Poster Sessions – Full Abstracts

Session 1: Monday May 21 2012

A - Development

1-A-55 Sim1 is required for proper migration and axon projection of V3 interneurons during development of the mouse spinal cord

Jake Blacklaws[1], Ying Zhang[1]

[1]Dalhousie University

The V3 interneurons in the vertebrate spinal cord are a group of excitatory, commissural neurons which play important roles in producing balanced and stable gaits. In the developing mouse spinal cord, V3 interneurons arise from the ventral-most progenitor domain, p3. V3 neurons enter the post-mitotic stage at embryonic day (E) 9.5, at which time they begin to express the transcription factor single-minded 1 (Sim1). The specific role of Sim1 during V3 development, however, is still largely unknown. In this study, we used several transgenic mouse models to knock out the expression of Sim1 and to examine the developmental fate of mutant V3 neurons. In Sim1 mutants, V3 neurons maintain their excitatory and commissural characteristics, but show a defective migration and axon projection pattern. After leaving the p3 domain, control V3 neurons migrate dorsally and laterally and eventually, at postnatal day 0 (P0), settle as clusters in Rexed's Lamina VIII, VII, and IV-V regions. In Sim1 mutants, however, the V3 neurons lack arganized migration. At P0, Lamina IV-V subgroup cells are mostly absent, while Lamina VII cells are significantly increased, showing that Sim1 mutant cells cannot correctly migrate to their dorsal position. We also found a drastic decrease in the number of cells with rostrally projecting axons in the Lamina VIII V3 domain. The the same time, expression of the calcium-sensing protein Calretinin decreased in a subgroup of Sim1 knockout neurons, which suggests that Sim1 may affect the development of spinal V3 neurons through some Ca signaling pathways.

1-A-56 NMDA receptor GluN2A subunits are required for normal dendritic development of dentate granule neurons

Joana Gil-Mohapel[1], Timal S Kannangara[1], Crystal A Bostrom[1], Andrea Flamank[1], Lee Thompson[1], Robyn M Cater[1], Brian R Christie[1]

[1]University of Victoria

N-methyl-D-aspartate (NMDA) receptors, primarily known for their involvement in synaptic plasticity and learning and memory, have also been shown to modulate adult hippocampal neurogenesis. However, the exact contribution of the GLuN2 subunits to neurogenesis is currently unknown. We used mice that lack the GluN2A subunit to determine its role in cell proliferation, neuronal differentiation, and maturation of dentate granule cells (DGCs) in the adult hippocampus. Immunohistochemistry against the endogenous cell cycle markers Ki-67 and proliferating cell nuclear antigen (PCNA) and the immature neuronal marker neurogenic differentiation protein (NeuroD) revealed that the deletion of the GluN2A subunit alone did not alter adult hippocampal cell proliferation and neuronal differentiation. However, a significant decrease in total dendritic length and dendritic complexity was observed in immature DGCs from GluN2A knock-out mice. Furthermore, immature cells that lacked GluN2A also showed a localized increase in spine density in the middle molecular layer, a region innervated by the medial perforant path. Interestingly, alterations in dendritic morphology and spine density were no longer seen in mature cells. These results indicate that although the GluN2A subunit does not play a role in the proliferation and differentiation stages of the neurogenic process, it is involved in the maturation of DGCs, contributing to the establishment of their neuronal morphology. They also suggest that loss of the GLuN2A subunit can impact synaptic physiology in DGCs.

1-A-57 Consequences of deletion of muscarinic receptors on functional organization of the mouse primary visual cortex

Marianne Groleau[1], Mattieu Vanni[1], Jurgen Wess[2], Christian Casanova[1], Elvire Vaucher[1]

[1] School of Optometry, University of Montreal, [2]Molecular Signaling Section, National Institute of Diabetes and Digestive and Kidney Diseases

Acetylcholine is released in response to visual stimulation in the primary visual cortex (V1) and modulates the neuronal activity through muscarinic receptors (mAChRs). It is also involved in the postnatal V1 maturation induced by visual experience. The aim of this study was to determine the involvement of the mAChRs in the functional organization of V1 of mice using mAChRs KO mice. The brain was imaged by optical imaging of intrinsic signals to measure the spatial frequency (SF) selectivity, contrast sensitivity and retinotopic maps in V1 of urethane anesthetized mice lacking M1 (n=7), M1/M3 (n=10) or M2/M4 (n=11) mAChRs and wild type animals (WT, n=15). Retinotopic maps along elevation and azimuth were measured using the continuous paradigm (Kalatsky and Stryker, 2003). Hemodynamic reponses for full-field sine wave gratings were also analyzed and amplitudes were measured as a function of contrast and SF. Finally, to evaluate the specificity of the connectivity, the "scatters" (Cang et al., 2005) of the phase were calculated. The size of V1 in elevation was decreased in M1 (LSD, p=0.032) KO compared to WT and an increase of the scatter along

azimuth was observed in M2/M4 KO mice compared to WT (t-test, p=0.017). The visual field along elevation was increased in M2/M4 KO mice compared to WT (t-test, p=0.017). Finally, the amplitude from the elevation retinotopic map was increased in M1 KO compared to M1/M3 KO (LSD, p=0.021). These in vivo results demonstrated that the lack of mAChRs leads to an altered retinotopic map and a change in the functional organization of V1.

1-A-99 Inhibition of histone deacetylases in neural stem cells and progenitors of the postnatal and adult CNS

Stacey Herzer[1], Stacey B Foti[1], Andrew D Moll[1], A. Jane Roskams[1]

[1]University of British Columbia

Chromatin remodeling is accomplished in part by histone deacetylases (HDACs) which promote histone-histone binding, and thereby prevents transcriptional activators from accessing DNA, consequently silencing gene activation. Neural development is contingent on multiple signaling events that drive progenitor cells through several discrete stages prior to terminal differentiation, and these transitions appear to require HDAC activity. Results from our lab suggest that HDACs 1 and 2 are key to regulating sequential shifts in state from neural progenitor cell to mature neuron or glial cell in the olfactory system. However, we have little understanding of how HDAC activity impacts distinct progenitor subtypes involved in postnatal neurogenesis, particularly in the adult subventricular zone (SVZ). We found that in vivo treatment with valproic acid (VPA) causes a significant decrease in proliferation in the anterior SVZ, rostral migratory stream, and olfactory bulb as measured by analysis of injected thymidine analogues. Similar effects were observed in vitro with both VPA and trichostatin A, resulting in the formation of fewer and smaller spheres. Subsets of neural stem cells (NSCs) are radial glia-like in morphology, and express several characteristic genes, including brain lipid binding protein (BLBP; aka FABP7) which may be epigenetically regulated. We are testing if BLBP positive cells are the main NSCs in the adult SVZ, and are treating these cells from postnatal and adult mice with HDAC inhibitors to manipulate their maintenance, proliferation, and differentiation in vitro.

1-A-100 Role of neogenin in olfactory epithelium development

Joseph Kam[1], David da Silva[1], Jean-Francois Cloutier[1]

[1]McGill University

The birth and differentiation of Olfactory Sensory Neurons (OSNs) is critical for our ability to detect and decode odorant information from the environment. To better define the molecular mechanisms that control neurogenesis in the Olfactory Epithelium (OE), we have examined the role of the transmembrane protein Neogenin in olfactory neurogenesis. Neogenin has been implicated in the regulation of diverse processes during development of the nervous system. Furthermore, multiple families of proteins can bind to the extracellular region of Neogenin including Netrins, Repulsive Guidance Molecules (RGM), and Bone Morphogenetic Proteins (BMP). Using various approaches, we have examined the pattern of expression of Neogenin and of its ligands in the OE. Our analyses have revealed that Neogenin is expressed at high levels in the basal progenitor cell region of the OE and in mature OSNs. In contrast, the expression of RGM-b, is restricted to immature OSNs. These patterns of expression suggest that RGM-b-Neogenin interactions may regulate the differentiation of progenitor cells into OSNs. To test this hypothesis, we have examined the development of OSNs in Neogenin mutant mice. Ablation of Neogenin expression leads to an increase in the number of proliferating cells, and to a decrease in the number of mature OSNs, indicating that Neogenin regulates the differentiation of progenitor cells into OSNs. We are currently assessing the role of RGM-b in this process by using both in vitro and in vivo approaches. Our findings therefore define a new role for Neogenin in olfactory neurogenesis.

1-A-101 Transcriptional regulation of the retinoblastoma family member p107 by Dlx homeobox genes during central nervous system development

Jamie Zagozewski[1], Molly Pind[2], [1]David D Eisenstat

[1]University of Alberta, [2]University of Manitoba

Sensory experiences exert powerful influences on developing brain by directing coordinated functional and morphological plasticity in order to form neural circuits optimized to process this sensory information. Previous work in our lab, using calcium imaging of 100s of neurons in the Xenopus tadpole retinotectal system, has demonstrated that different patterns of natural visual stimuli can either potentiate or depress visual evoked neuronal responses. It remains unknown, however, whether this plasticity in developing neural circuit functional responses is coupled to structural changes. Here, using rapid time-lapse two-photon imaging of neural network activity and single neuronal growth within the unanesthetized developing brain, we demonstrate morphological correlates of visual stimulation-induced functional plasticity. Further, we identify the transcription factor MEF2A/D as a central regulator of the metaplastic state determining neuronal response to plasticity inducing input. White noise (WN) visual stimuli that do not induce functional plasticity, yet alters response to plasticity-inducing stimuli transiently activates caspase-3, -7 and leads a rapid degradation of MEF2A/D through a NMDAR-dependent mechanism. Knocking down MEF2A/D alone is sufficient to induce a shift in functional and morphological outcomes responses

to visual experience driven plasticity. NMDAR-caspase-MEF2A/D represents a novel pathway regulating intrinsic properties of neuronal plasticity response to sensory experience.

1-A-102 JNK3 palmitolylation regulates neuron development

Xun Zhou[1], Guang Yang[1], Yuan Liu[1], Kaiyun Yang[1], Rui Liu[1], Shanshan Zhu[1], Ainsley Coquinco[1], Wendy Wen[1], Luba Kojic[1], William Jia[1], Max Cynader[1]

[1]University of British Columbia

The physiological and pathological roles of the c-jun N-terminal kinases (JNKs) have been intensively studied in the central nervous system. However, the mechanisms underlying the regulation of different JNK isoforms that allow them to fulfill their specific roles in brain development and neurological diseases are poorly understood. In our previous work, we found that zDHHC17/HIP14, a palmitoyl acyl transferase (PAT), regulates JNK activity in ischemic stroke. Here, we report an isoform-specific regulation of JNK3 by palmitoylation, a posttranslational modification, and the involvement of JNK3 palmitoylation in axonal development and post-excitotoxicity neuronal plasticity. Two cysteine residues at the COOH-terminus of JNK3 are required for dynamic palmitoylation, and a group of PATs is able to palmitoylate JNK3 expressed in the HEK293 cell line. Expression of palmitoylation-deficient JNK3 in cultured hippocampal neurons increases axonal branching, whereas expression of constitutively pseudo-palmitoylated JNK3 results in reduced axonal branches. The Wnt family member Wnt7a, a known modulator of axonal branching and remodelling, regulates the palmitoylation of JNK3. Interestingly, NMDA-induced excitotoxicity also induces JNK3 palmitoylation, which may contribute to the post-stress regulation of neuronal plasticity. Our results demonstrate that protein palmitoylation is a novel mechanism for isoform-specific regulation of JNK3 and suggests a potential role of JNK3 palmitoylation in neuronal development and post-stress neuronal plasticity.

B – Neural Excitiability, Synapses, and Glia: Cellular Mechanisms

1-B-1 Cellular prion protein regulates N-methyl D-aspartate receptors in hippocampal pyramidal neurons

Thomas Bartlett[1], [2]Changiz Taghibiglou, [1]Neil R Cashman, [1]Yu Tian Wang

[1]UBC, [2]University of Saskachewan

The cellular prion protein (PrpC) is expressed in many mammalian tissues and has been highly conserved through evolution. In prion diseases, a change in the folding of PrpC results in the beta-sheet-rich PrpSc isoform which causes drastic neurodegeneration. Disruption of PrpC native function is one hypothesized mode of action for PrpSc, implying that PrpC subserves a protective function in neurons. Consistent with other investigators, we have found an upregulation of N-methyl D-aspartate receptor (NMDAR) function in mice lacking PrpC (Prpnull). We find that PrpC normally interacts with NMDARs containing GluN2D/GluN2B subunits and hippocampal neurons from Prpnull mice have greater surface expression of these subunits. Consistent with these observations we see an increase in GluN2B-mediated NMDAR EPSCs in CA1 neurons of hippocampal slices and an increased total cellular expression of GluN2B in hippocampal tissue from Prpnull mice. Based on the importance of NMDARs containing GluN2B for the induction of synaptic plasticity, we undertook long-term potentiation and long-term depression experiments on hippocampal slices from 3-month old Prpnull, PrpC overexpressing (tg20) and wild-type mice. These experiments have allowed us to evaluate the importance of PrpC for synaptic plasticity in the hippocampus.

1-B-2 Nrf2 is epigenetically silenced in neurons

Karen Bell[1], Bashayer Al-Mubarak[1], Giles E Hardingham[1]

[1]University of Edinburgh, Scotland

We recently demonstrated that Nrf2, a transcription factor and master regulator of Phase II antioxidant defences, has a key role in neuronal ischemic preconditioning. Neuronal protection originated from astrocytes not neurons, as no Nrf2 activation occurred in pure neuronal cultures. We currently aim to determine whether the Nrf2 pathway is indeed paradoxically inactive in neurons. Specific overexpression of Nrf2 in neurons affords significant protection from ischemia and upregulates Nrf2 target products, suggesting the pathway can functionally respond when Nrf2 is present. Basal Nrf2 mRNA expression levels are significantly higher in astrocytes than in neurons, and Bach-2 a major repressor of Nrf2 activity is significantly increased in neurons, possibly suggesting that the inactivity within neurons stems from a decreased availability of Nrf2, as opposed to impaired downstream pathway function. ChIP experiments reveal a significantly lower association of acetylated histone H3 (a marker of transcriptional activity) at the Nrf2 promoter in neurons versus astrocytes. Application of histone deacetylase inhibitor TSA increases the association of acetylated histone H3 at the Nrf2 promoter in neurons and boosts neuronal Nrf2 mRNA expression. Fluorescent assisted cell sorting of mouse cortical tissue is currently underway to determine whether Nrf2 levels also differ in adult cell populations in vivo. Given that oxidative stress is a key pathological feature of numerous neurodegenerative diseases and stroke, an improved understanding of Nrf2 regulation in neurons is relevant.

