We found that the impact of noise exposure on A1 tuning selectivity, temporal processing of auditory signal and responses to oddball tones was almost indistinguishable from the effect of natural aging. Moreover, noise exposure resulted in a reduction in the population of parvalbumin inhibitory interneurons and cortical myelin as previously documented in the aged group. Most of these changes reversed after returning the rats to a quiet environment. Furthermore, we found that placing aged rats for 2 weeks in an enriched environment containing highly patterned auditory stimuli resulted in a partial selective recovery of some age-related A1 impairments. These results support the hypothesis that age-related changes in A1 have a strong activity-dependent component and indicate that the presence or absence of clear auditory input patterns might be a key factor in sustaining adult A1 function.

A26

LIVE-IMAGING OF BACE1 SECRETION FROM HEK-CELLS AND PRIMARY NEURONS

Filip Liebsch*, Mark Aurousseau, Derek Bowie, and Gerd Multhaup

Background:
The beta-secretase (also known as BACE1) is an aspartic acid protease that catalyzes the first step in the production of amyloid Abeta of Alzheimer disease. BACE1 is a type I transmembrane protein, which mainly localizes to hippocampal mossy fiber terminals (Kandalepas et al., 2013). BACE1 forms oligomers (Schmechel et al., 2004; Liebsch et al., manuscript in preparation) and cycles between the trans-Golgi network and the cell surface. While most BACE1 mainly exists membrane-bound, the BACE1 ectodomain is found in the extracellular space as a soluble catalytically active enzyme. Neither the exact mechanism nor the biological consequences have been explored.
Results:
BACE1 consists of a large extracellular domain with two active-site aspartates, a transmembrane sequence and a short cytosolic tail. We established a novel imaging technique in our lab using a pH-sensitive GFP-tag combined with Total Internal Reflection Fluorescence (TIRF) microscopy to precisely assess the surface expression of tagged membrane proteins. With this method, we found that the release of BACE1 into the extracellular space can be monitored in living cells in real-time.
Conclusion:
GFP-tagged BACE1 is released from cell-lines and primary hippocampal neurons. With the technique at hand and by biochemical methods, we are currently elucidating the biological meaning of BACE1 secretion.

Acknowledgements:
We thank the German National Academic Foundation and the International Copper Association for financial support.

References:
Kandalepas, P.C., Sadleir, K.R., Eimer, W.A., Zhao, J., Nicholson, D.A., and Vassar, R. (2013). The Alzheimer's beta-secretase BACE1 localizes to normal presynaptic terminals and to dystrophic presynaptic terminals surrounding amyloid plaques. Acta neuropathologica 126, 329-352.
Schmechel, A., Strauss, M., Schlicksupp, A., Pipkorn, R., Haass, C., Bayer, T.A., and Multhaup, G. (2004). Human BACE forms dimers and colocalizes with APP. The Journal of biological chemistry 279, 39710-39717.

A27

PTEN inhibition enhances myelination by amplifying IGF-1 signaling in rat and human oligodendrocyte progenitors

Marcio L. De Paula 1,2*, Qiao-Ling Cui 3, Shireen Hossain 1, Jack Antel 2,3 and Guillermina Almazan 1,2

1 Department of Pharmacology & Therapeutics, McGill University, Montreal, QC
2 Integrated Program in Neuroscience, McGill University, Montreal, QC
3 Neuroimmunology Unit, Montreal Neurological Institute, McGill University, Montreal, QC

Oligodendrocytes (OLGs) produce and maintain myelin in the central nervous system (CNS). In the demyelinating autoimmune disease multiple sclerosis, OLGs are damaged and those remaining fail to fully remyelinate CNS lesions. Therefore, current therapies directed to restrain the inflammation process with approaches that protect and reconstitute OLG density would be essential to pave the way of myelin repair. A critical signal for OLGs is insulin-like growth factor-1 (IGF-1), which promotes their development and ultimately myelin formation. PTEN inhibits the phosphoinositide 3-kinase (PI3K)/Akt signaling, a convergence downstream pathway for growth factors such as IGF-1. In this report, we temporarily inhibited PTEN activity by pharmacologically treating rat and human oligodendrocyte progenitors (OLPs) cultured alone or with dorsal root ganglion neurons (DRGNs) with bisperoxovanadium (phen) or knocking down PTEN mRNA expression using siRNA. Our findings show that phen potentiates IGF-1 actions by increasing proliferation of OLPs in a concentration-dependent manner, and caused a sustained and time-dependent activation of the main pathways: PI3K/Akt/mammalian target of rapamycin (mTOR) and MEK/ERK. At low concentrations, IGF-1 and phen stimulated the differentiation of rat and human OLPs. In addition, IGF-1 enhanced rat OLG differentiation by up-regulating earlier and late lineage markers when PTEN was knocked down by siRNA. Concordantly, the PTEN inhibitor synergized with IGF-1 to robustly augment myelin basic protein accumulation in rat newborn and human fetal OLGs co-cultured with DRGNs in a longer timeframe by promoting the elaboration of organized myelinated fibers as evidenced by confocal microscopy. Thus, our results suggest that a transient suppression of a potential barrier for myelination in synergy with other therapeutic approaches including growth factors may be promising to improve the functional recovery of CNS injuries.

A28

Differential DNA Methylation in Cocaine Addiction

Kathryn Vaillancourt*; Gang Chen; Gustavo Turecki

Background: Drug addictions create a substantial negative health impact on those who are affected by them and often have massive economic and social consequences. Like many complex mental health disorders, there is evidence that biological factors contribute to individual susceptibility to, and course of, cocaine abuse. Specifically, cocaine use is associated with epigenetic changes in multiple brain areas. These are chemical modifications to chromatin that affect gene expression without altering the genetic sequence. DNA methylation is a stable epigenetic mark that is associated with decreased gene expression. Using post-mortem brain tissue from the nucleus accumbens and dorsal striatum, regions known to be affected by cocaine use, we will examine changes in DNA methylation associated with cocaine addiction in humans.

Methods: First, we will use reduced representation bisulfite sequencing (RRBS) on samples from individuals with a history of cocaine dependence, and those without. This technique will identify multiple sites of differential methylation simultaneously and allow for genome-wide comparisons. Interesting findings will then be validated by fluorescence activated nuclei sorting (FACS) and targeted methylation analysis.

Implications: To our knowledge, this is the first study to examine genome-wide changes in DNA methylation associated with cocaine addiction in humans. By understanding how complex biological factors, including epigenetics, influence the development and course of cocaine addiction, we can develop more efficient screening and prevention programs as well as aid in the discovery of personalized treatment options.

A29

Cytoplasmic accumulation of ALS-linked FUS/TLS alters posttranslational modifications of histones regulating gene transcription

Michael Tibshirani*, Katie Mattina, Hongru Zhou, Wencheng Yang, Michael J. Strong, Lawrence Hayward, Heather Durham

Mutations in the DNA/RNA binding protein FUS/TLS have been linked to familial ALS type 6 (fALS6). This predominantly nuclear protein can accumulate in the cytoplasm of motor neurons of fALS6, sALS and other fALS cases. We have recently shown that asymmetric arginine dimethylation of FUS by PRMT1 is a post-translational modification that regulates it nucleocytoplasmic shuttling. In motor neurons of dissociated murine spinal cord cultures, we observed that the distribution of PRMT1 mirrored endogenous FUS and ectopically expressed human WT or mutant FUS. PRMT1 was depleted from the nucleus of neurons with cytoplasmic FUS. This study investigated the consequences of PRMT1 nuclear depletion on methylation of its nuclear substrates, specifically Arginine 3 on Histone 4, and downstream effects on transcription. In cultured motor neurons , nuclear depletion of PRMT1 was accompanied by decreased H4R3 methylation and H3 acetylation, a downstream consequence, and decreased RNA synthesis.

We also evaluated PRMT1 localization in neurons in a transgenic mouse model of ALS6 and in autopsy cases of sALS. Colocalization of PRMT1 and FUS was also observed in normal murine spinal motor neurons in vivo and in mice bearing FUSWT or FUSR495X transgenes. PRMT1 was depleted in nuclei of motor neurons of FUSR495X mice, similar to the culture model. However, PRMT1 was not stable postmortem in human autopsy tissue, nor in mouse tissue with comparable delay in processing.

A30

Complete reversal of neuropathic and inflammatory mechanical allodynia in pregnant mice.

Sarah F. Rosen*, Jeffrey S. Mogil

Women frequently report that chronic pain symptoms, such as migraines or lower back pain, dissipate during pregnancy, and often return after delivery (Kvisvik et al., 2011). In addition, during pregnancy, rats, mice and humans have been shown to display a significant increase in pain threshold prior to, and during labor, followed by an abrupt decrease in pain threshold after delivery. However, there has been little study of this phenomenon in animal models using modern assays of chronic pain. Here we show that female mice with spared nerve injury (SNI) or injected with complete Freund's adjuvant have a complete blockade of mechanical allodynia during pregnancy. Shortly after delivery the allodynia returns. Mice given a sham surgery also demonstrate a significant increase in mechanical pain threshold. Current experiments are examining the role of gonadal hormones in these phenomena.

A31

Optogenetic manipulation of cortical and subcortical circuits in non-human primates

Roberto A. Gulli*, Sebastien Tremblay, Nour Malek, Sylvain Williams, Antoine R. Adamantidis, Julio C. Martinez-Trujillo

Optogenetics is a burgeoning field, providing researchers a long sought-after tool to examine neural connectivity as well as the mechanistic role of genetically- and spatially-defined neuronal populations in generating behaviour. Here we report our experiments showing transduction of opsins in superficial and deep structures of the non-human primate brain.
We injected a lentiviral vector carrying excitatory opsin ChR2 (Lenti-hThy-1-ChR2(H134R)-eYFP) in the frontal eye field of an anaesthetized female Macaca fascicularis. Optical stimulation and in vivo electrophysiological recordings from the transduced region yielded up to 70-fold increases in firing rate while the monkey fixated on a visual target. Immunohistological analysis confirmed neuronal transduction of ChR2.
In a second female Macaca fascicularis, the right medial septum was injected with a lentiviral vector carrying the inhibitory opsin eNpHR3.0 (Lenti-hThy-1-eNpHR3.0-eYFP). These injections were carried out under general anaesthesia using an MRI-guided neuronavigation system. Immunohistology revealed dense transduction in putative neuronal and astrocytic cells. The left medial septum of this monkey was injected with ChR2 (Lenti-hThy-1-ChR2(H134R)-eYFP). However, no transduction was observed in immunohistological analyses.
In a third experiment, the inhibitory opsin ArchT (AAV2-CaMKIIa-ArchT-eYFP) was injected in the prefrontal cortex of an awake, behaving male Macaca mulatta. Injections were done through a recording chamber using a microinjectrode, allowing for functional verification of the injected region. Experimental manipulations are currently being carried out to assess opsin transduction.
These preliminary experiments show three methods to target both cortical and subcortical regions in optogenetics experiments. In accordance with published literature in rodents and monkeys, our results also suggest that opsin expression patterns may be nuanced between anatomical regions.

B1

Synapse-specific expression of Cp-AMPARs in neocortical inhibitory neurons

Lalanne Txomin*, Oyrer Julia, Chung Andrew, Farrant Mark & Sjostrom Per Jesper

Synaptic plasticity is critical for correct wiring and for constant reorganization of the brain's neuronal circuitry thought to underlie memory. Synaptic plasticity is diverse, varying between brain regions and cell types. Inhibitory neurons (INs) in particular present a striking diversity (1). The variability of synaptic plasticity is probably due to differences in post-synaptic molecular machinery. In the hippocampus, for example, calcium-permeable AMPA receptors (cp-AMPARs) govern plasticity of excitatory inputs to specific IN types (2). In general, little is known about synaptic plasticity of neocortical INs, probably because IN type is technically difficult to establish (3).
To understand IN plasticity rules, we sought to determine the presence of cp-AMPARs at pyramidal cell (PC) synapses onto basket cells (BCs) and Martinotti cells (MCs) in Layer 5 of mouse visual cortex acute slices. We combined paired recordings with 2-photon microscopy and mouse transgenics to identify INs by morphology, firing pattern, and expression of parvalbumin or somatostatin for BCs and MCs, respectively. With internal spermine, both miniature excitatory post-synaptic currents (mEPSCs) (rectification index 0.1±0.06, $p<0.001$) and PC-BC connections (0.104 ±0.04, $p<0.001$) rectified. Without internal spermine, however, mEPSCs did not rectify (0.97±0.09, $p=0.74$). In agreement, the selective Cp-AMPARs blocker Naspm reduced mEPSC amplitude in BCs (to 52±11%, $p=0.044$). In contrast, PC-MC connections did not rectify (1.408 ±0.15, $p=0.12$) in the presence of internal spermine.
In conclusion, our results indicate that cp-AMPARs are synapse-specifically expressed in visual cortex microcircuits, with a strong contribution to excitatory neurotransmission onto BCs but not onto MCs. This synapse-specific expression of cp-AMPARs is likely to differentially impact on both short and long-term plasticity, with implications for processing as well as for storage of information in cortical neural networks.

(1) Markram et al (2004) Nat. Rev. Neurosci . 5:793
(2) Lamsa et al (2010) Front. Syn. Neurosci. 2:8
(3) DeFelipe et al (2013) Nat. Rev. Neurosci. 14:203

B2

The Dopaminergic Ultradian Oscillator

I.D. Blum*, L. Zhu, K.-F. Storch

In rodents, chronic access to methamphetamine drives rhythms of rest and activity beyond the circadian range with the length of each cycle exhibiting dose dependency. These rhythms can be induced even after electrolytic lesion of the suprachiasmatic nucleus (SCN) or genetic inactivation of the circadian clock suggesting that the oscillator driving these rhythms does not rely on an intact circadian timing system. To date the substrate and location of this methamphetamine sensitive circadian oscillator (MASCO) is unknown. We observe that dopamine transporter (DAT) knock-out mice phenocopy the locomotor activity pattern seen in rodents given methamphetamine and responds similarly to specific pharmacological agents targeting dopaminergic transmission. This genetic model may provide the first inroad towards pinpointing the molecular basis and location of this oscillator. Interestingly, we observed that pharmacological interventions of the dopaminergic system similarly alter endogenous ultradian (~4 hour) behavioural rhythms and we provide the first evidence that the clocks underlying these two seemingly disparate processes are one and the same. We suggest that perturbing normal functioning of the dopamine transporter significantly lengthens the endogenous period of this oscillator from 4 to greater than twenty four hours. Finally, we propose that dis-regulation of this dopaminergic ultradian oscillator (DUO) may underlie certain aberrant behavioural patterns, e.g. sleep-wake disturbances, observed in specific psychiatric illnesses with underlying alterations in dopamine transmission.

B3

Endogenous beta-amyloid regulates memory stability

Peter S.B. Finnie*, Karine Gamache, Johnna Perdrizet, & Karim Nader

Although it is well-established that beta-amyloid plays a role in the pathogenesis of Alzheimer's disease, there is accumulating evidence for its role in physiological function, neuronal plasticity, and memory. For instance, beta-amyloid is released during neuronal stimulation (Kamenetz et al., 2003; Cirrito et al., 2005) and acute stress (Kang et al., 2007) and may promote memory consolidation (Garcia-Osta & Alberini, 2009). It has been demonstrated that the synaptic endocytosis of the NMDA-receptor subunit NR2B can be enhanced by application of beta-amyloid oligomers (Kessels et al., 2013). We have previously proposed that NR2B receptors in amygdala can control whether or not strong auditory fear memories undergo a process called reconsolidation following their reactivation (Wang et al., 2009). Thus, we predicted that strong training might increase beta-amyloid production, and that this might play a role in the downregulation of NR2B and hence the stability of these memories during retrieval. Specifically, reducing beta-amyloid production by inhibiting gamma-secretase activity would offset trafficking of NR2B from basolateral amygdala synapses, while also permitting strong memories to undergo reconsolidation following reactivation. However, our preliminary results support only the latter prediction, but crude-synaptic NR2B and NR1 levels were not influenced by infusion of the gamma-secretase inhibitor, LY-450139. Rather, we found that animals receiving strong training exhibited a dramatic elevation in crude-PSD expression of a calpain-cleaved NR2B subunit at approximately 115kDa, which was not observed in rats treated with LY-450139. Our working theory is that this cleavage may leave functional NR2B-containing NMDARs at the synapse (Simpkins et al., 2003) that might be incapable of C-terminal interactions with other membrane-associated proteins, such as CaMKII (see Leonard et al., 2002), which are important for memory stability.