1-B-3 Functional modulation of P2X4 receptor channels by UDP-activated P2Y6 receptors.

Louis-Philippe Bernier[1], Ariel Ase[1], Éric Boué-Grabot[2], Philippe Séguéla[1]

[1]McGill University, [2]Université Bordeaux

The microglial ATP-gated P2X4 receptor channel actively participates in central sensitization, making its functional regulation a key process in chronic pain pathologies. The P2Y6 GPCR, coupled to phospholipase C (PLC) via Gq/11 proteins, is also expressed in microglia, where it is involved in the initial response to nerve injury, triggering phagocytosis upon activation by UDP. Recent reports have shown that expression of both P2X4 and P2Y6 is upregulated in activated microglia following nerve injury. Here, we show that in primary mouse microglia, P2X4-mediated calcium entry is partially inhibited by P2Y6 activation. In resting microglia, pre-application of UDP also restricts the dilation of P2X4 into a large-conductance pore measured via YO-PRO-1 uptake assay. In LPS-activated microglia, both calcium and YO-PRO-1 entry through P2X4 were decreased following P2Y6 stimulation. We reconstituted this modulation in Xenopus oocytes: P2Y6 activation decreased P2X4 current amplitude, activation and desensitization rates, and reduced P2X4 permeability to the large cation NMDG. This functional interaction was blocked by U73122, a PLC inhibitor, but unaffected by PKC blocker staurosporine. This suggests that P2X4 inhibition relies on PLC-mediated hydrolysis of PI(4,5)P2, a membrane-bound phosphoinositide recently shown to directly potentiate P2X4 channel function. Extracellular levels of ATP and UDP in the spinal cord are increased following nerve injury, functional interactions between P2X4 and P2Y6 receptors are therefore critical in regulating pain-inducing microglial responses.

1-B-4 Characterizing synaptic CaMKII translocation following glutamate plus glycine stimulation in rat hippocampal neurons

Claire Bomkamp[1], Robert M Bragg[1], Jacqueline K Rose[1]

[1]Western Washington University

Previous studies report that bath application of glutamate glycine results in accumulation of calcium/calmodulin-dependent protein kinase II (CaMKII) at synapses. The role of synaptic CaMKII translocation remains unknown. To further characterize the effects of glutamate and glycine stimulation, two lines of investigation were pursued. First, the duration of CaMKII translocation was examined following glutamate plus glycine stimulation on rat hippocampal neurons. Immunocytochemistry was performed with antibodies to CaMKII and PSD-95 to measure synaptic CaMKII expression levels following either a 15- or 60-minute incubation after a one-minute 100 µM glutamate plus 10 µM glycine stimulation. Preliminary results indicate that the CaMKII synaptic association after 60 minutes persists at levels seen after 15 minutes. A second set of experiments examined if the plasticity potential of neurons previously stimulated with 100 µM glutamate plus 10 µM glycine was altered by administering a chemical induction of LTP (cLTP) protocol following glutamate and glycine stimulation. Surprisingly, immunocytochemistry revealed that neurons previously stimulated with glutamate and glycine showed a decrease in synaptic CaMKII localization following cLTP. By comparison, when cLTP induction preceded glutamate glycine application, high levels of synaptic CaMKII were observed suggesting that order of stimulation may influence the direction of plasticity.

1-B-5 Palmitoylation of δ-catenin is essential for activity-dependent regulation of synapse adhesion and structure

Stefano Brigidi[1], G. Stefano Brigidi[1], Yu Sun[1], Shernaz X Bamji[1]

[1]University of British Columbia

Cadherin adhesion molecules are known to be essential players in activity-dependent changes in synaptic structure as well as the maintenance of LTP. However, it is unclear how fluctuations in neuronal activity translate to changes in cadherin-based adhesion at the synapse. Here we demonstrate that activity increases the palmitoylation of δ-catenin, a brain-specific component of the cadherin adhesion complex, enhancing its recruitment to synapses and its binding to N-cadherin. Neuronal activity significantly stabilizes N-cadherin at the synapse. However, knockdown of δ-catenin or expression of δ-catenin mutants that disrupt its palmitoylation or its binding to cadherin, abolishes activity-induced stabilization of N-cadherin at synapses. Importantly, this translates to an abolishment of activity-induced changes in synapse density and maturation. We propose that regulation of palmitoylation-dependent association between δ-catenin and N-cadherin underlie activity-induced changes in synapse structure and efficacy.

1-B-6 Anti-inflammatory effects of statin-class drugs on primary cultured microglia

Matthew Churchward[1], Kathryn G Todd[1]

[1]University of Alberta

As the primary immune cells of the central nervous system, microglia play critical roles in defence against foreign agents and clearance of cellular debris, as well as a myriad of lesser explored roles in development, homeostatic regulation, and synaptogenesis. In some neurodegenerative conditions inflammatory activation of microglia can exacerbate the underlying disease state, accelerating or causing further damage to afflicted tissue.

The statin class of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors have been proposed to have anti-inflammatory and neuroprotective effects in addition to their well-characterized effects at lowering serum cholesterol. We have sought to investigate the anti-inflammatory mechanism of simvastatin, a commonly prescribed HMG-CoA reductase inhibitor in primary cultured microglia from neonatal rats. We have found that simvastatin both reduced cellular cholesterol levels and inhibited activation of microglia in response to potent inflammatory stimuli in a dose dependent manner. This was accompanied by increased cell survival and reduced secretion of pro-inflammatory cytokines (including TNFα and IL1β) and nitric oxide. In contrast, direct manipulations of cholesterol levels using the biochemical reagent methyl-β-cyclodextrin rendered microglia more reactive to inflammatory stimuli and allowed us to resolve cholesterol-dependent and independent effects of HMG-CoA reductase inhibition.

1-B-7 Alterations in HCN channel function contribute to neuropathic pain processing in medial prefrontal cortex

Steven Cordeiro Matos[1], Zizhen Zhang[1], Philippe Seguela[1]

[1]Montreal Neurological Institute - McGill University

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels regulate a broad range of critical neuronal properties including dendritic integration and synaptic transmission. Recent data suggest that cationic currents mediated by HCN channels (Ih) also play a role in peripheral and spinal pain pathways by facilitating ectopic firing and hyperexcitability. Consistently, mice in which HCN2 channels were specifically deleted in Nav1.8-positive DRG nociceptors showed no neuropathic pain in response to mechanical and thermal stimuli. However, little is known regarding the role of Ih in supraspinal pain pathways. The medial prefrontal cortex (mPFC) is known to be involved in affective aspects of chronic pain and exhibits high HCN channel expression. Using the rat spared nerve injury (SNI) model of chronic neuropathic pain and whole-cell patch-clamp recordings in layer II/III pyramidal neurons of the mPFC, we observed changes in HCN channel properties characterized by a hyperpolarizing shift in the voltage-dependent activation in SNI neurons, indicating nerve-injury associated alterations of HCN channel function. Consistently, SNI pyramidal neurons exhibited increased excitability and membrane input resistance (Rin) compared to sham pyramidal neurons. Interestingly, the current amplitude and density of the HCN channels were not significantly different between sham-operated and SNI rats. Further experiments investigating changes in intracellular HCN channel modulators and/or in the expression profile of HCN isoforms in neuropathic mPFC pyramidal neurons are in progress.

1-B-8 Neurotrophic actions of TCAP-1 in hippocampal cells: Regulation of actin fibres and antagonism with corticotropin releasing factor

Louise de Lannoy[1], Dhan Chand[1], Lifang Song[1], David A Lovejoy[1]

[1]University of Toronto

Teneurin C-terminal associated peptide-1 (TCAP-1) has a number of neurotrophic actions on hippocampal cells. In vitro, TCAP-1 increases axon fasciculation in immortalized hippocampal cells. In vivo, TCAP-1 increases spine density in the CA1 and CA3 of the hippocampus and modulates dendritic arborization in the CA3. Common to all of these projections is actin, the primary component of the cytoskeleton. Here we explore the pathway by which TCAP induces outgrowth, with a focus on the role of actin fibres. We observe that: 1) TCAP-1 treated E14 immortalized hippocampal cells show an increase in the localization of phalloidin-stained actin fibres and 2) TCAP treatment leads to a change in the localization of elongation factor-1α (EF-1α), the second most abundant component of the cytoskeleton and an actin-crosslinking protein. This neurotrophic effect is also neuroprotective against stress. Treatment of E14 hippocampal immortalized cells with corticotropin releasing factor (CRF) leads to a decrease in immunolabeling for actin stress fibres. These results suggest that the pathway by which TCAP-1 induces neural outgrowth involves the stabilization of actin fibres by cross-linking proteins including EF-1α in a manner that differs from that of CRF.

1-B-9 Cav3-KCa3.1 complex enhances detection of facilitating parallel fiber inputs in cerebellar Purkinje cells

Jordan Engbers[1], Ray W Turner[1]

[1]Hotchkiss Brain Institute

The ability for a neuron to distinguish important input signals from background noise is essential for proper circuit function. Cerebellar Purkinje cells process thousands of parallel fiber (PF) synaptic inputs and postsynaptic mechanisms must exist that allow the detection of granule cell bursts carrying sensory information while filtering out background input. Recently, we demonstrated that Cav3 calcium channels form a complex with intermediate conductance calcium-activated potassium channels (KCa3.1) in Purkinje cells. Using a combination of modeling and experimental methods, we now show that the Cav3-KCa3.1 complex enhances signal detection capabilities by selectively suppressing non-facilitating background inputs. PARA We simulated facilitating and non-facilitating EPSPs in a single compartment model containing the Cav3-KCa3.1 complex and IH. The Cav3-KCa3.1 complex reduced the summation of both sets of inputs, with

facilitating inputs achieving a higher degree of summation. The model showed that facilitation counteracted the increase in KCa3.1 activation during an input train, allowing EPSPs to summate to higher voltages. Current-clamp recordings revealed a Ni2 -sensitive, supralinear increase in the rate of EPSP decay during repetitive PF input trains, confirming that the Cav3-KCa3.1 complex dynamically changes temporal summation. PARA Our results demonstrate that the Cav3-KCa3.1 complex controls summation of synaptic inputs to suppress background noise while allowing facilitating inputs from single PF sources to summate and generate spike output in Purkinje cells.

1-B-10 The native serotonin 5-HT5A receptor: electrophysiological characterization in rodent cortex and 5-HT1A-mediated compensatory plasticity in the knockout mouse

Nathalie Goodfellow[1], Craig D Bailey[1], Evelyn K Lambe[1]

[1]University of Toronto

The 5-HT5A receptor is the least understood serotonin (5-HT) receptor. Here, we electrophysiologically identify and characterize a native 5-HT5A receptor current in acute ex vivo brain slices of adult rodent prefrontal cortex. In the presence of antagonists for the previously-characterized 5-HT1A and 5-HT2 receptors, a proportion of layer V pyramidal neurons continue to show 5-HT-elicited outward currents in both rats and mice. These 5-HT currents are suppressed by the selective 5-HT5A antagonist, SB-699551, and are not observed in 5-HT5A receptor knockout mice. Further characterization reveals that the 5-HT5A current is activated by submicromolar concentrations of 5-HT, is inwardly rectifyingwith a reversal potential near the equilibrium potential for K ions, and is suppressed by blockers of Kir3 channels. Finally, we observe that genetic deletion of the inhibitory 5-HT5A receptor results in an unexpected, large increase in the inhibitory 5-HT1A receptor currents. The presence of functional prefrontal 5-HT5A receptors in normal rodents along with compensatory plasticity in 5-HT5A receptor knockout mice testifies to the significance of this receptor in the healthy prefrontal cortex.

1-B-11 Functional coupling between BDNF and NMDA receptors in spinal cord lamina I neurons following peripheral nerve injury

Michael Hildebrand[1], Yi Li[1], Simon Beggs[1], Xue Jun Liu[1], Michael W Salter[1]

[1]Hospital for Sick Children

Aims: We have previously discovered that release of brain-derived neurotrophic factor (BDNF) from activated spinal cord microglia induces behavioural hypersensitivity in the peripheral nerve-injury (PNI) model of neuropathic pain. Specifically, BDNF disinhibits spinal cord dorsal horn (lamina I) neurons through a TrkB receptor-mediated downregulation of the chloride transporter, KCC2. Separately, we have discovered that upregulation of excitatory NMDA receptors (NMDARs) is also critical for pain hypersensitivity after PNI. We aim to explore whether the BDNF disinhibition and NMDAR facilitated excitation pathways are functionally coupled. Methods: The activity of synaptic NMDARs was measured through voltage-clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) in visually identified lamina I neurons of acute spinal cord slices from both naïve and PNI adult rats. Results: We find that NMDARs but not AMPARs are reversibly potentiated through a BDNF/TrkB-mediated pathway following PNI. In support, incubating naïve spinal cord slices with chronic BDNF induces the same potentiation of NMDARs that is observed in the nerve-injured state. Conclusions: We are the first to directly explore the functional properties and modulation of synaptic NMDARs in lamina I neurons and find that BDNF causes a potentiation of NMDARs in a rat model of neuropathic pain. This pathological coupling between disinhibition and facilitated excitation spinal cord signalling pathways has significant implications for the potential design of novel pain therapeutics for man.

1-B-12 Calcium entry via L-Type calcium channels or NMDARs mediates early odor preference learning in neonate rats

David Jerome[1], Qinlong Hou[1], Qi Yuan[1]

[1]Memorial University of Newfoundland

L-Type Calcium Channels (LTCCs) are involved in synaptic plasticity. Calcium entry through these channels links excitatory synaptic input with intracellular transcriptional events. Here we investigate the role of LTCCs in early odor preference learning in rats. This learning model provides a simple method for studying learning and memory since the memory encoding occurs in the olfactory bulb itself. Bulbar infusion of the β-adrenoceptor agonist isoproterenol during odor training induces a preference for the odor in rat pups 24 h later. Learning results in an enhanced output from Mitral Cells (MCs). MC-bound NMDA receptors (NMDARs) are critically involved in plasticity at the olfactory nerve-MC synapse. We hypothesized that LTCCs can be activated following NMDAR activations to mediate early odor preference learning. Using immunohistochemistry, we demonstrated that LTCCs are present in the MC, located primarily on the MC proximal apical dendrites in neonatal rats. Inhibiting LTCC function via nimidopine infusion blocked isoproterenol-induced learning. Activation of LTCCs via BayK-8644 infusion rescued isoproterenol-induced learning from a D-APV block. Interestingly, the infusion of BayK by itself was not sufficient to induce learning without isoproterenol. Synapsin 1 phosphorylation was reduced following BayK infusion, suggesting BayK reduces olfactory nerve releases, which may compromise the effect of LTCC activation in MCs. Finally, in a

disinhibition learning model via gabazine bulbar infusion, learning was not blocked by nimodipine, but prevented by co-infusion of D-APV.

1-B-13 The effects of hyperglycemia on microglia activity

Sara M Kenawy[1], Kathryn G Todd[1]

[1]University of Alberta

Hyperglycemia is a major consequence of Diabetes Mellitus (DM) and an important factor in the development of diabetes-induced complications. Hyperglycemia can cause neuronal injury directly and indirectly through glial cells. However, the effects of hyperglycemia on microglia, the innate immune cells of the brain, remain unclear. Microglia play pivotal roles in the development or the progression of many CNS disorders including diabetes induced neuropathy and retinopathy. Thus, the aim of this study was to investigate the effects of hyperglycemia on microglia. To achieve this aim, purified microglial cultures were grown in three glucose concentrations (0, 17.5 and 30mM). Microglial phagocytotic activity was determined by assaying consumed fluorescent beads and survival was measured using MTT assays. Microglial release of NO, the pro-inflammatory cytokines IL-1â and TNFá and brain-derived neurotrophic factor (BDNF) were determined using ELISA. Our results showed that microglia were activated in high glucose concentrations (HG=30mM) whereas microglia from glucose deprived cultures (GD=0mM) were not. Specifically, microglia in HG released significantly more NO, IL-1â, TNFá compared to GD microglia. Additionally, microglia in HG showed more phagocytic activity and BDNF release than those in the other glucose concentrations. Interestingly, microglia deprived of glucose survived in greater numbers compared to those in other glucose conditions.Our data suggest that hyperglycemia activates microglia and this activation could influence the fate of neurons in conditions such as DM.

1-B-14 Hydrogen sulfide influences paraventricular nucleus neurons

Sahara C Khademullah[1], Alastair V Ferguson[1]

[1]Queen's University

Hydrogen sulfide (H2S) has been shown to have various effects on neurons in a number of CNS regions, and enzymes responsible for the production of H2S have been identified in the paraventricular nucleus of the hypothalamus (PVN). We have used whole cell patch clamp techniques to examine the effects of sodium hydrogen sulfide (NaHS), a hydrogen sulfide donor on the excitability of PVN neurons. Bath application of 50µM NaHS influenced 93% of the cells tested (n=15), with 23% depolarized (n=3, 9.1±4.6mV) and 77% hyperpolarized (n=10, -14.9±2.3). Similar application of 10µM NaHS influenced 100% of the cells tested (13/13), with a significantly larger proportion (62%) being depolarized (n=8, 5.5±1.9mV) and 38% hyperpolarized (n=5, -11.7±3.8mV). Similar predominantly depolarizing responses to NaHS were observed at concentrations of 1µM (82% depolarized, n=9, 3.4±2.3mV; 18% hyperpolarized n=2, -17.7±1.2mV), 100nM (86% depolarized, n=6, 5.1±1.4mV; 14% hyperpolarized n=1, -8.8mV), while 1nM NaHS only influenced one of 12 neurons tested. These findings shed light on the effects that NaHS has on the membrane potential of neurons in the PVN, suggesting a potential role for this gasotransmitter in the PVN. Funded by CIHR

1-B-15 PDZ domain interactions with the carboxy-terminus of connexin36 localize the effector and scaffolding proteins AF6 and MUPP1 to gap junctions that form electrical synapses in rodent brain

Bruce D Lynn[1], Xinbo Li[2], James I Nagy[1]

[1]University of Manitoba, [2]Oregon Health and Science University

Electrical synapses formed by interneuronal gap junctions composed of connexin36 (Cx36) occur in most major structures in the mammalian CNS. These synapses link ensembles of neurons and influence their network properties. Little is known about the macromolecular constituents of neuronal gap junctions or how transmission through electrical synapses is regulated at the level of channel conductance or gap junction assembly/disassembly. Knowledge of the molecular organization and regulation of neuronal gap junctions is required to gain understanding of the roles of electrical synapses in neuronal circuitry. Gap junctions are somewhat like tight and adhesion junctions in that all three are found at close plasma membrane appositions, and therefore may associate with a similar repertoire of structural and regulatory proteins. Previously we reported that the tight junction-associated protein zonula occludens-1 (ZO-1) interacts with Cx36 and is localized at gap junctions. Here we demonstrate that two additional proteins known to be associated with tight and adherens junctions, namely AF6 and MUPP1, are components of neuronal gap junctions in rodent brain. By immunofluorescence, AF6 and MUPP1 were co-localized with Cx36 in many brain areas. Co-immunoprecipitation and pull-down approaches revealed an association of Cx36 with AF6 and MUPP1 that required the C-terminus PDZ domain interaction motif of Cx36 for interaction with the single PDZ domain of AF6 and with the 10th PDZ domain of MUPP1. This work was supported by grants to JIN from CIHR, NSERC and NIH.

1-B-16 Synaptic plasticity and reversal learning are impaired following B-catenin stabilization in hippocampal neurons

Fergil Mills[1], Thomas Bartlett[1], Marta Wisniewska[2], Jacek Kuzniki[2], Yu Tian Wang[1], Shernaz Bamji[1]

[1]University of British Columbia, [2]The International Institute of Molecular & Cell Biology

β-catenin, the intracellular partner of the cadherin family of cell adhesion molecules, has been shown to play a critical role in memory consolidation, and is essential for synapse assembly and plasticity. β-catenin levels are tightly regulated in cells, but in a number of neurological disorders β-catenin turnover is disrupted, leading to the accumulation of β-catenin in the brain. We demonstrate that conditional overexpression of β-catenin in the adult hippocampus results in deficits in synaptic morphology and transmission. Increasing β-catenin also abolishes long-term depression (LTD), a form of long-lasting synaptic plasticity associated with memory extinction. In accordance with this, β-catenin-overexpressing mice displayed substantial deficits in the reversal learning phase of the Morris water maze, exhibiting aberrant perseveration of a previously-learned platform location following platform reversal. These results demonstrate that increased levels of β-catenin can have a significant effect on synapse function and plasticity, and may contribute to cognitive impairments observed in neurodegenerative disorders where elevated levels of β-catenin have been reported.

1-B-17 Connexin36 at nerve terminals in the vestibular, cochlear and hippocampal systems: mixed chemical/electrical transmission in mammalian CNS

James I Nagy[1], Wendy Bautista[1]

[1]University of Manitoba

Electrical synaptic transmission via gap junctions has become an accepted feature of neuronal communication in the mammalian brain, and occurs often between dendrites of interneurons in many CNS regions. Gap junctions also form at nerve terminals, where they contribute the electrical component of mixed chemical/electrical transmission. Mixed synapses occur widely in lower vertebrates, but have rarely been described in mammalian CNS. We used immunofluorescence detection of the gap junction forming protein connexin36 (Cx36) to examine its association with nerve terminals in rodent brain. In the vestibular nuclei, one of the few regions where terminal with gap junctions have been described, immunolabelling for Cx36 was widely distributed and often co-localized with the terminal marker vesicular glutamate transporter-1 (vglut-1). In the ventral cochlear nucleus, Cx36 was heavily concentrated on neurons, which were richly invested with vglut-1-positive terminals, resulting invariably in Cx36/vglut-1 co-localization. In the hippocampus, a high density of fine, punctate immunolabelling for Cx36 was found in the stratum lucidum in the ventral hippocampus of rat brain. A high percentage of these Cx36-positive puncta was localized to mossy fiber terminals labelled for the terminal marker vglut-1, as well as with other proteins highly concentrated in, and diagnostic markers of, these terminals. These results suggest that mixed chemical/electrical synapses occur abundantly in some forebrain and brainstem structures of rodent CNS. Supported by grants from CIHR, NIH and NSERC.

1-B-18 Aquaporin 4: A target for drug discovery in brain edema prevention

Caroline Perronnet[1], Geoffroy Noël[2], Hakima Moukhles[1]

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

Brain edema, defined as the abnormal accumulation of water in the brain, is a major clinical complication associated with nervous system disorders including stroke. Recently, a role for the water-permeable channel aquaporin 4 (AQP4) in the development of brain edema has been established. AQP4 is the principal water channel in the brain and is clustered at the perivascular astrocyte endfeet abutting blood vessels. We have shown that the polarized distribution of AQP4 is regulated via the interaction of the dystroglycan complex with perivascular laminin. Indeed, mice lacking components of this complex or in which the laminin/dystroglycan interaction is disrupted show a delayed onset of brain edema due to a redistribution of AQP4 away from astrocyte endfeet. Current treatments to reduce brain edema are limited and it is of major importance to develop new therapeutic strategies to prevent brain edema. Modulators of AQP4 clustering may have a therapeutic application to reduce edema by blocking water influx in astrocytes at early stages of stroke. In the present study, we characterized four inhibitors of the laminin-induced clustering of AQP4 in vitro in astrocytes cultures. Each of these drugs was tested in vitro to evaluate its toxicity. Ongoing in vivo studies will determine the impact of these drugs on the perivascular distribution of AQP4. The drugs that will prove to be efficient in distributing AQP4 away from astrocyte endfeet will be used subsequently to study their ability to prevent the deleterious effects caused by brain swelling in rat models of cytotoxic brain edema.

1-B-26 alpha-2 adrenoceptor mediates a cAMP-independent signalling during early odor preference learning in rats

Amin Shakhawat[1], Qi Yuan[1]

[1]Memorial University of Newfoundland

Norepinephrine release to the olfactory bulb has been shown to be critical for early odor preference learning in rats. Previous research has highlighted the role of β-

adrenoreceptor activation in this learning. β-adrenoceptor agonist isoproterenol systemic injection, or local bulbar infusion, is sufficient to induce learning. Isoproterenol-induced learning is dependent on cAMP/pCREB signalling in mitral cells of the olfactory bulb and demonstrates an inverted-U dose curve. Recently, however, it has been proposed that α2-adrenoreceptor may also be involved in early odor learning by disinhibiting mitral cells from granule cells. PARA Here we showed that inhibition of α2-adrenoceptors by yohimbine bulbar infusion blocked odor learning induced by an odor shock paradigm, while selective activation of α2-adrenoreceptors by clonidine dose-dependently induced learning. To test the disinbition hypothesis, we measured pCREB levels in the mitral cells following unilateral infusions of either clonidine, or a GABAa antagonist gabazine during training. We observed a significant increase in pCREB in both compared to vehicle infused bulbs. Furthermore, cAMP expression was measured from same animals that were tested for pCREB. We found no change in mitral cell cAMP levels, suggesting that α2-adrenoreceptors activation may trigger different intracellular cascades than the cAMP pathway. Finally, we showed that α2-adrenoreceptors act synergistically with β-adrenoreceptors in early odor learning. Co-applications of clonidine with suboptimal doses of isoproterenol enabled learning.

1-B-27 Molecular dissection of NMDA receptor GluN2B subunit by rescue in GluN2B -/- neuron culture: Glutamate binding is required for surface trafficking, and intracellular determinants control synaptic recruitment of CaMKIIα

Kevin She[1], Jacqueline Rose[2], Ann Marie Craig[1]

[1]University of British Columbia, [2]Western Washington University

NMDA receptors mediate many forms of synaptic plasticity. Trafficking of these receptors to the neuron surface and their molecular interactions at synapses are critical for proper brain function and activity dependent plasticity. Here, we used a rescue approach expressing YFP-GluN2B constructs in GluN2B -/- (KO) hippocampal neurons in culture to address two fundamental questions. (1) Recent evidence suggests that surface trafficking of other ionotropic glutamate receptors requires ligand binding for exit from the endoplasmic reticulum. By expressing a panel of GluN2B ligand binding site point mutants with varying glutamate efficacy, we show that surface expression of GluN2B correlates with glutamate efficacy. These results suggest that surface delivery of NMDA receptors is controlled by intracellular ligand binding. (2) GluN2B is proposed to bind and recruit calcium-calmodulin dependent protein kinase II (CaMKII) to synapses to mediate multiple forms of synaptic plasticity. Indeed, we find that accumulation of CFP-CaMKIIα at synapses is induced in wild-type but not in KO neurons by bath stimulation of NMDA receptors or by a chemical long-term potentiation (cLTP) protocol. Stimulated synaptic accumulation of CFP-CaMKIIα was rescued in KO neurons by YFP-GluN2B or chimeric GluN2A/2B tail but not by GluN2A, chimeric GluN2B/2A tail, or GluN2B with point mutations in the CaMKII binding site. Thus, activity-regulated synaptic aggregation of CaMKII is dependent on the cytoplasmic CaMKII binding site of GluN2B and not on differential permeation properties between GluN2B and GluN2A.