B4

Investigating the role of Fragile X-related Protein 1 (FXR1P) in Brain Plasticity

Erin Nuro* , Denise Cook, Emma V. Jones, Haider F. Altimimi, Edith Hanna, W. Todd Farmer, David L. Nelson, Joseph Rochford, David Stellwagen, Jean-Claude Béïque, Keith K. Murai

Title: Investigating the role of Fragile X-related Protein 1 (FXR1P) in Brain Plasticity

Fragile X Related Protein 1 (FXR1P) is one of two autosomal homologues of the Fragile X Mental Retardation Protein (FMRP), a protein whose expression is significantly reduced in Fragile-X syndrome. Like FMRP, FXR1P is an mRNA binding protein that is implicated in regulating the synthesis of specific target proteins (Siomi et al., 1995). However, in comparison to FMRP, little is known about the function of FXR1P in brain function.
Our lab has recently discovered that FXR1P co-localizes with translational machinery near synapses suggesting that it could play a role in locally controlling the levels of proteins involved in synaptic plasticity, learning and memory, and autism spectrum disorders (ASD).
To test this we have generated a conditional knockout mouse model where the FXR1 gene is conditionally ablated from neurons in the forebrain, including the hippocampus (a brain region important for learning and memory). Using this model we are investigaiting the role of FXR1P in learning, memory, and ASD.

B5

Long-term dance training changes gray matter structure

Falisha Karpati*, Chiara Giacosa, Virginia Penhune, Nicholas E.V. Foster, Krista L. Hyde

Introduction:
Studying individuals with specialized training, such as music and dance, offers a unique window to study human brain plasticity and the interaction with behavior. Both music and dance involve long-term and intensive practice of sensorimotor skills, and the type and duration of training can be quantified. However, while several studies have investigated the neural correlates of musical training (Wan and Schlaug, 2010), only one study (Hänggi et al., 2010) has examined the structural brain differences between dancers and non-dancers and did not include a behavioural component. The goals of the present study were to identify the effects of long-term dance training on cortical thickness and to correlate cortical thickness with performance on a dance task.

Methods:

Participants:
We tested 15 professional dancers and 15 controls. The two groups were matched in age. Subjects were carefully screened via a detailed questionnaire on their dance and music background. Dancers were recruited from professional modern dance schools where they danced full-time, and had 10 or more years of professional training in dance, but no formal training in music. Controls had no formal experience in dance, music, figure skating, aerobics or any competitive sport. Participants had no past or current neurological, psychiatric, or neuropsychological problems, and no illegal drug use. The study was approved by our local ethics committee and written informed consent was obtained from all participants.

Scanning Protocol, Morphometric Analyses and Behavioural Testing:
T1-weighted MR sequences were obtained for all subjects on a 3 Tesla Siemens scanner. The T1 anatomical MRIs for all subjects were submitted to “CIVET” (Ad-Dab'bagh et al., 2006) for processing and cortical thickness analyses. Each T1-weighted image volume was corrected for signal intensity nonuniformity, linearly transformed into standardized stereotaxic space, and segmented into gray and white matter, cerebrospinal fluid and background. The gray and white matter surfaces were then fitted using deformable models, resulting in two surfaces with 81920 polygons each. Next, a cortical thickness map was calculated for each subject, where cortical thickness was measured at every point (or vertex) on the cortical mantle, and then blurred with a 20 mm surface based blurring kernel (Lerch et al., 2005). Statistical analyses were performed at every point on the cortical mantle to test for region specific group differences in cortical thickness using SurfStat software (http://www.math.mcgill.ca/keith/surfstat/). Age and gender were included as covariates. Results were thresholded over the whole-brain at $p<0.001$ uncorrected.
Participants also underwent behavioural testing including a battery of auditory, motor and cognitive tasks. The main task of interest in the present study is a dance video game (Dance Central for Xbox Kinect) where participants viewed an avatar dancing and were asked to imitate the movements. Correlations between cortical thickness and performance on this dance task were analyzed.

Results:
Dancers had thicker cortex in the right parahippocampal gyrus, right superior parietal lobule, right superior temporal gyrus and left medial superior frontal gyrus. Regions found to be positively correlated with performance on the dance task included the right parahippocampal gyrus, right superior parietal lobule and left superior temporal gyrus.

Conclusions:
Our results expand on previous findings by correlating brain structure in dancers to behaviour. The next step is to examine the specificity of dance training by comparing our results in dancers to those of musicians. This research will advance our knowledge on the specificity of brain-behavioral relationships in dance and music in typical development, and may have applications for future therapies in motor disorders.

B6

Pitch and time processing in speech versus music: effect of musicianship and attention

Anastasia Sares* (1,2),
Nicholas E.V. Foster (1,2),
Kachina Allen(3),
Krista L. Hyde (1,2)

1Faculty of Medicine, Montreal Children's Hospital and Montreal Neurological Institute, McGill University
2Center for Brain Language and Music (CRBLM) and International Laboratory for Brain Music and Sound (BRAMS)
3 Department of Psychology, Rutgers University, NJ

Musical training enhances pitch processing in music and speech, but it is less clear whether musicians show enhanced time processing in speech, or how attention affects performance. There is also debate on whether pitch processing in speech and music rely on distinct or overlapping neural resources. Here we investigated the effect of musicianship and attention on both pitch and time processing in speech versus music, as well as the underlying structural neural correlates. We tested adult musicians versus non-musicians on a battery of prosody tests developed in our lab. Participants heard short sentences or their tonal analogues in which they had to detect parametric changes in pitch or time in a directed or non-directed attention condition. Unlike previous work, here we used the same set of stimuli to test pitch and time changes, thus allowing direct comparison of results across conditions. Structural MRI scans were also obtained for participants. Participants performed better with larger pitch and time changes in both speech and music, and better in directed attention in general, but musicians outperformed non-musicians overall. We are currently examining the brain structural MRI data in relation to these behavioural results to test for expected brain-behavioral correlations in auditory cortex. These results promise to shed light on the brain-behavioural basis of pitch and time processing in speech and music, and how these are shaped by musical training and attention. Our findings set the stage for future studies in our lab on prosody processing in typically-developing children and those with autism.

B7

Brain and behavioral correlates of auditory-motor synchronization in children with autism spectrum disorder

*Ana Tryfon1,2, Nicholas E.V. Foster1,2, Tia Ouimet1, Krissy Doyle-Thomas3, Evdokia Anagnostou3, Alan C. Evans2, Lonnie Zwaigenbaum4, Krista L. Hyde1,2, & NeuroDevNet ASD imaging group5

1 Montreal Children's Hospital, McGill University, Montreal, Canada
2 Montreal Neurological Institute, McGill University, Montreal, Canada
3Holland Bloorview Kids Rehabilitation Hospital, Toronto, Canada
4 Glenrose Rehabilitation Hospital Edmonton, Canada
5 http://www.neurodevnet.ca/research/asd

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that is characterized by impaired social interaction and communication, as well as atypical sensory perception. Individuals with ASD generally have diminished processing of complex, verbal and social material (e.g. speech), but can have enhanced processing of basic, non-verbal and non-social material (e.g. music). (Ouimet et al., 2012) Auditory-motor synchronization is critical to both speech and music, but has not been studied much in ASD. Here, we investigated basic auditory-motor synchronization in ASD children and how performance maps onto brain structure. We studied 34 children with ASD and 40 TD age-matched controls (mean age across groups: 12.04, SD: 2.7, range: 6-16 years). Participants were tested on an auditory-motor synchronization task in which they tapped in synchrony with auditory rhythms of varying metrical complexity. (Chen et al., 2008) Performance was calculated in terms of the participant's ability to reproduce time intervals between each sound event in a sequence. T1-weighted brain anatomical MR images were acquired for all subjects and cortical thickness maps were generated. Statistical analyses were performed at every point on the cortical mantle to test for significant correlations between cortical thickness and performance on the auditory-motor task. All children (both ASD and TD) performed worse on more complex rhythms, but children with ASD showed better performance on the most complex rhythms. Cortical thickness in bilateral motor areas was correlated with performance on the most complex rhythms. These findings are consistent with current models of enhanced processing of basic, non-verbal and non-social stimuli in ASD. (Mottron et al., 2006)