1-B-28 Neuroprotection by peptides designed to block the extracellular interaction between AMPA receptors and secreted neuronal activity regulated pentraxin

Carlo Smith[1], Max Cynader[1]

[1]University of British Columbia

Prolonged stimulation of N-methyl-D-aspartate type glutamate receptors (NMDARs) results in internalization of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptors (AMPARs) and long-term depression (LTD) of post-synaptic response, termed NMDAR-LTD. This process has been causally linked to neuronal death. Neuronal activity regulated pentraxin (NARP) is a secreted immediate early gene that binds to and clusters AMPARs in response to normal and pathological synaptic activity, and is associated with excitatory synaptogenesis and synaptic plasticity; including LTD. We hypothesize that NMDA-induced NARP facilitates NMDAR-LTD and cell death by clustering AMPARs at sites of regulated endocytosis. Here we show that NARP is up-regulated 4-8 hours after treatment of primary cortical neurons with 50uM of NMDA and 10uM glycine for 1 hour (which causes 47% cell death 24 hours later). Using peptide arrays, we then developed four peptides that mimic sites on AMPARs that bind NARP. A mixture of all four peptides significantly reduced NMDA-induced cell death at 0.5, 5 and 10uM. When tested individually, only three out of the four peptides were robustly protective at 10uM. We also found that the peptide mixture prevents NMDA-induced AMPARs endocytosis. In summary, peptides that block interaction between NARP and AMPARs prevent NMDAR-induced endocytosis of AMPARs and cell death.

1-B-29 Gabapentin, calcium channels and neurotransmitter release

Peter Smith[1], James E Biggs[1], Yishen Chen[1], Patrick L Stemkowski[1], Sascha R Alles[1], Aquilino L Garcia[2]

[1]University of Alberta, [2]Universidad de Cantabria

The gabapentinoids, pregabalin and gabapentin are used to treat neuropathic pain. They are actively transported into neurons where they bind to the á2ä subunit of voltage gated Ca2+ channels. This impairs the trafficking of pore forming á subunit of Ca2+ channels to nerve terminals. In primary afferents, this is thought to reduce the release of

glutamate and to thereby restrict the flow of nociceptive information into the spinal cord. To test this hypothesis, we used a defined medium organotypic culture of rat spinal cord to examine long term actions of gabapentinoids on glutamatergic transmission. In confirmation of the idea that gabapentinoids decrease neurotransmitter release, 5 days exposure to 10ìM pregabalin, reduced the amplitude of spontaneous EPSC's by 20% in excitatory neurons of the superficial dorsal horn yet did not affect those in inhibitory neurons. However, other data question the relationship between this observation and pregabalin's effects on Ca2+ channels. This is because 200ìM Mn2+ has little effect on evoked EPSC's in spinal dorsal horn but this low concentration of Mn2+ all but eliminated Ca2+ channel currents in the cell bodies of primary afferent neurons. Mn2+ (200ìM) also reduced K+ evoked Ca2+ responses in spinal cord by 82.5±0.9% (n=7). It is therefore unlikely that pregabalin can produce sufficient suppression of presynaptic Ca2+ channel current to account for its effect on neurotransmitter release. This implies that additional processes may be involved. for example, gabapentinoids may interact directly with the transmitter release process.

1-B-30 Slitrk3 selectively controls inhibitory synapse development via trans-synaptic interaction with axonal PTPdelta

Hideto Takahashi[1], Kei-ichi Katayama[2], Kazuhiro Sohya[2], Hiroyuki Miyamoto[2], Tuhina Prasad[1], Yoshifumi Matsumoto[2], Maya Ota[2], Hiroki Yasuda[3], Tadaharu Tsumoto[2], Jun Aruga, Ann Marie Craig[1]

[1]University of British Columbia, [2]RIKEN Brain Science Institute, [3]Gunma University Graduate School of Medicine

Balanced development of excitatory and inhibitory synapses is required for normal brain function, and their imbalance may underlie pathogenesis of neuropsychiatric disorders. Compared with many identified trans-synaptic adhesion complexes that organize excitatory synapses, little is known about synaptic organizers specific for inhibitory synapses. Here we report Slit and NTRK-like family member 3 (Slitrk3) as a postsynaptic adhesion molecule that selectively regulates inhibitory synapse development via trans-interaction with axonal tyrosine phosphatase receptor PTPd. Slitrk3 expressed in fibroblasts triggers only inhibitory presynaptic differentiation in contacting axons of cocultured hippocampal neurons. Recombinant Slitrk3 preferentially localizes to inhibitory postsynaptic sites. Slitrk3-deficient mice exhibit decreases in inhibitory, but not excitatory, synapse number and function in hippocampal CA1 neurons. Intriguingly, these mice also exhibit increased susceptibility to chemoconvulsant-induced seizures and occasional spontaneous behavioral and electrographical seizures, presumably as a consequence of the selective decrease in the number of functional inhibitory synapses. Further, we identified PTPd as the high-affinity presynaptic functional receptor of Slitrk3. PTPd knockdown experiments revealed that Slitrk3 requires trans-interaction with axonal PTPd to induce inhibitory presynaptic differentiation. These results identify Slitrk3-PTPd as an inhibitory-specific trans-synaptic organizing complex required for normal functional GABAergic synapse development.

1-B-31 Large-scale analysis of adult human brain gene expression patterns

Patrick Tan[1], Leon French[1], Paul Pavlidis[1]

[1]University of British Columbia

We present results of analyzing a recently-released detailed atlas of gene expression in the human brain. Our motivation is the complexity of the transcriptome and cellular content of the brain (over 200 cell types which collectively express over 80% of the genes in the genome). The use of detailed genome-wide data sets can provide insight into evolutionary, developmental and functional processes governing the observed structures. Previously we showed that in the mouse brain, the dominant expression pattern across the brain is characterized by variance in the expression of known cell type markers, suggesting an inverse relationship between local glial and neuronal content (French et al. (2011) Frontiers in Neuroinformatics 5:12). In the current work we analyzed two high-resolution human gene expression profiles provided by the Allen Institute for Brain Science. This constitutes data on the RNA levels of genes across hundreds of brain regions. We applied principal component analysis and other multivariate approaches to these data and report that the patterns found in the mouse are largely conserved in human. We provide evidence that this pattern can also be found within individual brain regions across donors. An intriguing aspect of data is a small number of genes which show strongly discordant patterns between mouse and human. Our findings may be used to identify functionally important differences between the mouse and human brain, and to identify new cell-type-specific genes associated with the patterns.

1-B-32 Dynamin and the dystroglycan complex regulate the polarized distribution of aquaporin-4 in astrocytes

Daniel Tham[1], Hakima Moukhles[1]

[1]University of British Columbia

The type-4 water-permeable aquaporin channel (AQP4) is expressed in a highly polarized manner in astrocytes. Whereas high concentrations of AQP4 are found at perivascular astrocyte endfeet, little is present in other cellular domains. The receptor dystroglycan (DG) is similarly localized, and its close association with the basal lamina allows it to interact with its ligand, laminin. Evidence suggests that the asymmetric distribution of AQP4 is necessary for proper cerebral osmostasis. We therefore sought in the present study to determine the role of the DG complex in the regulation of AQP4 endocytosis in

astrocytes. Using primary rat astrocyte cultures, we first determined that laminin increases the expression of AQP4 at the plasma membrane in a DG-dependent manner, and further demonstrate that this occurs via the inhibition of AQP4 internalization. We also show that caveolin 1 and dynamin II are associated with the DG complex, and that dynasore, an inhibitor of the GTPase activity of dynamin, decreases dynamin-DG associations, suggesting that DG preferentially binds to GDP-bound dynamin. We found that dynamin inhibition increases the expression of AQP4 at the cell surface by reducing channel endocytosis, revealing a role for dynamin in AQP4 polarization. Finally, we show that laminin treatment and the silencing of DG expression both result in changes to the size distribution of AQP4 aggregates in astrocytes. These data indicate that interactions between DG, the extracellular matrix and endocytic components may regulate AQP4 polarization in astrocytes.

1-B-33 Calcium permeability and modulation of a cholinergic current in Aplysia bag cell neurons

Sean White[1], Neil S Magoski

[1] Queen's University

The bag cell neurons of Aplysia translate brief synaptic input into a prolonged afterdischarge and subsequent hormone release culminating in egg laying. Afterdischarge can either be brought about through direct afferent stimulation or the application of acetylcholine. Based on calcium imaging of fura PE3-loaded bag cell neurons, acetylcholine produced limited direct calcium influx through the cholinergic ionotropic channel. Similarly, increases in intracellular calcium fail to alter either the magnitude or dynamics of the current. However, the acetylcholine-evoked depolarization result in calcium elevation due to subsequent activation of voltage-gated calcium current. Following completion of the afterdischarge, the bag cell neuron cluster enters a ~18 hour refractory period of reduced excitability, where synaptic input no longer generates an afterdischarge, Acetylcholine application to refractory clusters also fails to depolarize individual bag cell neurons. The refractory period is associated with an increase in tyrosine phosphorylation, while the afterdischarge appears to require lower levels. Appropriately, application of the general tyrosine kinase inhibitor, genistein, as well as specific SRC antagonist, PP1, reduced the acetylcholine current. Collectively, these data suggest that once the acetylcholine current depolarizes the neurons to permit calcium entry, tyrosine dephosphorylation suppresses further activation and aberrant excitation.

1-B-34 Pannexin 1 regulates neural stem and progenitor cell proliferation and forms a novel interaction with phosphoglycerate dehydrogenase

Leigh Wicki-Stordeur[1], Adrian D Dzugalo[1], Rose M Swansburg[1], Jocelyne M Suits[1], Leigh Anne Swayne[1]

[1]University of Victoria

We have recently discovered expression of the pannexin 1 ion and metabolite channel in ventricular zone neural stem and progenitor cells. By manipulating pannexin 1 expression and activity in Neuro-2a neuroblastoma cells and primary neurosphere cultures, we have demonstrated that pannexin 1 positively regulates cell proliferation, in part through release of adenosine triphosphate (ATP). To further elucidate the mechanism by which pannexin 1 regulates proliferation, we used an unbiased proteomic approach to identify putative protein interaction partners. We uncovered a novel interaction with phosphoglycerate dehydrogenase, an enzyme that redirects glycolytic flux towards the production of serine. Up-regulation of serine metabolic pathways confers growth advantages and stimulates proliferation through multiple mechanisms. Therefore we hypothesize that this newly-identified interaction between pannexin 1 and phosphoglycerate dehydrogenase represents an additional mechanism by which pannexin 1 regulates neural stem and progenitor cell proliferation. These novel findings have important implications for the generation of new neurons in both healthy and injured brain.

C – Disorders of the Nervous System

1-C-19 Transplantation of skin-derived precursors differentiated into schwann cells (SKP-SCs) after chronic spinal cord injury.

Peggy Assinck[1], Shallee Dworski[2], Joseph Sparling[1], Gregory J Duncan[1], Di L Wu[1], Yuan Jiang[1], Jie Liu[1], Brian K Kwon[1], Freda Miller[2], Wolfram Tetzlaff[1]

[1]ICORD/UBC, [2]Hospital for Sick Children/University of Toronto

Cell transplantation has emerged as a promising candidate therapy for spinal cord injury, however, the best candidate cell remains a matter of intense debate. Our laboratories have previously shown that Schwann cells differentiated from skin-derived precursors (SKP-SC), when transplanted 7-days after contusion injury promote histological and functional recovery in rats. SKPs are potentially suitable for autologous transplantation, however this would require 6-8 weeks to grow sufficient cells and the therapeutic potential of SKP-SC in the chronic injury environment has not yet been investigated. Here, we transplanted one-million cells into the lesion site of rats at 8 weeks post T9/T10 contusion injury and allowed survival until week 29. Behavioral data

indicate that SKP-SC transplantation prevented the decline of forelimb/hindlimb stride length (Catwalk) and elicited a continuous recovery of the open field locomotor scores, which reached significance by 19 and 21 weeks post injury. Cellular bands of SKP-SC bridged the lesion and were massively filled with axons ensheathed by P0-positive (Schwann cell) myelin of endogenous as well as transplant origins. This success with a cell transplantation approach in a chronic stage of SCI is rare and may open the door for autologous SKP-SC transplantation as a possible clinical treatment for SCI, while obviating the need for immunosuppresion and minimizing the risk of tumors from stem cell transplantation. Supported by the Stem Cell Network, Rick Hansen Foundation and CIHR of Canada.

1-C-20 Motor and non motor outcomes of the MAO inhibitor phenelzine in mice with EAE

Curtis Benson[1], Grace Wong[1], Travis Musgrave[1], Gustavo Tenorio[1], Gail Rauw[1], Glen B Baker[1], Bradley J Kerr[1]

[1]University of Alberta

Multiple sclerosis (MS) is an autoimmune degenerative disease of the central nervous system (CNS). MS is associated with motor and non-motor symptoms. Many of these symptoms can be related to changes in the levels of key neurotransmitters. Using the animal model experimental autoimmune encephalomyelitis (EAE), we have described the benefits of treatment with the monoamine oxidase (MAO) inhibitor phenelzine. Daily PLZ treatment caused substantial behavioural improvements. However, not all of the positive outcomes were maintained with daily PLZ treatment. To determine whether modifying the dose of PLZ could better sustain the motor and non-motor improvements, PLZ treatment was given on alternating days. Alternating PLZ treatment reduced the severity of EAE clinical signs, improved exploratory behaviours and reversed EAE-induced deficits in an assay of learning and memory, the novel object recognition test. Similar to daily PLZ treatment, PLZ treatment on alternating days resulted in a significant elevation of 5-HT within the ventral horn of the spinal cord. However, in contrast to daily PLZ treatment, giving PLZ every other day lead to significantly higher levels of GABA upon completion of the experiment. Examination of the MAO enzyme activity showed that with alternating treatment there was statistically less inhibition of MAO compared to mice treated daily, possible accounting for the difference in GABA levels. These results demonstrate that providing PLZ every second day is able to sustain GABA increases and better maintain positive behavioral outcomes in the EAE model.

1-C-21 WNK1/HSN2 mutations causing human HSAN type 2 deregulate KCC2 function in zebrafish.

Valérie Bercier[1], Edna Brustein[1], Meijiang Liao[1], Patrick A Dion[1], Guy A Rouleau[1], Pierre Drapeau[1]

[1]Université de Montréal

Hereditary sensory and autonomic neuropathy type 2 (HSAN2) is a rare human pathology characterized by the early loss of sensory perception. HSAN2 arises from expression of autosomal recessive mutations confined to exon HSN2, an alternatively spliced exon conferring nervous system specificity to the WNK1 (with-no-lysine protein kinase 1) serine-threonine kinase. In zebrafish embryos, WNK1/HSN2 is expressed in the neuromasts of the posterior lateral line (PLL), a mechanosensory system composed of individual sensory organs called neuromasts. Defects in the development of this system, both in the number of individual neuromasts and of the hair cells they possess, are observed upon knockdown of the WNK1/HSN2 isoform. We investigated interactions between the WNK1 kinase and the neuronal potassium chloride co-transporter 2 (KCC2) in the context of HSAN2, as KCC2 has been implicated in promoting neurogenesis. WNK1 is known to phosphorylate KCC2, regulating its activity and possibly its expression levels. We found that KCC2 is expressed in mature neuromasts and observed an increased level of KCC2 RNA in WNK1/HSN2 knockdown embryos. Additionally, overexpression of human KCC2 RNA in embryos replicated the WNK1/HSN2 knockdown phenotype. Interestingly, it was possible to rescue the specific phenotype upon simultaneous knockdown of the zebrafish KCC2 and WNK1/HSN2. We suggest that the loss-of-function mutations in WNK1/HSN2 linked with HSAN2 lead to an imbalance in the levels of activated KCC2, deregulating its levels of transcription and hindering its timely role in neuronal maturation.

1-C-22 The receptor for advanced glycation end products (RAGE) underlies hyperglycemia-induced autonomic malfunction: implications in diabetic autonomic neuropathy

Andrew Chandna[1], Christine Chang[1], Paul Pennington[1], Darrell Mousseau[1], Veronica A Campanucci[1]

[1]University of Saskatchewan

Diabetic autonomic neuropathy (DAN) is among the most common and troubling complications of diabetes leading to abnormalities in the cardiovascular system and predisposing patients to asymptomatic ischemia and sudden cardiac death. Recently, a new model for the onset of diabetic autonomic neuropathy has been proposed, where hyperglycemia causes the elevation of cytoplasmic reactive oxygen species (ROS) leading to the inactivation

of nicotinic acetylcholine receptors (nAChRs) in sympathetic neurons. Because nAChRs drive sympathetic function, their inactivation results in the depression of fast synaptic transmission and the onset of autonomic neuropathy in diabetic mice. Yet, the source of ROS in autonomic neurons during hyperglycemia remains unclear. RAGE has been implicated in the development of sensory abnormalities in diabetes through oxidative stress; however, its effect on sympathetic neurons during hyperglycemia has not been investigated. Here we propose RAGE as a major source of ROS in DAN. We show for the first time the expression and up-regulation of RAGE in cultured sympathetic neurons exposed to hyperglycemia. Consistent with these findings, direct activation of RAGE by its natural ligands (e.g. AGEs, S100 and HMGB-1) increased cytoplasmic ROS, which in turn induced the inactivation of nAChRs in sympathetic neurons. Remarkably, impairing RAGE function with the help of a neutralizing antibody prevented the hyperglycemia-induced inactivation of nAChRs. Therefore, these results support a pivotal role for RAGE in DAN.

1-C-23 Neurocarta: an online platform for integration and sharing of phenotypic and genomics information

Carolyn Ch'ng[1], Elodie Portales-Casamar[1], Frances Lui[1], Nicolas St-Georges[1], Anton Zoubarev[1], Paul Pavlidis[1]

[1]University of British Columbia

This work is part of neuroinformatics core activities developed in support of NeuroDevNet, a large Canadian research network funded by the Networks of Centres of Excellence, devoted to the study of brain development with the goal to translate this knowledge into improved diagnosis, prevention and treatment of neurodevelopmental disorders. As part of the NeuroDevNet Neuroinformatics Core mission, we are developing an online platform that integrates information on genes for the analysis and interpretation of neurodevelopmental data produced within the network. The core concept of Neurocarta is to allow users to track information about genes in a flexible way, but with rich connections to other data. It is developed as an extension of Gemma, a database and software system for the meta-analysis of functional genomics data. Our system currently hosts 17,000 evidence lines linking 4,560 genes to 1,556 different phenotypes, annotated from the literature and public databases. Preliminary analysis of the data content have provided some insights on the underlying character of the system. For instance, curated disease genes in Neurocarta exhibit a 'multifunctionality' bias. Future investigations will be directed towards building on existing and new knowledge emerging from the network to increase our understanding of neurodevelopmental disorders and the relationships among them at the genetic and phenotypic levels.

1-C-24 Rescue from tau-induced neuronal dysfunction produces insoluble tau oligomers

Catherine Cowan[1], Sarah Hands[1], Douglas W Allan[2], Amritpal Mudher[1]

[1]University of South Hampton, [2]University of British Columbia

We have found that Drosophila which express wild-type human 3-repeat tau (hTau0N3R) neuronally provide an interesting model of tauopathies such as Alzheimer's disease, and have provided insights into the mechanisms by which tau can cause neuronal dysfunction (Williams et al., 2000; Mudher et al., 2004; Chee et al., 2005; Cowan et al., 2010). The phenotype is one of early neuronal dysfunction, due to breakdown of microtubules and reduction in axonal transport, before any formation of insoluble tau or neuronal death. This work and others (Wittman et al., 2001) indicated that formation of insoluble tau is not necessary for tau toxicity. PARATreatment with lithium chloride ameliorated all aspects of this phenotype. We show here that a more specific GSK-3â inhibitor, AR-A01448, which also rescues the neuronal dysfunction in hTau-expressing Drosophila, acts via the same mechanism of restoring microtubule integrity. Thus inhibition of GSK-3â is sufficient to rescue the tau-induced neuronal dysfunction. PARAHere we show that both of the GSK-3â inhibitors that we have used to rescue the tau-induced phenotype, LiCl and AR-A01448, also have the remarkable unexpected effects of increasing total levels of hTau protein, and producing small insoluble tau species in the form of granular tau oligomers. This indicates that there are species of insoluble tau that are, at minimum, non-toxic; and raises the possibility that such species may even be protective against tau toxicity.

1-C-25 Myelin gene regulatory factor knockout delays functional recovery from spinal cord injury

Gregory Duncan[1], Jason R Plemel[1], Brett J Hilton[1], Jie Liu[1], Meagan J McKeen[1], Julian Kim[1], Steven Mao[1], Wolfram Tetzlaff[1]

[1]Uuniversity of British Columbia

Spinal cord injury (SCI) results in the death of oligodendrocytes and demyelination of axons. Following SCI, myelin replacement occurs to restore conductance and potentially protect axons from degeneration. The role of this regenerative process and its contribution to functional motor recovery following spinal cord injury (SCI) has, however, never been tested directly. Removing a transcriptional regulator, known as myelin gene regulatory factor (MRF), necessary for the maturation of OPCs into myelinating oligodendrocytes, should inhibit efficient remyelination. To remove MRF, we injected a cre-expressing adeno-associated virus 5 (AAV5) into the spinal

cord of mice that were either heterozygous or homozygous for floxed MRF two weeks prior to a thoracic crush injury. AAV5 was capable of infecting OPCs, as indicated by co-labelling of virally expressed GFP with OPC markers PDGFRα and Olig 2 at the time of injury. After injury, MRFfl/fl mice infected with Cre-expressing AAV5 had a delayed motor behavioural recovery when assessed on the Basso Mouse Scale. When the density of mature oligodendrocytes near the lesion was assessed using the marker CC1 there was no difference in their density, or the proportion of viral cells colabelling with CC1. MRF knockout did not induce pronounced oligodendrocyte apoptosis four weeks following injury as assessed by cleaved caspase 3. The delayed behavioural recovery suggests an important role for MRF in the spontaneous motor recovery following spinal cord injury.

1-C-35 Peripheral neuropathy in diabetes is associated with mitochondrial dysfunction in dorsal root ganglia neurons via impaired AMP-activated protein kinase signaling.

Paul Fernyhough[1], Darrell Smith[2], Ali Saleh[2], Jason Schapansky[1], Suzanne Gomes[1], Eli Akude[1], Dwane Morrow[2], Nigel A Calcutt[3], Subir Roy Chowdhury[2]

[1]University of Manitoba, [2]St. Boniface Hospital Research, [3]UCSD

Mitochondrial dysfunction in sensory neurons contributes to distal axonopathy in diabetic neuropathy. The AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor gamma coactivator-1a (PGC-1a) signaling axis senses metabolic demands of cells and regulates mitochondrial function. We tested the hypothesis that deficits in AMPK/PGC-1a signaling in sensory neurons underlie impaired axonal plasticity, sub-optimal mitochondrial function and development of neuropathy in rodent models of diabetes. Phosphorylation and expression of AMPK/PGC-1a and mitochondrial respiratory chain complex proteins were down-regulated in dorsal root ganglia (DRG) of both streptozotocin (STZ)-diabetic rats and db/db mice. Adenoviral-mediated manipulation of endogenous AMPK using mutants modulated neurite outgrowth in cultures of sensory neurons. In sensory neurons derived from STZ-induced diabetic rats resveratrol elevated AMPK, enhanced neurite outgrowth and normalized mitochondrial polarization. The bioenergetics profile (maximal oxygen consumption rate, coupling efficiency, respiratory control ratio and spare respiratory capacity) was aberrant in sensory neurons from STZ-diabetic rats and was corrected by resveratrol. Finally, resveratrol treatment for the last 2 months of a 5 month period of diabetes reversed thermal hypoalgesia and attenuated foot skin intra-epidermal nerve fiber loss. These data suggest that the development of distal axon loss in sensory neuropathy is linked to nutrient excess and mitochondrial dysfunction via defective signaling of AMPK/PGC-1a.

1-C-36 Antidepressants differentially increase cell proliferation in the dentate gyrus of intact and ovariectomized adult female rats

Michelle Foisy[1], Meighen M Roes[1], Stephanie E Lieblich[1], Melissa YM Chan[1], Liisa AM Galea[1]

[1]University of British Columbia

Major depression is two to three times more common in females than in males. Furthermore, depressive symptoms are often more severe in females, and there are sex differences in antidepressant efficacy. Females are especially likely to develop depression after giving birth and during perimenopause, when ovarian hormone levels decrease dramatically. Research also suggests that ovarian hormones may affect antidepressant efficacy. In this experiment, we examined the effect of ovarian hormone status on antidepressant-induced neurogenesis, a putative neural marker of depression, in the dentate gyrus of adult female rats. Intact (Sham) and ovariectomized (OVX) adult female rats received 21 days of injections with vehicle, the selective serotonin reuptake inhibitor fluoxetine, or the tricyclic antidepressant imipramine. Animals were then perfused, adrenal glands were extracted, and brains were examined for cell proliferation using the cell cycle marker Ki67 and immature neurons using doublecortin. Preliminary results indicate that adrenal ratio (adrenal weight/body weight) was significantly higher with antidepressant treatment in the OVX, but not Sham, rats. Cell proliferation was increased with antidepressant treatment in Sham rats and to a lesser extent OVX rats, and imipramine increased cell proliferation to a greater extent than fluoxetine. These results suggest that ovarian hormones affect the ability of different antidepressants to alter adrenal function and cell proliferation in the dentate gyrus and may provide insight into the treatment of major depression in females.

1-C-37 Enhanced calpain and striatal-enriched tyrosine phosphatase (STEP) activation contribute to increased extrasynaptic NMDA receptor localization in Huntington disease.

Clare Gladding[1], Marja D Sepers[1], Jian Xu[2], Lily Y Zhang[1], Austen J Milnerwood[1], Paul J Lombroso[2], Lynn A Raymond[1]

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

The neurodegenerative disorder Huntington disease (HD) is caused by a CAG repeat expansion in the HD gene encoding the mutant form of huntingtin (mhtt). HD is associated with striatal degeneration and excitotoxicity, in part due to altered NMDA receptor (NMDAR) trafficking and signaling. Deleterious extrasynaptic NMDAR (Ex-NMDAR) localization and signaling are increased early in

yeast artificial chromosome mice expressing full-length mhtt with 128 polyglutamine repeats (YAC128). Here, we investigate whether GluN2B calpain cleavage and/or STriatal Enriched tyrosine Phosphatase (STEP)-mediated dephosphorylation of GluN2B Y1472, contribute to increased YAC128 striatal Ex-NMDAR expression. Striatal PSD and non-PSD fractions were isolated from WT and YAC128 mice, or from cortico-striatal slices treated with the calpain inhibitor calpeptin or a STEP inactivating TAT-STEP C-S peptide. Non-PSD cleaved, full-length and surface GluN2B levels were significantly increased in YAC128 mice compared to WT which were reduced significantly by calpeptin. Furthermore, increased YAC128 synaptic STEP61 activity correlated with decreased GluN2B Y1472 phosphorylation. Moreover, synaptic GluN2B expression was significantly increased by STEP inactivation. In conclusion, elevated YAC128 STEP61 activation promotes a shift of synaptic NMDARs to extrasynaptic sites, whereas increased calpain activity enhances Ex-NMDAR expression. Understanding the mechanisms underlying elevated Ex-NMDAR expression could be beneficial for developing HD therapeutics.

1-C-38 Characterization of the caspase-6 interactome identifies novel substrates that play a role in the pathogenesis of HD

Rona Graham[1], Sean-Patrick Riechers[2], Stefanie Butland[3], Yu Deng[3], Niels Skotte[3], Jenny Russ[2], Vinayagam Arunachalam[2], Erich Wanker[2], Michael R Hayden[3]

[1]Univerity of Sherbrooke, [2]Max Delbruck Centrum, [3]CMMT/University of British Columbia

Caspase-6 has emerged as an important player in Huntington disease (HD), Alzheimer disease (AD) and stroke, where it is activated early in the disease process. Preventing the proteolytic processing of mutant huntingtin (mhtt) or APP in the case of HD and AD, respectively, or targeted deletion of casp6 has been beneficial in these conditions. Using Y2H screening we identified a high confidence list of 89 potential casp6 interactors of which 58% are predicted to contain a casp6 recognition site. Bioinformatic approaches were used to prioritize the characterization of casp6 interactors involved in the pathogenesis of neurodegenerative diseases. Currently, 7 proteins have been assessed and 5 demonstrated to be novel casp6 substrates. STK3, a proapoptotic kinase and validated casp6 substrate identified was then assessed in an acute model of HD. Immortalized striatal cells with and without mhtt were serum starved and assessed for STK3 expression. A significant increase in full-length levels of STK3 is observed in mhtt expressing neurons at baseline ($p<0.05$). A significant increase in fragment levels of STK3 is observed in neurons expressing mhtt vs. WT post serum starvation ($p<0.0001$). A total of 37 proteins, not containing a predicted casp6 site, were assessed for interaction using LUMIER and 51% further validated. Current studies include validating other potential substrates and performing CO-IP on LUMIER-verified hits. Characterization of casp6 interactors will provide critical information regarding key pathways involved in the pathogenesis of neurodegenerative diseases.