B8

Intentional binding and sense of responsibility: Temporal (un-)binding of actions and effects when inflicting pain on others

Anne Loeffler*, Marcel Brass, Jelle Demanet, Lize DeCoster, Dorit Wenke

The sense of agency (SoA) refers to the experience of controlling one's actions, and through them, events in the outside world. Although SoA appears to be closely related to the concept of responsibility, hardly any studies so far have investigated SoA in situations in which one's own actions have (negative) consequences for others. In the present study subjects inflicted electric shocks of supposedly different pain intensities on somebody else (a confederate). SoA was measured by intentional binding – the subjective compression of the time interval between one's own actions and their effects. Our results indicate that intentional binding was significantly reduced in a strong-pain condition compared to a no-pain and a weak-pain condition. This suggests that people tend to dissociate themselves from own actions that produce strongly negative consequences for others. The decrease in intentional binding, and hence SoA, over strong pain was particularly pronounced in participants with high empathic concern for unfortunate others.

B9

Intracortical Inhibition Alterations In Multiple Sclerosis

NANTES J.C.*, WHATLEY B., YUSUF A., TAYLOR-SUSSEX R., & KOSKI L.

Multiple sclerosis (MS) is a chronic neurological disease characterized by aberrant immune-mediated neuroinflammation and excitotoxicity. Symptoms, including motor and cognitive dysfunction, often improve spontaneously despite the persistence of tissue damage during remission phases. Prior research has established that GABA-mediated intracortical inhibition (ICI), as measured with transcranial magnetic stimulation (TMS), is relatively high during remission phases. Among MS patients in remission, we sought to estimate the extent to which ICI (short-interval ICI (sICI) and cortical silent period (cSP)) is related to white and grey matter damage, and predicted that higher ICI would be correlated with more severe brain damage.

Fifteen remission-phase patients with MS participated, as did age and sex-matched healthy participants. Conventional magnetic resonance images were acquired, and normalized brain volumes were quantified. TMS of the primary motor cortex was also used to measure sICI and cSP durations, biomarkers for GABA(A) and GABA(B) receptor-mediated ICI, respectively. Compared to controls, patients had significantly lower volumes of white matter ($t = -2.25$, $p < .05$) and cortical grey matter ($t = -2.21$, $p < .05$), as well as longer cSP durations ($t = 2.10$, $p < .05$). Lower white matter volume was correlated with longer cSP ($r = -0.630$, $p < .05$), although grey matter volume did not correlate with cSP ($p > .05$). sICI of patients did not differ from controls ($p > .05$), nor was sICI correlated with brain damage among patients ($p > .05$).

Our data suggest that during the remission phase of MS, cSP prolongation occurs in proportion to white matter atrophy. As cSP is related to GABA(B) activity, it would be advantageous to investigate whether this neurotransmitter receptor affects MS pathophysiology and/or symptom remission.

B10

Amyloid beta peptide generation is determined by key residues within the Amyloid Precursor Protein transmembrane sequence

Oestereich, Felix*; Bittner, Heiko; Weise, Chris; Hildebrand, Peter; Multhaup, Gerhard; Munter, Lisa

The amyloid precursor protein (APP) plays a central role in Alzheimer disease. APP is cleaved into amyloid-beta peptides (Abeta), which vary in length. It has been show that Abeta42 is neurotoxic and is thought to cause Alzeimer disease. However, it is unclear why this pathogenic form of Abeta is generated. We hypothesize that dimerization of the APP transmembrane sequences influences the Abeta peptide generation.
The transmembrane sequence of APP contains two homo-interaction motifs: a GxxxG and a TVxxIT motif. To test our hypothesis, we exchanged the central Glycine33 of the GxxxG motif with each standard amino acid, using a mutagenesis approach. We then accessed the effects on dimerization and its effect on APP processing. We found that weakening of dimerization by 30-35 % led to a 70 % decrease of neurotoxic Abeta42 peptides generation. To investigate the TVxxIT interaction motif, the Threonine residues were exchanged for Valine. We found that the mutant T43V strongly increases Abeta42 levels whereby T48V has inverse effects on Abeta42 generation.
We conclude that APP transmembrane sequence interactions influence formation of neurotoxic Abeta peptides. These sites may be promising targets to reduce neurotoxic Abeta generation and might prevent Alzheimer disease.

B11

Acute moderate hypoxia reduces the number of activated Hypocretin/Orexin neurons in chicken embryos.

JP. Landry*, S. Wiebe, D. Sheen, M. Pompeiano

In birds and mammals, acute mild hypoxia increases autonomic functions such as heart rate, while more extreme hypoxia decreases them (Mortola, 2009; Butler, 1967). Orexin/Hypocretin peptides (H/O) regulate arousal states, feeding, energy metabolism, thermogenesis, and autonomic function. In rodents, H/O neurons receive projections from brainstem nuclei related to cardiovascular and respiratory function (Sakurai et al., 2005), and in turn send projections back to brainstem areas involved in autonomic activity. H/O has been shown to increase heart rate (HR), Arterial Blood Pressure (AP) and medullary sympathetic outflow (Zhang, Fukuda, & Kuwaki, 2005); H/O knockout mice exhibited reduced AP under anesthesia or while awake (Kayaba, et al., 2003). We wanted to determine whether activation of H/O neurons in the chicken embryo hypothalamus is differentially affected by acute mild and moderate hypoxia, and to confirm activation in brainstem areas related to cardiovascular and respiratory function.

White Leghorn chicken eggs were incubated for 18 days (hatching is at 21 days) and then treated for four hours to one of three conditions: moderate hypoxia (10% O2), mild hypoxia (15% O2), and normoxic controls (21% O2). The embryos were anesthetized in ovo, intracardially perfused, and brains were frozen and sectioned using a cryostat. Double florescent immunohistochemical staining for Orexin peptides and c-Fos protein (as a marker of neural activation) was carried out. Brainstem neuronal activation was evaluated using chromogenic immunohistochemical staining of Egr-1 protein (another marker of neural activation).

In all conditions, activated H/O neurons were found primarily in the Paraventricular Hypothalamic Nucleus, with few activated H/O neurons in the Posterior or Lateral Hypothalamic Nuclei. Mild hypoxia did not alter the number of activated O/H neurons, whereas moderate hypoxia reduced the number of activated H/O neurons, particularly in the Paraventricular Nucleus. In the brainstem, moderate hypoxia-induced activation of areas related to cardiovascular and respiratory function was found, namely in the Nucleus of the Solitary Tract, the Dorsal Motor Nucleus of Vagus, and the medullary Ventral Lateral Reticular Formation.

These results suggest that, in chick embryos, moderate levels of acute hypoxia activate brainstem areas that maintain sufficiency in blood O2 levels and reduce metabolism, thermogenesis, and heart rate partly through inhibition of the H/O neuronal population.

B12

Neural network synchronization in the limbic system and its contribution to temporal lobe epilepsy

Zahra Shiri*, Charles Behr*, Shabnam Hamidi*, Rochelle Herrington, Pariya Salami, Maxime Lévesque, Massimo Avoli

Temporal lobe epilepsy (TLE) is one of the most common forms of partial epilepsy whereby recurrent seizures appear in early adulthood. There is often a history of an initial brain insult (such as febrile convulsions, encephalitis, or status epilepticus) years prior to the onset of seizures. Symptoms consist of recurrent partial or secondarily generalized seizures which mostly originate from the hippocampus, the amygdala or entorhinal cortex and are often refractory to medication. In our laboratory, we aim to better understand the cellular mechanisms of TLE using in vivo and in vitro electrophysiological tools in the pilocarpine animal model. Specifically, our studies are exploring: (i) the role of GABAA receptor-mediated mechanisms in ictogenesis and epileptogenesis; (ii) how this latter process is influenced by neurosteroids; and (iii) the involvement of high-frequency oscillations (80-500 Hz) and the activity of single cells during epileptogenesis and ictogenesis.