1-C-39 Bnip3 up-regulation and mitochondrial dysfunction in PARP-1 induced neurotoxicity

Amit Kamboj[1], Ping Lu[1], Spencer B Gibson[1], Christopher M Anderson[1]

[1]St. Boniface Hospital Research Centre/University of Manitoba

The nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) causes neuron death in brain ischemia by inducing mitochondrial permeability and nuclear translocation of apoptosis-inducing factor (AIF). The mechanisms of mitochondrial damage by PARP-1 are poorly understood. Bcl-2/adenovirus E1B 19 kDa-interacting protein (Bnip3) mediates neuron death in hypoxia by permeabilizing mitochondrial membranes, and Bnip3 transcription is regulated by factors shown previously to be influenced by PARP-1. We thus hypothesized that PARP-1 causes Bnip3-mediated mitochondrial dysfunction and neuron death. We used primary cortical neuron cultures to examine neuron death and mitochondrial integrity in hypoxia, which is a model that produces Bnip3-dependent cell death, and following treatment with the DNA alkylator, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), which is a direct normoxic PARP-1 activator. Hypoxic neuron survival (48 hrs) was significantly enhanced by the parp-1-/- and bnip3-/- genotypes, compared to control hypoxic wildtype cells, indicating both PARP-1 and Bnip3 are important contributors to neuron death. Hypoxic Bnip3 expression and mitochondrial integration was blocked by deletion of PARP-1; while Bnip3 expression, mitochondrial integration and nuclear AIF translocation was enhanced by normoxic PARP-1 activity. MNNG (PARP-1)-induced neuron death was accompanied by mitochondrial permeability transition and membrane potential collapse, which was significantly attenuated by deletion of Bnip3. These results implicate a role for PARP-1 in Bnip3 expression and mitochondrial activity.

1-C-40 Evaluating the impact of versican isoforms on oligodendrocyte maturation and remyelination

Michael Keough[1], Lorraine W Lau[1], Axinia Doering[1], V. Wee Yong[1]

[1]University of Calgary

The extracellular matrix lesion environment impacts remyelination following a CNS insult. We previously reported that mice lacking the remodeling enzyme MMP-9 have reduced remyelination capacity, partly by an inability to clear an inhibitory chondroitin sulfate proteoglycan (CSPG) called NG2. More recently, we found that CSPGs

are upregulated after demyelination and are cleared during remyelination; an inhibitor of CSPG synthesis, xyloside, promoted repair. Here we have tested the hypothesis that particular CSPG members are candidates for remyelination failure in chronic demyelination. Using the dorsal column lysolecithin demyelination model in mice, we found an isoform of the CSPG versican (V2) increased during demyelination and decreased during remyelination. Unexpectedly, another isoform (V1) was slightly increased during demyelination but most robustly expressed during remyelination, suggesting that it may be growth promoting. We found that the sources of versican in lysolecithin injury are predominantly monocytoid lineage cells; indeed, macrophages produce versican when stimulated in culture. We also found that CSPGs reduce adhesion and maturation of oligodendrocyte precursor cells (OPCs). The impact of versican isoforms on OPC maturation is presently unknown and we are purifying V1 and V2 isoforms from bovine CNS for use as substrates for OPCs in culture. If versican isoforms have opposing functions on OPCs, we will attempt strategies to enforce regulation of the versican gene to reshape the extracellular environment to that which is more conducive for myelin repair.

1-C-41 The role of FoxO3a in PARP-1-induced Bnip3 signaling pathway during hypoxia

Ping Lu[1], Amit Kamboj[1], Christopher M Anderson[1]

[1]The University of Manitoba

The nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) plays a critical role in mitochondrial dysfunction and neuron death in cerebral ischemia, but the mechanisms of PARP-1-induced mitochondrial damage remains unclear. Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (Bnip3) is a pro-apoptotic protein that causes dysfunction of mitochondria and can be induced epigenetically by transcription factor, FoxO3a (Forkhead box O 3a). We found previously that PARP-1-induced mitochondrial dysfunction is dependent on Bnip3 expression and that hypoxic Bnip3 expression in cortical neurons is dependent on PARP-1 expression. The objective of the present study was to define how PARP-1 controls Bnip3 expression by focusing on nuclear activation of FoxO3a. We demonstrated that hypoxia significantly increased Bnip3 mRNA in cultured cortical neurons. This affect was attenuated by the PARP-1 inhibitor, PJ34 and genetic deletion of PARP-1. Hypoxic PARP-1 activation resulted in reduced intracellular NAD levels, leading to postulation that control of Bnip3 transcription by NAD - dependent histone deacetylase (Sirt1) may be inhibited by PARP-1. We found a direct interaction between Sirt1 and FoxO3a and that PARP-1 inhibition significantly reduced acetylation of FoxO3a enhanced by hypoxia. Moreover, nuclear translocation of FoxO3a in response to hypoxia was inhibited by PARP-1 inhibition. These data demonstrate that hypoxia leads to PARP-1-induced NAD depletion, which in turn, enhances acetylation of FoxO3a. Further work is required to show that FoxO3a directly drives Bnip3 promoter activity.

1-C-42 Meta-analyses of gene expression patterns associated with schizophrenia in the postmortem human brain

Meeta Mistry[1], Jesse Gillis[1], Paul Pavlidis[1]

[1]University of British Columbia

Schizophrenia is a severe psychiatric illness for which the etiology remains unknown. Numerous studies have examined gene expression profiles in postmortem human brain samples from individuals with schizophrenia compared with healthy controls, to gain insight into the molecular mechanisms of the disease. Although some findings have been replicated across studies, there is a general lack of consensus on which genes or pathways are affected. Here, we present the most comprehensive analysis to date of expression patterns in schizophrenia by assembling a data set of 153 affected and 153 control individuals across seven independent studies. Remarkably, our combined differential expression analysis revealed a signature of genes showing differences in the schizophrenic brain associated with various aspects of neuronal communication. Using the same seven datasets, we created aggregated coexpression networks for control and schizophrenia cohorts separately. We investigated network properties of our differentially expressed 'schizophrenia genes', to reveal a shared relationship between genes. Moreover, we show that the observed behaviour of network properties of our schizophrenia genes is distinct from other functionally defined gene sets and other disease gene groups. Our results provide evidence for a common underlying expression signature in this heterogeneous disorder, and demonstrate the utility of network analysis of coexpression in the control and schizophrenic human brain.

1-C-43 Altered sensory function and cognitive deficits are independent of disease severity in a chronic-relapsing model of experimental autoimmune encephalomyelitis (EAE)

Camille Olechowski[1], Gustavo Tenorio[1] , Bradley J Kerr[1]

[1]University of Alberta

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that leads to severe neurological disabilities. In addition to the impairments in sensory and motor function, MS is also associated with a high incidence of depression, cognitive impairments and neuropathic pain. Previously, our lab demonstrated that neuropathic pain behaviours are present before and at disease onset in an animal model of MS, experimental autoimmune encephalomyelitis (EAE). We

have now monitored changes in cognitive ability to determine if altered pain sensitivity is also associated with behavioural signs indicative of cognitive impairment in this model across different EAE disease severities. Additionally, we have used the β-lactam antibiotic Ceftriaxone that has been reported to be capable of upregulating glutamate transporter expression in the CNS and monitored its effects on sensory and cognitive changes in EAE mice.

1-C-44 The contribution of HIP14 and HIP14L to the synaptic dysfunction observed in Huntington's Disease

Matthew Parsons[1], Austen J Milnerwood[1], Roshni R Singaraja[1], Liza M Sutton[1], Michael R Hayden[1], Lynn A Raymond[1]

[1]University of British Columbia

Palmitoylation, a post-translational modification mediated by palmitoyl acyltransferases (PATs), helps to anchor proteins within the plasma membrane. Many synaptic proteins are palmitoylated, suggesting that palmitoylation deficits may result in synaptic dysfunction. Interestingly, Huntington's Disease (HD), a neurodegenerative disease severely affecting normal cognitive and motor function, is associated with both a palmitoylation deficit and early synaptic dysfunction. These alterations may contribute to the acute vulnerability of striatal medium spiny neurons in HD. To begin to investigate the contribution of PAT signalling to the synaptic dysfunction observed in HD, we performed electrophysiological recordings from acute striatal and hippocampal slices from mice lacking either HIP14 or HIP14L - two PATs implicated in HD - and compared their cellular and synaptic properties to known measures affected by the huntingtin mutation. We found that while HIP14-/- mice displayed many striatal and hippocampal deficits that mimic various HD mouse models, HIP14L-/- mice did not differ from their wild-type littermates in a number of electrophysiological measures. Neither knockout alone could account for the increase in extrasynaptic-NMDA receptor pool that occurs in early HD. Our data suggest that a palmitoylation deficit in HD can at least partially explain the key synaptic modifications associated with the disease state. Thus, targeting certain PATs to alleviate these synaptic changes may prove beneficial as a treatment to delay or prevent cell death and disease symptoms in HD.

1-C-45 Gap junction blockers promote neuroprotection in retinal degeneration induced by mechanical trauma.

Vera Paschon[1], Guilherme S Higa[1], Alexandre H Kihara[1]

[1]University of São Paulo – Brazil

Previous studies showed that connexin (Cx) channels in the gap junctions (GJ) are involved in neurodegeneration after injury. The aim of this study was to elucidate the role of GJ communication in a focal lesion induced by mechanical trauma of the retina, a model that allows spatial and temporal definition of the lesion with high reproducibility, permitting visualization of the focus, penumbra and adjacent areas. Cx36 maintained mRNA expression and protein levels throughout neurodegeneration. Cx43 mRNA was upregulated in the 7-day lesioned retinas (+182%, $P<0.05$), as well as protein levels (+27%, $P<0.05$). The functional role of cell coupling was assessed employing GJ blockers and openers combined with lactate dehydrogenase (LDH) assay, a method for evaluating cell death/viability. LDH in vitro assays were performed in 1-day lesioned retinas. Carbenoxolone (CBX), a broad-spectrum GJ blocker, reduced LDH release after 4hours (-22%, $P<0.01$) whereas quinine, a Cx36-channel specific blocker, decreased LDH release after 1h (-31%, $P<0.01$), 2h (-28%, $P<0.01$) and 4h (39%, $P<0.01$). Analysis of dying cell distribution confirmed that the GJ blockers reduced apoptosis spread. Blockade of GJ communication during neurodegeneration with quinine caused downregulation of initial and effector caspases. To summarize, we observed specific changes in Cx expression indicating their participation in acute neurodegeneration processes. Our results revealed that the GJ channels permeability may take part in reliable neuroprotection strategies aimed to fast treatment of mechanical trauma in the retina.

1-C-46 Pain and cellular activation in experimental autoimmune encephalomyelitis (EAE)

Liam Potter[1], Bradley J Kerr[1], Ian Winship[1], Curtis A Benson[1]

[1]University of Alberta

Experimental autoimmune encephalomyelitis (EAE) is an animal model that possesses similarities to Multiple Sclerosis (MS) in its underlying pathology. Previous work done in our lab has shown increased sensitivity to pain and signs of cognitive or affective deficits in the EAE model, similar to those seen in MS patients. We have also shown that, compared to non-diseased controls, EAE mice exhibit elevated levels of c-Fos, a marker of cellular activation, in the CNS. We are now investigating how FOS levels are affected in the CNS of mice with EAE in response to different types of sensory stimulation (noxious and non-noxious) as well as after treatments that can modify the disease course. We find that ongoing FOS expression is significantly decreased in the ipsilateral superficial dorsal horn of the spinal in response to tactile stimulation with von Frey Hairs. Similar effects are seen in the hindlimb somatosensory cortex. Treatment with the MAO inhibitor phenelzine (PLZ), that we have previously shown can decrease the severity of clinical signs in EAE mice and improves gross locomotor function in the disease, was associated with a significant increase in cFOS expression in the ventral horn of the spinal cord. However, basal levels of FOS in the superficial dorsal horn were not affected in

EAE mice treated with PLZ. Experiments are now underway using in vivo functional brain imaging techniques to assess how these changes in FOS levels relate to patterns of activation in cortical regions using different forms of somatosensory stimulation.

1-C-47 Minocycline inhibits matrixmetalloproteinase activity after acute ischemic stroke with hyperthermia

Shakib Rahman[1], Kathryn G Todd[1], Ashfaq Shuaib[1]

[1]University of Alberta

Hyperthermia is known to exacerbate the damage caused by a stroke, but the exact mechanism is unknown. Matrix metalloproteinase (MMP) expression and activity, which is important in maintenance of the cerebrovasculature, is increased after hyperthermia according to recent work from our lab. Minocycline, a neuroprotective agent, has been shown to reduce infarct size in ischemic stroke. We predict that this effect is due to the MMP inhibition by minocycline which should reduce MMP activity and subsequent basal lamina degradation. We treated animals with either minocycline (3mg/kg,iv) or saline one hour after induction of ischemia using an embolic stroke model. Animals were subjected to hyperthermia (39ºC) or normothermia (37ºC) for two hours before recovering for two hours. Brain tissue was analyzed for MMP2 and MMP9 protein expression and activity and laminin degradation was detected at four hours after stroke onset. Our data demonstrates that minocycline treatment significantly decreases MMP2 but not MMP9 protein expression after upregulation via hyperthermia and ischemia, but there was no effect on protein expression in normothermia. MMP2 and MMP9 activity was significantly reduced, as was laminin degradation in ischemia with hyperthermia after minocycline treatment. Taken together our data provides a potential mechanism for the neuroprotective effect of minocycline in hyperthermia. Our data also demonstrates that minocycline is effective in reducing MMP activity and subsequent degradation of laminin and is a potential therapy for ischemic stroke during hyperthermia.

1-C-48 The role of receptor for advanced glycation end products (RAGE) in sensory neurons isolated from normal rats

Ali Saleh[1], Darrell R Smith[3], Lori Dunn[1], Corina Mertens[1], Randy Van der Ploeg[1], Cory Toth[2] , Douglas W Zochodne[2], Paul Fernyhough[3]

[1]St. Boniface Hospital Research Centre, [2]Dept of Clinical Neurosciences and the Hotchkiss Brain Institute, University of Calgary, [3]Dept of Pharmacology & Therapeutics, University of Manitoba, St. Boniface Hospital Research Centre

The Receptor for Advanced Glycation End-products (RAGE) is a multi-ligand signaling system implicated in chronic diseases such as diabetes and neurodegenerative disorders. In diabetes, the up-regulation of function of RAGE has been associated with cellular perturbation and tissue injury. However, in the PNS, knockout or blockade of RAGE was associated with impaired nerve regeneration. We tested the hypothesis that RAGE signaling modulated neurotrophin-induced neurite outgrowth in cultured sensory neurons. Dorsal Root Ganglia (DRG) neurons from normal rats were cultured and infected with lentivirus carrying shRNA to RAGE and neurite outgrowth analyzed. The effects of various RAGE ligands, signal transduction inhibitors and function blocking anti-RAGE IgG were also tested. Finally, neurons were transiently transfected with different RAGE promoter reporter constructs and impact of cytokines studied. ShRNA or anti-RAGE IgG blockade of RAGE inhibited neurotrophin-induced neurite outgrowth by 60-90% ($P<0.05$). RAGE ligands including human glycated albumin (HGA), S100B and HMG-1 in the presence of neurotrophins elevated neurite outgrowth at least 2-fold ($P<0.05$). HGA enhanced neurite outgrowth via NF-kB, PI-3K and MAPK pathways. IL-1â elevated RAGE promoter activity. In adult sensory neurons RAGE signaling is an important mediator of neurotrophin-dependent neurite outgrowth. Early in type 1 diabetes RAGE expression is impaired in DRG possibly due to lowered cytokine expression, a finding that may impact on early sensory neuron dysfunction.

1-C-49 Amyloid-beta oligomers induce tau-independent disruption of fast axonal transport via calcineurin in cultured hippocampal neurons

Michael Silverman[1], Michael A Silverman, Elisa Ramser, Kathlyn J Gan, Emily Y Fan, Matthew M Suzuki

[1]Simon Fraser University

Fast axonal transport (FAT) defects are suggested to play a crucial role in neuronal dysfunction in Alzheimer's disease (AD). We have previously shown that amyloid beta oligomers (AβOs), a causative agent of AD, impede FAT of dense core vesicles (DCVs) in cultured hippocampal neurons. AβOs induce hyperphosphorylation and fragmentation of the axonal MAP tau; however, it remains unclear how these modifications disrupt transport. To determine whether tau is required for AβO-induced transport defects, we imaged fluorescently-tagged brain-derived neurotrophic factor (BDNF), a DCV cargo, in living neurons from WT and tau-/- mice treated with low nM concentrations of AβOs. BDNF bidirectional transport was similarly reduced in AβO-treated WT and tau-/- neurons, suggesting that tau is not involved in AβO-induced BDNF transport defects. We also show that the integrity of the microtubule network and tubulin modifications are unaffected in the presence of AβOs, eliminating the possibility that transport defects were caused by cytoskeleton instability. We hypothesized that AβO-disrupted transport results from tau-independent

dysregulation of signaling cascades implicated in AD. For example, the activation of calcineurin (CaN), a Ca2 /calmodulin-dependent phosphatase, which is upregulated in the presence of AβOs. CaN inhibition using FK506 in neurons from WT and tau-/- mice not only rescued, but reversed AβO-induced FAT defects. Our results indicate that AβO-induced FAT disruption is independent of tau and microtubule destabilization, and that this dysregulation is mediated by CaN.

1-C-50 Therapeutic effects of ganglioside GM1 administration in the R6/2 model of Huntington disease

Simonetta Sipione[1], Melanie Alpaugh[1], Alba Di Pardo[1], Luis Carlos Morales[1], Mel Horkey[1]

[1]University of Alberta

Huntington disease (HD) is a neurodegenerative disorder that results in motor, cognitive and psychiatric deficits. The disease is caused by the expansion of a polyglutamine stretch in huntingtin, a ubiquitous protein with still unclear functions. The molecular mechanisms underlying neurodegeneration in HD are complex and include transcriptional dysregulation, mitochondrial dysfunction, impaired intracellular and axonal transport, as well as aberrant signaling and neurotransmission. PARA We recently demonstrated that the synthesis of ganglioside GM1, a lipid molecule involved in cell signaling, is also impaired in multiple HD models. We further demonstrated that restoring normal levels of GM1 abrogates motor symptoms in 6 month-old YAC128 mice, a model of HD that expresses the entire human HD gene. The beneficial effects of GM1 lasted for at least 14 days after discontinuation of the treatment, suggesting that, in addition to treating disease symptoms, GM1 might slow down disease progression and neurodegeneration. PARA To test this hypothesis and to measure the effects of GM1 on neurodegeneration we used R6/2 mice, which express an N-terminal fragment of mutant huntingtin and display more severe and accelerated neurodegeneration than the YAC128 model. Intraventricular infusion of GM1 for 28 days improved motor behaviour in R6/2 mice, decreased brain weight loss and prolonged R6/2 mice lifespan. There results confirm that GM1 exerts neuroprotective effects in vivo and slows down disease progression. They also suggest that GM1 administration could be a therapy for HD.

1-C-51 Pirenzepine, a muscarinic receptor antagonist reverses sensory neuropathy and corrects deficits in mitochondrial protein expression and function in dorsal root ganglia of streptozotocin-induced diabetic rodents.

Darrell Smith[1], Subir K Roy Chowdhury[1], Katie Frizzi[2], Lakshmi Kotra[3], Nigel A Calcutt[2], Paul Fernyhough[1]

[1]St. Boniface Research Centre, [2]University of California San Diego, [3]Department of Pharmaceutical Sciences, University of Toronto

Muscarinic acetylcholine type 1 receptor (M1R) antagonism enhances neurite outgrowth in embryonic and adult sensory neurons. We tested the hypothesis that treatment with a M1R selective antagonist, pirenzepine (PZ), would prevent or reverse intraepidermal fiber (IENF) loss in streptozotocin (STZ)-induced diabetic rodents (type 1 diabetes model). We also determined if reversal of neuropathy was associated with correction of deficits in mitochondrial function in dorsal root ganglia (DRG) of diabetic rodents. Male Swiss Webster mice or Sprague Dawley rats were made diabetic with STZ and maintained for 22 weeks. Sensory neuropathy was confirmed by the presence of thermal hypoalgesia. Thereafter, mice and rats were treated with daily sc injections of 10 mg/kg PZ. Treatment for 2 months restored thermal sensitivity and reversed a diabetes-induced reduction of IENF levels. DRG were analyzed for expression of mitochondrial-related gene expression and activity of electron transport system (ETS) complexes. In the DRG of diabetic mice AMP kinase, PGC-1α and various ETS components exhibited a 50% or greater reduction in expression that was significantly reversed by PZ. The drug also normalized ETS complex activity in the DRG of diabetic mice and corrected reduced rates of respiratory chain activity (measured as rate of oxygen consumption) in the DRG from STZ-diabetic rats. M1R antagonism effectively reversed sensory neuropathy in rodents and this was accompanied by modulation of signal transduction pathways associated with enhanced mitochondrial biogenesis and activity.

1-C-52 Endothelial cells regulate p53-dependent apoptosis of neural progenitors after irradiation

Shun Wong[1], Fred Lu[1], Yu-Qing Li[1], Isabelle Aubert[1]

[1]Sunnybrook Health Sciences Centre

Endothelial cells represent an important component of the neurogenic niche, and may regulate self-renewal and differentiation of neural progenitor cells (NPCs). Whether they play a role in determining the apoptotic fate of NPCs after stress or injury is unclear. NPCs are known to undergo p53-dependent apoptosis after ionizing radiation, whereas endothelial cell apoptosis after irradiation is dependent on membrane acid sphingomyelinase, and is abrogated in smpd1 (gene that encodes acid sphingomyelinase) deficient mice. Here, we found that p53-dependent apoptosis of NPCs in vivo after irradiation was inhibited in smpd1 deficient mice. NPCs cultured from mice wild type or knock out of the smpd1 gene however demonstrated no difference in apoptosis radiosensitivity. NPCs transplanted into the hippocampus of smpd1 knockout mice were protected against apoptosis after irradiation compared to those transplanted into smpd1 wildtype mice. Intravenous administration of basic fibroblast

growth factor, which does not cross the blood-brain barrier, and known to protect endothelial cells against apoptosis after irradiation also attenuated the apoptotic response of NPCs. These findings provide evidence that endothelial cells may regulate p53-dependent apoptosis of NPCs after genotoxic stress, and add support to an important role of endothelial cells in regulating apoptosis of NPC after injury or in disease.

1-C-53 Selective ih modulator ZD7288 produces anxiolytic-like effects in neurophysiological and behavioural models of anxiety

Michelle Yeung[1], Dallas Treit[1], Clayton T Dickson[1]

[1]University of Alberta

Hippocampal theta rhythms have been associated with a number of behavioural processes, including learning and memory, spatial navigation, and more recently, anxiety. Recently it has been argued that the suppression of evoked hippocampal theta frequency is a reliable neurophysiological signature of anxiolytic drug action. This model is based on the observation that all clinically proven anxiolytic drugs (e.g. benzodiazepines) reduce the frequency of reticularly-elicited hippocampal theta brain rhythm, while drugs that do not selectively affect anxiety (e.g. antipsychotics and procognitive drugs) do not modulate theta frequency. While considerable pharmacological evidence supports this model, there is emerging evidence that other classes of clinically proven drugs (e.g. anticonvulsants - Phenytoin, see Yeung et al., 2011), which previously were believed not to possess anxiolytic effects, can also suppress hippocampal theta and, surprisingly, act as an anxiolytic in behavioral animal models of anxiety. In this study, we provide evidence which suggests that ZD7288, a bradycardic agent which indirectly modulates hippocampal theta oscillatory activity through the regulation of the hyperpolarization-activated inward current ih-- but has no known involvement in anxiety-- also suppresses evoked hippocampal theta frequency and reduces anxiety in animal behavior models (the Elevated Plus Maze) after intra-hippocampal microinfusions. Taken together, our results provide strong converging evidence of the predictive validity of the theta suppression model of anxiolytic drug action.

1-C-54 Iron accumulation and expression of iron homeostasis proteins in the spinal cord in experimental autoimmune encephalomyelitis (EAE)

Juan Zarruk Serrano[1], Katrin Schulz[1], Jennifer L Berard[1], Samuel David[1]

[1]McGill University Health Center

The mechanism underlying iron accumulation in the brain in MS is not known. We used EAE, a widely used animal model of MS, to study iron homeostasis in the spinal cord in relapsing-remitting (RR) and chronic (CH) forms of EAE. Mice were sacrificed at the onset, peak and remission stages of EAE as well as 8-9 weeks after induction ('end stage'). A significant increase in iron was detected at the end stage of RR and CH EAE using the ferrozine assay. Increased ferritin immunoreactivity, was detected earlier in EAE lesions starting at the peak stage in RR and CH EAE, and was localized mainly to macrophages. At the end stage, ferritin was significantly higher in CH EAE versus RR EAE. The mRNA expression of molecules involved in iron homeostasis was assessed. The heavy chain of ferritin increased 1.5-fold at onset, 2.5-fold at peak, and returned to normal in remission in RR and CH EAE. Ferritin light chain increased 5-fold at peak in both forms of EAE and appeared to be higher in remission in CH EAE versus RR EAE. There was a 7-fold increase in ceruloplasmin at the onset and peak in RR and CH EAE, while hephaestin was increased about 1.7-fold only in RR EAE. A small (30%) increase in DMT1 was seen in onset of RR and CH EAE. No differences were detected in transferrin receptor 1. These results suggest that iron accumulation is seen in the later stages of EAE. As increased ferritin immunoreactivity in macrophages is seen before signs of increased iron accumulation, it is possible that this early increase in ferritin expression may be regulated by oxidative stress or cytokines.

D – Sensory and Motor Systems

1-D-89 Non-invasive peripheral stimulation allows the re-expression of endogenous locomotor pattern in adult rats with complete thoracic spinal cord transection

Olivier Alluin[1], Hugo Delivet-Mongrain[1], Serge Rossignol[1]

[1]Université de Montréal

Numerous studies have demonstrated the spontaneous re-expression of locomotion in adult cats 2-3 weeks after complete thoracic spinal cord injury (SCI). However, such re-expression of hindlimb locomotion without pharmaco- or electrostimulation has not been fully documented in adult rats after a complete thoracic SCI. In our study, a complete SCI was made at T9 in 5 adult female Wistar rats. Rats were then trained on a treadmill 10 minutes daily, 5 days a week at different velocities for 14 weeks using only manual perineal stimulation. Throughout this period, movements were recorded using conventional video or fluoroscopy (X-ray) for kinematic analyses and electromyography of selected hindlimb muscles was acquired. Our results show the recovery of locomotor capability in all the adult paraplegic rats. Two days after SCI, strong perineal stimulation was necessary to elicit some slight flexion/extension of the hindlimbs. Then, in the course of the next 3 weeks the locomotor pattern gradually improved

while the stimulation intensity needed to produce locomotion decreased. Three to five weeks after SCI, all rats were able to perform more than 10 consecutive, well-defined, alternating and coordinated step cycles with plantar placements. Locomotion was executed in a natural posture (horizontal) using only moderate external perineal stimuli. Thus, the spinal network of adult paraplegic rats can re-express hindlimb locomotion on a treadmill without requiring pharmacological or other types of electrostimulation although non-invasive afferent stimulation is required.