B13

Transcultural Neuropsychiatry: Considerations for Clinical Research and the RDoC

Daina Crafa*

Neuropsychiatric research commonly uses brain imaging techniques to identify differences and disruptions in neural connectivity and processing in patients. This trend is likely to increase considering the recent push by the U.S. National Institute of Mental Health to develop new ways of classifying psychopathology according to neurobiological properties. This endeavor can be quite useful but it risks essentialism by reducing disorders to a closed set of biomedical traits. There are many reasons to avoid such over-specificity. In particular, it conflicts with findings from cultural neuroscience, which show substantial systematic heterogeneity between culturally diverse populations. These studies show cross-cultural and belief-based differences in brain activity of healthy controls. Many of the brain regions these differences have been observed in are also areas of known disruption in certain disorders. Cultural differences likely exist in the neural processes of patients as well, and any system defining disorder needs to account for this variability. However, culture is an abstract term that is difficult to defined and contains many uncontrolled variables. Current models for operationalizing culture in neuroscientific research risk reliance on stereotypes and can be inappropriate when working with the sensitive patient population. A clinical framework for describing the interaction between brain, culture, and disorder is needed. Building on evidence from current literature, the Culture-Brain-Behavior Interaction (CBB) Model provides a framework for thinking about individual heterogeneity in the brain. Specifically, the CBB Model offers an approach that avoids essentialism when describing psychopathology and extends this approach to human neural diversity. After a brief literature review, the CBB Model will be described. Its relevance to neuropsychiatric research and particularly the RDoC will be examined in detail. Implications for transcultural psychiatry and directions for future research are also discussed.

B14

Bank of Standardized Stimuli (BOSS)- the impact of normative variables and image modality on episodic memory.

Mary O’Sullivan
Martin Lepage
Mathieu B. Brodeur
Bianca Lapierre*

Cognitive processes are complex and difficult to study, since multiple variables can interact and confound with the effect of the independant variable. Therefore, stimuli that are used in experiments require rigourous control and must be normalized. The Bank of Standardized Stimuli (BOSS) was created to provide all researchers normative visual stimuli initially based upon seven variables (name, category, familiarity, visual complexity, object's typicality, manipulability and orientation). There was previous evidence suggesting that these variables influence memory performance, but knowing how it affects episodic memory retrieval would demonstrate the neccessity of controlling these variables in order to prevent confounding effects in memory tasks. Thus, two studies were conducted using BOSS photos in wich performance was analysed as a function of the norms and as a function of the stimulus visual modality (i.e., color photo, greyscale photo, line drawings). Distinct influences were found as name agreement, familiarity, visual complexity, object agreement, viewpoint agreement and color diagnosticity all changed the memory performance by biasing responses. Memory was also found to be modulated by the stimulus visual modality.

B15

The effect of context on recognition of objects

Tatiana Ruiz*
Melissa Maguire
Mathieu B. Brodeur

Recognition of objects is largely influenced by contextual surrounding. To receive insight into the brain activities underlying this context effect, event-related potentials (ERPs) to 20 subjects were recorded while recognizing ambiguous and non-ambiguous objects appearing in consistent and inconsistent scenes. ERPs to non-ambiguous objects were unaffected by context consistency. ERPs to ambiguous objects in consistent scenes were similar to those of unambiguous objects whereas those to ambiguous objects in inconsistent scenes were significantly different over the frontal and posterior areas. Context therefore alters the recognition process of ambiguous objects by bringing it close to that of the non-ambiguous objects.

B16

The characterization of a novel subtype of photoreceptor cell in the mammalian retina

Adele Tufford*, Pierre Mattar, Alanna Watt, Michel Cayouette

Vision depends on the ability of our photoreceptors, both rods and cones, to translate the striking of photons on a specialized pigment into an electrical code. Recently, a third type of photosensitive cell has been identified in the retina, an intrinsically photosensitive retinal ganglion cell (ipRGC). ipRGCs use the photopigment melanopsin, and reside in an entirely separate layer from conventional photoreceptors, making it the third retinal cell type known to directly transmit light. Although small in number, ipRGCs are able to exert powerful control over non-image forming vision – namely our circadian rhythms and pupillary light reflex. Recent work suggests that they also modulate learning, memory, mood, as well as conventional image-forming vision. We have recently discovered and are profiling a novel cone (colour-sensitive) photoreceptor which resides in the ganglion cell layer of the mouse and human retina, and expresses photopigments for both red/green light sensitivity, and blue light sensitivity. We have described their numbers, distribution, and molecular profile over early post-natal development and into adulthood. Our data suggests that these photoreceptors preferentially form synapses with ipRGCs, suggesting they play a functional role in the intrinsic retinal light response. We have also shown that the distribution of these photoreceptors are drastically changed in melanopsin knockout mice. These ganglion-cell layer cones also express all of the classical photoreceptor protein machinery needed to transduce a light signal, leading us to believe that they functionally respond to light. We are now attempting to record intrinsic light-evoked responses from these cones via 2-photon targeted whole cell recordings, from a transgenic mouse line wherein cone photoreceptors are selectively labeled.

B17

Fragmentation of circadian locomotor activity in the Sandy mouse model of schizophrenia

Katarina Stojkovic*, Sanjeev K. Bhardwaj, Lalit K. Srivastava, Nicolas Cermakian

It is well established that patients suffering from schizophrenia often exhibit disrupted sleep and circadian rhythms. In this regard, the study of mouse models of schizophrenia can help elucidate the origin and impact of these disruptions. Sandy mice have a natural loss-of-function mutation in dysbindin-1, a gene associated with schizophrenia in humans. Our aim is to determine whether the dysbindin-1 mutation in Sandy mice leads to alterations in circadian locomotor behaviour, consistent with human symptomatology.

To study circadian locomotor behaviour, we monitored the activity of 8 wild-type and 8 Sandy mutant (Sdy) 10-14-week-old male mice in running wheels. All mice were subjected to different lighting conditions: 12h-light, 12h-dark, constant darkness and constant light (20 lux, then 200 lux). We calculated several parameters such as circadian free-running period, the strength of the rhythm (power of the fast Fourier transform (FFT)), general activity and the distribution of activity over the 24h day, and measures of rhythm stability and fragmentation.

We found that activity bout duration and the number of counts/activity bout, as well as overall activity, were reduced in Sdy mice across all light conditions. Conversely, Sdy mice have more activity bouts/day and lower interdaily stability, indicating more dispersion of activity and more unstable rhythms in the mutants. Accordingly, the power of the FFT was lower in mutants, indicating weaker circadian periodicity. Finally, we found that constant light induced a longer free-running period and more activity outside of the active phase in the Sdy mice.

Taken together, our data show more dispersed activity and weaker rhythms in Sdy mice, suggesting increased fragmentation of circadian locomotor activity in mutants, reminiscent of the sleep and circadian disruptions seen in schizophrenia patients.

B18

The circadian clock in B16 melanoma cells controls tumor growth in vitro and in vivo.

Silke Kiessling*
Nathalie Labrecque
Nicolas Cermakian

Circadian disruption is associated with cancer. Cancer cells exhibit uncontrolled fast cell division. Since tumour suppressor and key cell cycle genes are regulated by circadian clocks, circadian dysfunction might be responsible for increased tumour proliferation. Here we address the possibility that improving circadian rhythms in tumour cells would control their cell cycle and thereby reduce proliferation.
First we characterized the circadian clock in B16 mouse melanoma cells. We found rhythmic clock genes expression in cultured B16 cells only when stimulated by serum, forskolin and dexamethasone shock. Although we were able to induce rhythmic gene expression with dexamethasone in cultured Bmal1-/Per2-LUC transfected B16 cells, rhythms quickly dampened, demonstrating a functional but instable clock in cells. However, when B16 cells were injected in mice and develop tumors, clock gene expression was suppressed and arrhythmic. However, by repeated intra-tumoral dexamethasone treatment we were able to induce rhythmic clock gene expression in vivo.
To test whether melanoma cell growth and tumor growth is regulated by their clock, we measured their proliferation rate after induction of circadian rhythms and tumor growth in vivo. Already two days after single dexamethasone or forskolin treatment we counted ~50% less cells. This effect was even more pronounced after repeated treatments. Moreover, in vivo tumor volumes were reduced by more than 50% after 6 days of dexamethasone treatment.
Together, these results show that the tumour clock can be manipulated to regulate tumour growth. This strategy might become a new, innovative way to slow down cancer progression and thereby improve the outcome of established anti-cancer therapies.

B19

Anatomical Localization of Membrane Progesterone Receptors in Brainstem Respiratory Areas

Karim Habbal*MSc, Vincent Joseph PhD

Progesterone is a potent respiratory stimulant, but the implication of progesterone receptor subtypes on this effect are not known. Progesterone has two main types of receptors, the "classical" nuclear receptor, and the recently identified membrane progesterone receptors. While it has been shown that the nuclear progesterone receptor is expressed in the nucleus tractus solitatrius, a brainstem nuclei involved in respiratory control, much less is known relatively to the expression of membrane progesterone receptors in this area. Accordingly, we used immunohistochemistry to determine the localization of membrane progesterone receptors (mPR) in respiratory-related areas in the brainstem of adult male mice. Serial slices were incubated with antibodies against alpha and beta mPR (mPR-alpha and mPR-beta). A prominent staining for mPR-alpha, and mPR-beta appeared in caudal and rostral parts of the nucleus tractus solitarius (NTS), X and XII nuclei, but while mPR-alpha stained cell bodies, mPR-beta stained fibers. With double fluorescence labeling and confocal microscopy we showed that mPR-alpha is co-localized in cCatecholaminergic neurons (TH+) in NTS. mPR-beta is expressed in TH+ fibers in these regions. Furthermore, 3-beta- hydroxysteroid dehydrogenase (3-beta-HSD), which is involved in progesterone synthesis, was also densily expressed in these regions. These results suggest that mPR-alpha and mPR-beta may play a role in respiratory control, and that local progesterone synthesis may modulate respiratory function.

B20

Impact of freezing of gait on self-perceived balance confidence for functional activities in people with Parkinson’s disease

Lorena R. S. Almeida*, Rebecca A. Gruber, Jan Goldstein Elman, Nádja Negreiros, Guilherme Valença, Elen Beatriz-Pinto, Jamary Oliveira-Filho

Objective: To identify associations of freezing of gait (FOG) and fear of falling in people with Parkinson's disease (PD); and to compare self-perceived balance confidence between patients with and without FOG.
Methods: One hundred and three subjects in two countries with a diagnosis of PD were included in this study. In addition to collecting demographics and clinical data, we assessed patients with the Unified Parkinson's Disease Rating Scale (activities of daily living and motor sections), Freezing of Gait Questionnaire (FOG-Q) and Activities-Specific Balance Confidence Scale (ABC). Subjects were classified as freezers if they scored one or more on the FOG-Q, item three. Mann-Whitney and Pearson Chi-Square tests were performed. Results are shown as median (Q1; Q3).
Results: The sample had a median age of 70 years and 60 subjects (58.3%) were male. Forty-one (39.8%) subjects were classified as freezers. Freezers had longer disease duration, and increased disease severity and functional limitations ($p<=0.001$). Median score on the ABC for freezers was 60 (31.87; 82.19); for non-freezers median score was 75.94 (49.22; 93.12) ($p=0.017$). Median scores were significantly lower for freezers on items: walking around the house, reaching on tiptoes, standing on chair to reach, sweeping the floor, walking across a parking lot/in the mall/being bumped/on a slippery surface ($p<=0.037$). We found borderline p value for the following tasks: walking outside ($p=0.071$), walking down/up a ramp ($p=0.086$) and riding an escalator without holding the rail ($p=0.062$).
Conclusions: Our data suggest that freezers have lower self-perceived balance confidence on tasks that are more challenging to their postural control and that have been described as likely to elicit FOG. These results suggest that people with PD who freeze know when they are at risk of falling, and support the validity of the self- report ABC scale for measuring fear of falling in this group.

B21

Creative, curiosity driven research question generation and project development around Parkinson’s Disease, at an international Parkinson’s Disease Summer School, report on 4 years process improvement.

Paul de Roos(1)*, Krzysztof Nesterowicz(2), Mohammad Fereshtehnejad(3)
1) Uppsala University, Department of Neurosciences, Uppsala, Sweden
2) Jagiellonian University, Medical College, Faculty of Pharmacy, Krakow, Poland
3) Karolinska Institutet, Department of NVS, Stockholm, Sweden

Objective: create an international, multi-professional learning and working environment where research questions are generated and ideas are exchanged which lead participants as a team to create relevant research plans in 9 days on Parkinson's disease (PD).
Background: evidence on factors influencing creativity, team and individual performance were combined with insights from educational literature on learning styles, learning in a workplace and on human motivation. The way traditional academic organizations operate is not aligned optimally with evidence from these areas of science. Here is a possible space for improvement.
Methods: an agile development approach with fast improvement cycles was used for prototyping an environment to deliver the desired objective. A team of trainers with experience in development of learning processes and soft skills, movement disorder specialists and external peer reviewers worked to improve the conceptualization of the program. Formal evaluation and informal non-structured feedback from perspectives of all stakeholders fueled improvement.
Results: A total of 65 multi-professional participants from 22 countries with the age range of 19-33 year participated in 4 Parkinson's Disease Summer Schools (PDSS) during 2009-2012. In total 16 research plans were developed to answer knowledge gaps in different issues on PD. Variation in pre-existing scientific and methodological knowledge,teamwork skills as well as language skills posed the largest challenges.
Conclusions: Introduction of e-learning prior to the Summer School, input of people with PD, assessment, and reduction of differences in academic competency improve the quality of PDSS. Organizational independence, continuous support of partners and growth of our support community have allowed us to develop rapidly a new educational concept which combines problem based and experiential learning principles together with external peer review and involvement of people with PD. Our stated objective has not been achieved yet, but current program improvements may change this.

B22

Gender differences in non-motor symptoms in early, drug naïve Parkinson’s disease

Marcello Moccia*, Marina Picillo, Marianna Amboni, Roberto Erro, Katia Longo, Carmine Vitale, Roberto Allocca, Anna De Rosa, Giuseppe De Michele, Lucio Santoro, Giuseppe Orefice, Maria Teresa Pellecchia, Paolo Barone

Objective: Gender differences in brain structure and function may lead to differences in the clinical expression of neurological diseases, including Parkinson's disease (PD). Few studies reported gender-related differences in the burden of non-motor symptoms (NMS) in treated PD patients, and this matter has not been previously explored in drug-naïve patients. In consideration of this, we assessed gender differences in NMS frequency in a large sample of early, drug-naïve PD patients compared with age-matched healthy controls.
Methods: Two hundred early, drug-naïve PD patients and sixty age-matched healthy controls were included in the study. Frequency of NMS was evaluated by means of NMS Questionnaire. The difference in gender distribution of NMS was evaluated with the X2 exact test; multiple comparisons were corrected with Benjamini-Hockberg method.
Results: Male PD patients complained of problems having sex and taste/smelling difficulties significantly more frequently than female PD patients. Furthermore, men with PD complained more frequently of dribbling, sadness/blues, loss of interest, anxiety, acting during dreams, and taste/smelling difficulties as compared to healthy control men, while female PD patients reported more frequently loss of interest and anxiety as compared with healthy control women.
Discussion: This study shows specific sex-related patterns of NMS in drug-naïve PD. In contrast with previous data, female PD patients did not present higher prevalence of mood symptoms as compared to male PD patients. Comparison with healthy controls showed that some NMS classically present in premotor and early stage of disease (i.e. acting during dreams, taste/smelling difficulties) are more frequent in male than in female patients.

B23

DWT Analysis of the Photopic Hill Reveals New Diagnostically Relevant Features of the Human ERG.

Mathieu Gauvin*, M Hébert, R.K. Koenekoop, J.M. Little, Jean-Marc Lina and Pierre Lachapelle

Purpose: Analysis of clinical electroretinograms (ERG) are usually limited to peak-time and amplitude (Time-Amplitude Domain: TAD) measurements of the a- and b-waves. However, it is well known that retinopathies can also markedly modify the morphology of the ERG signal, a feature potentially of diagnostic significance if it can be quantified in an objective, reliable and reproducible way. We examined if analysis of the ERG in the Time-Frequency Domain (TFD), such as that offered with the discrete wavelet transform (DWT), could meet this challenge.

Method: Photopic ERGs obtained from normal subjects [n=25; 21 flash intensities: -2.23 to 2.84 log cd.sec.m-2 (to obtain the photopic hill: PH); background: 30 cd.m-2] and patients presenting with a variety of retinal disorders (n=10; flash intensity: 0.64 log cd.sec.m-2; background: 30 cd.m-2) were analysed using novel DWT descriptors (20 Hz a, 20 Hz b, 40 Hz a, 40 Hz b, 80 Hz ops, 160 Hz ops, sigma Energy, delta Variance and the Hölder exponent) and the resulting TFD PHs were compared [Pearson's correlation coefficient (r), ANOVAs] to the more traditional TAD PH (i.e. b-wave amplitude).

Results: The luminance-response functions generated using the TFD descriptors all presented with a photopic hill-like shape that was nearly identical to the classical PH obtained using b-wave amplitude measurements, with a match that was nearly perfect is some instances (r values varying between 0.82 to 0.98; p<.001), suggesting that the selected TFD descriptors appraised attributes of the cone ERG that co-varied with those obtained with TAD measures. Of interest, the variability (coefficient of variation: CV) of DWT measurements was found to be significantly less compared to TAD measures of the same ERGs. For a 92% reduction in the signal to noise ratio (SNR) of ERGs, the CV of TAD measures increased by more than 100% compared to less than 50% for DWT measures. Finally, analysis of pathological ERGs showed that one (or more) TFD descriptor could be affected first (or significantly more) depending on the type of retinopathy and/or stage of.

Conclusion: This study demonstrates, for the first time, that TFD analysis of the photopic ERG, such as that achieved with DWT derived descriptors, can be used to quantify the ERG beyond what can be accomplished using a TAD approach. Furthermore, our results also raise the possibility that a given retinopathy may impair more specifically one (or more than one) DWT descriptors, thus offering an alternative approach to identify (early diagnosis), classify and possibly stage the different pathophysiological processes that impair retinal function; an information that will be highly relevant especially in diagnostically challenging cases where the retinal disorder has not yet impaired TAD measures of the ERG. Funded by the CIHR and the Réseau-Vision of the FRQS.

B24

Identifying regulatory mechanisms of long term memory

Josephy, S.*, Aboulkassim, T., Maira, M., Pirvulescu, I., Menard, C., Quirion, R., Saragovi, U.

The process of memory formation has intrigued scientists for decades, but the nature of memory and mechanisms of acquisition, storage and retrieval remain obscure. In broad terms, memory can be divided into two types: short-term memory (STM) and long-term memory (LTM). In LTM information is acquired, processed, and stored; and the stored memory is retrieved to become functional when required.

We studied the role of the TrkA receptor for Nerve Growth Factor (NGF) in memory. TrkA receptors belong to the family of Trk tyrosine-kinase receptors, and are expressed in the cholinergic neurons responsible for memory. Ligand activation of TrkA is involved in the survival, differentiation, phenotype, synaptic strength and plasticity, and proper function of cholinergic neurons; plus it plays a key role in memory. In pathological states activation of TrkA enhances the cholinergic phenotype, induces neurotrophic activities, and rescues function. This has been published for age-associated memory impairment, and more recently for a model of memory impairment, the APP-overexpressing transgenic mouse.

A small molecule partial agonist of TrkA (termed D3) was very efficient at rescuing memory. D3 proved to be effective at improving short-term memory and long term memory in ageing animal models, and short-term memory in the APP models. These findings led to the hypothesis that drug-based activation of TrkA (with the D3 partial agonist of TrkA) might have an impact on memory acquisition, processing, storage or retrieval.

Unexpectedly, when D3 was tested in normal young mice, it had no effect on short term memory but it conveyed persistent long term memory impairment, in the absence of toxicity. Our goal now is to conduct further research in finding an explanation for the aforementioned LTM impairment in the wild type mice.

We measured molecular pathways that are known to be involved in LTM including CaMKII, CREB, MAPK, PKC/PKM and AKT. Such molecules are studied in the aforementioned D3-treated WT mice as in aged memory impaired mice. We are also testing the effect of the compound on synaptogenesis and neurogenesis in hippocampal primary neurons in vitro. By elucidating the etiology of the conveyed LTM impairment we hope to reveal unknown mechanisms in the process of memory formation.

B25

Role of RUNX1 in Glioblastoma Tumour-Initiating Cells

Karen Hei Man Fung* and Stefano Stifani

Glioblastoma (GBM) is the most malignant and common adult brain cancer. Despite the advances in treatment, the median survival of GBM patients remains less than two years due to therapeutic resistance and cancer recurrence after surgery. GBM displays a cellular complexity consisting of a hierarchy of poorly differentiated neural cells, including GBM tumour-initiating cells (TICs). Since TICs are the proposed culprit of the inevitable GBM recurrence, this particular subpopulation has the potential to be key targets for new GBM therapeutic approaches. However, the molecular mechanisms of TIC tumorigenic potential remain poorly defined. We have observed that the transcription factor RUNX1 is expressed in a subpopulation of GBM cells with stem-like features in both GBM surgical specimens and primary cultures of GBM-derived TICs. More importantly, inhibition of RUNX1 activity impairs the proliferation of TICs, leading to increased cell cycle exit. These results identify RUNX1 as a factor important for TICs behaviour and suggest that RUNX1 and/or some of its target genes may represent potential therapeutic targets for GBM therapies.

B26

A method for Measuring Cross-Frequency Phase Amplitude Coupling in Neural Oscillations

Soheila Samiee*, Sylvain Baillet

Neural oscillations with different frequencies can interact in several ways. Recent evidence suggests that this interaction, which is called cross frequency coupling (CFC), may serve as a functional role in neuronal communication, computation and memory. Among possible cross frequency coupling types, modulation of amplitude of high frequency oscillations by the phase of a lower frequency rhythm has been received a particular interest. Several methods for measuring this coupling have been introduced in the last decade; however, no single method has been chosen as a gold standard so far. In the present work, a method for assessing cross frequency phase amplitude coupling with higher temporal resolution than traditional measures is presented. In this method, the low frequency that modulates the amplitude of a higher oscillation is detected automatically using power spectrum of high frequency envelope, and the coupling strength of this pair is measured using mean vector length algorithm. The results of evaluating our contribution using the synthesized data show that the measure defined in this way is well suited for assessing the intensity of coupling, as well as finding the dominant coupled frequencies in signal. Finally, the result of applying the proposed measure to actual MEG data of median nerve stimulation is presented, which shows an emerging coupling in gamma band around 90 Hz right after stimulation in relevant primary somatosensory cortex.

B27

Inhibition of spinal PKM zeta reverses persistent referred allodynia induced by injection of acidic saline in the rat thigh

Hibatulnaseer Nasir*, Stephanie Ding, André Laferrière, Terence Coderre

Background. The transition from acute to chronic pain relies on central sensitization occurring in the spinal cord dorsal horn. While there are several mechanisms contributing to the initiation of nociceptive plasticity, a unique and pivotal role has recently been ascribed to the persistently active atypical protein kinase M zeta (PKM zeta) in the maintenance of nociceptive neuroplasticity in the spinal cord dorsal horn (SCDH). PKM zeta is upregulated in SCDH during persistent nociception, and its inhibition reverses short-lasting allodynia that depend on SCDH neuroplasticity.

Methods. We produced long-lasting persistent referred allodynia in the rat hind paw using two injections of low-pH saline into the gastrocnemious muscle. The myristoylated protein kinase zeta – pseudosubstrate inhibitor (ZIP), its inactive control peptide scr-ZIP, the PKC inhibitor NPC and a control vehicle were injected intrathecally either at 24 h, or one week after the second acidic saline injection. Rats were tested for mechanical paw withdrawal thresholds using von Frey filaments up to 5 weeks following drug treatment. In addition, PKM zeta, and PKC zeta levels were measured using Western blot.

Results. Inhibition of PKM zeta using ZIP reversed mechanical allodynia in acidic saline-treated rats when injected either 24 h or one week after pain induction. Importantly, a non-specific PKC inhibitor and control peptides are unable to yield this effect. PKM zeta protein level is significantly decreased in ZIP treated compared to scr-ZIP treated animals either at 24h or one week after the drug.

Conclusion. We provide novel evidence for the contribution of PKM zeta, but not PKC, to the maintenance of persistent, SCDH-dependent nociceptive plasticity, and long-lasting referred allodynia created by repeated injections of low-pH saline into the gastrocnemious muscle of the rat. These results add to the significance of PKM zeta in the maintenance of maladaptive neuroplasticity contributing to persistent pain.

B28

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

Cornea V.M *., Jong Y-J. I., Ribeiro-da-Silva A., O'Malley K.L. and Coderre T.J.

Metabotropic glutamate 5 receptors (mGluR5) play a key role in the modulation and plasticity of pain especially in dorsal horn spinal cord where they represent a promising therapeutic target. mGluR5's are G protein coupled receptors (GPCR's) that have been shown to be expressed on cell surface and intracellular membranes (including the nuclear membranes) of striatal neurons in culture and in visual cortex. While it has been shown in neuronal cultures that the activation of plasma membrane versus nuclear membrane mGluR5 stimulates different signalling pathways leading to the activation of different genes, the physiological role of the nuclear-located receptors of the GPCR family remains unknown.
Quantitative immunogold electron microscopy was used to show for the first time that mGluR5s are also localized on the nuclear membrane of neurons in rat spinal cord dorsal horn. Moreover, we found that mGluR5s on the nuclear membrane are significantly increased in superficial dorsal horn neurons from neuropathic rats (seven days after spared nerve injury: SNI) versus control rats, without detectable changes in the plasma membrane-, intracytoplasmic-, or intranuclear areas. Western blots of subcellular fractions from dorsal horn spinal cord demonstrate that mGluR5 protein is increased 1.6 fold in the nuclear fraction from neuropathic rats versus control rats, while no changes are detected in the plasma membrane fraction. The neuronal glutamate transporter protein, EAAT3, level remained unchanged after SNI. In agreement with striatal mGluR5 studies, downstream signalling molecules induced by intracellular mGluR5 activation, such as phosphorylated extracellular signal-regulated kinase (ERK) and activity-regulated cytoskeletal-associated protein (Arc), showed a significant increase in the nuclear fraction of neurons from the spinal dorsal horn of neuropathic rats. We used immunocytochemistry to extend and confirm these findings, showing that intrathecal glutamate-induced activation of spinal dorsal horn mGluR5s led to an increase in Fos labelling. Increased Fos staining was prevented by previous injection of an inhibitor of neuronal glutamate transporters.
In sum, anatomical and biochemical evidence from spinal cord ex vivo studies support and extend mGluR5 studies in dissociated striatal cultures. This is the first evidence that nuclear-located mGluR5s in spinal dorsal horn neurons are functionally active during neuropathic pain. Moreover, this is the first model which shows the physiological function of a nuclear GPCR.

Funded by CIHR Strategic Training Program in Pain: Molecules to Community award to VC and CIHR grants to TJC and ARdS and NIH grants to KLO.

B29

A Computational Model of Systems Memory Reconsolidation

*Peter Helfer
Thomas Shultz
Oliver Hardt
Karim Nader

Memory reconsolidation, the re-stabilization of consolidated memories after reactivation-induced destabilization, has received considerable attention in recent years. Nevertheless, the neural processes underlying the phenomenon remain elusive. With the aim of contributing to the development of a theory in this area, we here present a computational model of reconsolidation at the “systems” level. The model is an extension of TraceLink, which has previously been used to account for a range of memory phenomena related to consolidation.

B30

Netrin-1 function in myelin maintenance assessed using an oligodendrocyte precursor cell-organotypic slice culture transplant model

Jenea M. Bin*, Sarah-Jane Bull, and Timothy E. Kennedy

Netrin-1 and its receptor deleted in colorectal cancer (DCC) are required for the maintenance of paranodal axoglial junctions in myelin. Neurons and oligodendrocytes express netrin-1, but it has not been determined whether paranode maintenance requires netrin-1 made by the oligodendrocytes, the axon, or both. To address whether cell-autonomous expression of netrin-1 by oligodendrocytes is required for myelin maintenance, we transplanted oligodendrocyte precursor cells (OPCs) from netrin-1 knockout pups into organotypic cerebellar slices from shiverer mice. Shiverer mice lack myelin basic protein (MBP), allowing myelin produced by transplanted cells to be visualized using immunohistochemical staining for MBP. Preliminary results indicate that the paranodes of myelin generated by both netrin-1+/+ and netrin-1-/- oligodendrocytes initially form normally; however, nine weeks post-transplant the caspr immunoreactive domain is significantly longer in slices transplanted with netrin-1-/- OPCs. This phenotype mimics that previously observed in netrin-1-/- organotypic slices and provides evidence that the defect in paranode maintenance arises from loss of netrin-1 expression by mature myelinating oligodendrocytes. Our results illustrate the utility of this transplant model to isolate both short- and long-term functional requirements of specific proteins expressed by oligodendrocytes, with particular use for studying mutations for which no conditional knockout mice are available, or mutations that are lethal before myelination is complete in vivo.

B31

Protein Digital Nanodot Gradients with Adjustable Reference Surfaces to Investigate Axonal Migration in Response to Nanopatterned Cues

S. G. Ricoult*, G. H. Thompson-Steckel, G. Ongo, J. P. Correia, T. E. Kennedy, and D. Juncker

Cell navigation operates in response to inhomogeneous distribution of extracellular cues. There is thus an incentive to create deterministic protein patterns in vitro to address how the density and distribution of these cues directs cell migration. Although many experimental results were reported, (i) the gradients are often limited in range, produced as continuous patterns, and difficult to quantify; moreover (ii) the reference surface (RS) – the area surrounding the patterns – is often not well controlled nor characterized, and in fact methods to adjust it are missing. Here, we address these two points by introducing digital nanodot gradients (DNGs) and a RS with tunable affinity.

We present lift-off nanocontact printing using a high resolution, low cost photopolymer stamp, to pattern proteins as millions of 100 nm dots according to any predefined pattern in 5 min. Tens of DNGs composed of 200 nm dots with two-dimensional unidirectional increase in spacing were designed so as to form surface gradients with a dynamic range of up to 4400. The slope of the DNGs can follow linear, quadratic, or exponential slopes that can be quantified with deterministic spot localization, thus offering new opportunities to study cell navigation.

To control cell-surface affinity, a range of RSs composed of different ratios of poly-D-lysine (PDL), with high cell-surface affinity, and polyethylene glycol (PEG) with low cell-surface affinity, were produced. The response of a cell to a patterned cue with a these RSs revealed the effect of the RS on cell navigation. High affinity RSs (high % PDL) rendered neurons insensitive to the DNGs, while low affinity RSs (high % PEG) resulted in cells responding non-specifically to the gradient. In contrast to these extremes, we identified intermediate RS-affinities in which cells respond to patterned proteins via appropriate cell-type specific signal transduction and where axons migrate towards the higher density portion of the protein gradient. Our findings indicate that choices made by cells in response to patterned protein depend strongly on the response to the RS.

Substrate-bound protein gradients with adjustable RSs will facilitate the study of cell migration by enabling the attribution of movement to patterned protein and the creation of complex gradient geometries. These studies will provide a better understanding of development and help us design better therapies for regeneration.