1-D-90 Distribution of connexin36 in juvenile and adult rodent spinal cord: Co-localization with vglut-1 suggests primary afferent terminals form mixed chemical and electrical synapses

Wendy Bautista[1], David A McCrea[1], James I Nagy[1]

[1]University of Manitoba

Neuronal gap junctions in mammalian CNS are formed primarily by connexin36 (Cx36) and represent the ultrastructural correlate of electrical synapses. Although the presence of electrical coupling between rodent spinal cord neurons has been described,a comprehensive analysis of the distribution of Cx36 in developing or mature cord has not yet been reported. We used immuno¬histochemical approaches to examine Cx36 localizationin the cervical, thoracic and lumbar spinal cord at various developmental stages. The anti-Cx36 antibodies used have been validated for specificity by showing absence of Cx36 detection in Cx36 knockout mice. As elsewhere in the CNS, immunolabelling for Cx36 in spinal cord was exclusively punctate in appearance, which presumably reflects localization to sites of gap junctions. Cx36 was densely distributed throughout spinal cord gray matter during the second postnatal week. Although Cx36 expression was reduced in adult cord, it persisted in deep laminae of the dorsal horn and some regions of the ventral horn including areas containing motoneurons. Most striking at all spinal levels were densely distributed Cx36-positive puncta on cell bodies and initial dendrites of neurons located near the central canal at the transition between the dorsal and ventral horn. Our observations suggest that the majority of Cx36-positive puncta in adult spinal cord is co-localized with the nerve terminal marker vglut-1, considered to be localized mainly in the terminals of myelinated primary afferents these terminals form mixed chemical/electrical synapses.

1-D-91 Effects of pregabalin on nociceptive behaviour and evoked glutamate release in a trigeminal neuropathic pain model

Terence Coderre[1], Naresh Kumar[1], Pavel S Cherkas[2], Ye Cao[2], Hua Wang[2], Chen-Yu Chiang[2], Jonathan O Dostrovsky[2], Barry J Sessle[2]

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

Pregabalin is effective in treating many neuropathic pain conditions, but it is unclear if it has analgesic effects in animal models of orofacial pain. The aim of the present study was to examine if pregabalin reduces nociceptive behaviour in a rat model of trigeminal neuropathic pain and also affects glutamate release in the medulla. Mechanical nociceptive withdrawal thresholds were tested with von Frey filaments applied to the face pre-operatively and for 7 days post-operatively in rats with infraorbital nerve transection (IONX) and in control rats. In other rats, an in vivo microdialysis fibre was inserted at post-operative day 7 into the exposed medulla, microdialysis samples were collected, and glutamate release evoked by tooth pulp stimulation was determined by HPLC. The effects of pregabalin (1-100 mg/kg, i.p.) were examined on the behaviour and medullary glutamate release at post-operative day 7. The facial mechanical withdrawal thresholds in IONX rats were dramatically decreased bilaterally from post-operative day 1 to 7, at which time pregabalin significantly and dose-dependently reversed the decreased thresholds when compared with saline ($p < 0.05$, ANOVA). Compared with saline, pregabalin also significantly and dose-dependently attenuated evoked medullary glutamate release in IONX rats. This study demonstrates that pregabalin dose-dependently attenuates the mechanical allodynia and medullary release of glutamate in this trigeminal neuropathic pain model, and indicates that it may be useful clinically for treating orofacial neuropathic pain states.

1-D-92 Distinct cortical circuit mechanisms for complex forelimb movement and motor map topography

Thomas Harrison[1], Oliver G Ayling[1], Timothy H Murphy[1]

[1]University of British Columbia

Cortical motor maps are the basis of voluntary movement, but they have proven difficult to understand in the context of their underlying neuronal circuits. We applied light-based motor mapping of Channelrhodopsin-2 mice to reveal a functional subdivision of the forelimb motor cortex based on the direction of movement evoked by brief (10 ms) pulses. Prolonged trains of electrical or optogenetic stimulation (100-500 ms) targeted to anterior or posterior subregions of motor cortex evoked reproducible complex movements of the forelimb to distinct positions in space. Blocking excitatory cortical synaptic transmission did not abolish basic motor map topography, but the site-specific expression of complex movements was lost. Our data suggest that the topography of movement maps arises from their segregated output projections, whereas complex movements evoked by prolonged stimulation require intracortical synaptic transmission.

1-D-93 Asymmetries in human smooth pursuit eye movements

Sally Ke[1], Dinesh K Pai[1], Miriam Spering[1]

[1]University of British Columbia

Smooth pursuit eye movements are the key effector response to moving visual objects. Although these movements are generally quite accurate, some studies have reported asymmetries in the direction of smooth pursuit. Here we study smooth pursuit systematically across stimulus directions, speeds, and locations in the visual field to assess asymmetries and to investigate their potential perceptual consequences. Stimuli were small spots of light moving along one of four cardinal or diagonal axes at speeds ranging from 2 to 45 deg/s. Binocular eye movements were recorded in a large sample of 20 subjects. We found a strong pursuit bias towards downward motion with fewer catch-up saccades, faster initial eye acceleration, and higher peak eye velocity than towards upward motion. Pursuit was also better in horizontal than in vertical directions. Asymmetries along all axes were stronger for fast than for slow speeds. Our finding of a strong up-down asymmetry is consistent with human imaging results, where activation in the cerebellar flocculus is greater during downward than upward pursuit, as well as behavioral and neurophysiological studies in monkeys. These asymmetries could reflect the influence of prior experience on smooth pursuit - object motion along the horizontal axis or downward occurs more frequently than object motion in other directions. The functional significance of better downward pursuit could be to anticipate trajectories of falling objects due to gravity.

1-D-94 In vivo large-scale cortical mapping using channelrhodopsin-2 stimulation in transgenic mice reveals asymmetric and reciprocal relationships between cortical areas

Diana Lim[1], Majid H Mohajerani[1], Jeffrey LeDue[1], Jamie Boyd[1], Shangbin Chen[1], Timothy H Murphy[1]

[1]University of British Columbia

We have used in vivo voltage sensitive dye (VSD) imaging and arbitrary point channelrhodopsin-2 (ChR2) stimulation in transgenic mice for large-scale functional mapping of cortical activity. Photostimulation of ChR2-expressing neurons in primary sensory cortices evoked cortical activity patterns that were similar to activity patterns evoked by peripheral sensory stimulation, suggesting that point stimulation can evoke downstream cortical activity and that optogenetic stimulation can be used to map prototypical cortical activity. We used ChR2 point stimulation to elicit cortical activity at a number of cortical areas including associational areas, which are difficult to access through peripheral stimulation, and we used VSD imaging to image cortical activity over a large scale. Using graph theory and network analysis, we created a network diagram which displays the regions of ChR2 point stimulation as network nodes and the evoked cortical responses as network edges. We identified asymmetrical connection weights between nodes and identified the parietal association cortex as a network hub. We identified uneven connection strength between primary (S1) and secondary sensory (S2) areas, with the connections from S1 to S2 areas being significantly stronger than connections from S2 to S1. Using ChR2 stimulation and VSD imaging, we can investigate large-scale circuit organization and deduce functional relationships between a number of cortical areas. We anticipate this will be a useful approach for mapping cortical activity in various models of human disease, such as stroke.

1-D-95 A hierarchically organized sound discrimination pathway in auditory cortex

Stephen Lomber[1], Amee J McMillan[1], Andres Carrasco[1], Paul Cornwell[1]

[1]Western University

In this study we tested the hypothesis that ventral, but not dorsal, areas of cat temporal lobe have greater specificity for complex acoustic stimuli. The animals concurrently learned to discriminate three sound classes: tones, narrow-band bursts, and conspecific vocalizations. With criterion performance of 70% correct, we identified that conspecific vocalizations were learned the fastest, while tonal discriminations often required twice the time to master. After training, cooling loops were bilaterally placed over primary auditory cortex (A1), second auditory cortex (A2), temporal cortex (area T), and insular cortex (area IN) to permit their temporary and reversible deactivation. The animals were tested while each area was bilaterally or unilaterally deactivated. Presentation of the three classes of stimuli was randomly presented within each testing session. Bilateral deactivation of A1 resulted in discrimination deficits on all three stimulus classes. Bilateral deactivation of A2 caused deficits only for the narrow-band burst and conspecific vocalization classes. Bilateral deactivation of area T resulted in deficits restricted to the conspecific vocalizations. Unilateral deactivation of left, but not right, area T caused deficits during conspecific vocalization discriminations. These findings provide evidence for the lateralization of conspecific vocalization discrimination in the left hemisphere. The results of this study indicate a "what" processing pathway in auditory cortex of the cat that arises in primary auditory areas and radiates down the temporal lobe.

1-D-96 Effects of histone deacetylation inhibition on corticospinal tract function and regeneration: in vitro and in vivo

Tao Meng[1], Audrey Petit[1], Christie McShane[1], Jie Liu[1], Greg Duncan[1], Wolfram Tetzlaff[1], Jane Roskams[1]

[1]University of British Columbia

The corticospinal tract (CST) is important for descending motor control, but has proven recalcitrant to regenerate in both rodents and humans after spinal cord injury (SCI). This could be partially due to the fact that CST neurons terminally differentiate into a non-regenerative state following synaptogenesis-with a final shift into maturity mediated, in part, by histone deacetylation. We are thus testing if it is possible to a transiently de-repress the mature neuronal state of CST neurons using histone deacetylase inhibitors (HDACi) to reactivate developmental expression programs that may facilitate repair and regeneration. To test HDACi that may stimulate CST regeneration, we recently developed an in vitro model of "regenerating" postnatal YFP-positive CST neurons that have already established spinal targets. We have used a combination of Cellomics for survival kinetics and morphometric analysis for outgrowth patterns to test different HDACi and found that both Trichostatin A (TSA) and Tubastatin can augment outgrowth of CST neurons in vitro. We are also testing if in vivo HDACi will help lesined motor neurons to display an enhanced capacity for outgrowth, leading to the production of novel circuitry after CST lesion. We administered TSA daily for 1 week after SCI in Thy1-YFP mice used to create CST neurons in vitro. These data suggest that transiently inhibiting HDAC activity with TSA immediately after lesion may enhance CST neuron capacity for outgrowth, enhace behavioural recovery and reduce lesion volume.

1-D-97 Reference frames for visual and motor responses in the Superior Colliculus during head unrestrained gaze shifts

Morteza Sadeh[1], Hongying Wang[1], Gerald P Keith[1], John Douglas Crawford[1]

[1]York University

Micro-stimulation of the superior colliculus (SC) evokes coordinated eye-head gaze shifts toward an eye-centered goal (e.g., Klier et al. Nat. Neurosci. 2001), and head-unrestrained recordings suggest that SC neurons primarily code target location in eye coordinates during saccades directly to visual targets (DeSouza et al. J.Neurosci.2011). Here, we asked if visual and motor responses in the SC (Wurtz and Goldberg, 1971) code different spatial information during head-unrestrained gaze shifts. We recorded neurons of SC in the head-unrestrained monkey during a task which involved a variable memory delay (550-625 ms) between visual target presentation and the saccade 'go' signal (extinguished fixation light). 3-D eye and head rotations were recorded, and receptive field data were analyzed in multiple frames using a statistical method reported previously (Keith et al. J. Neurosci. Meth. 2009). To date 71 neurons were recorded from the left and right SC of two monkeys, and have been analyzed. 19 of these only showed a visual response, 16 neurons had a motor response and the remainder showed visual (after target presentation) motor (around the gaze saccade) responses. Visual receptive fields were both 'closed' (N = 37) and 'open' (20) with a kernel bandwidth of 2-10º. Corresponding motor receptive fields were either 'closed' (11) or 'open' (41), with a kernel bandwidth of 3-11º, always in the contralateral hemifield. Majority of the cells showed optimal (and sometimes significant) coherence of activity in an eye-centered frame in both responses..

E – Homeostatic and Neuroendoctrine Systems

1-E-73 Effects of REM sleep deprivation on hypothalamic and hippocampal glucocorticoid receptors expression and emotional and cognitive behavior in rats

Patricia Barra de la Tremblaye[1], Marianne LeBreton[2], Megan Dunbar[1], Idu Azogu[1], Nathalie LeMarec [1], Hélène Plamondon[1]

[1]University of Ottawa, [2]Université du Québec à Montréal

REM sleep deprivation (RSD) has been implicated in impairment of hippocampal-dependent memory and increased emotionality linked to HPA axis hyperactivity. Glucocorticoids represent a main regulator of allostasis in response to various stressors. Among brain regions, the paraventricular nucleus of the hypothalamus (PVN) and the CA1 hippocampal pyramidal neurons are rich in glucocorticoid receptors (GR). Glucocorticoid plasma levels are elevated after RSD but alterations in GR expression in the brain following RSD remains to be characterized. The current study aims to investigate the impact of acute (ARSD) and chronic (CRSD) REM sleep deprivation, using the platform-over-water method, on locomotion, emotional arousal and memory performance of rats using the open field, the Y Maze and the Morris Water Maze tests. We also assessed expression of GR in the paraventricular nucleus of the hypothalamus (PVN) and the CA1 of the hippocampus by immunohistochemistry. The results show an increased locomotion in the periphery of the open field that was observed in CRSD rats while ARSD animals showed increased exploration of the central zone. These findings suggest altered arousal and/or anxiety level in sleep deprived animals. Interestingly, ARSD led to enhanced retention of an aversive stimulus, as well as enhanced spatial memory. Immunohistochemical analysis revealed that GR-ir is markedly increased in CA1 neurons following CRSD compared to ARSD and control rats. GR-ir in the PVN is being analysed.

1-E-74 Linking neuronal survival and cytoskeletal dynamics: A novel mechanism associated with C-terminal region of teneurin-1 (TCAP-1) and integration through a dystroglycan-associated, ERK-p90RSK-dependent signaling pathway in the mouse hippocampus.

Dhan Chand[1], Lifang Song[1], David A Lovejoy[1]

[1]University of Toronto

Teneurin C-terminal associated peptides (TCAPs) are a new family of bioactive peptides encoded on the terminal exon of the teneurin genes and are highly expressed in the central nervous system. Previous studies indicate that TCAP-1 regulates axon fasciculation and dendritic spine density in the rodent hippocampus. Moreover, TCAP-1 is neuroprotective under oxidative stress and blocks corticotropin-releasing factor (CRF)-mediated behaviours and c-fos synthesis in the hippocampus. Thus, new studies were aimed at understanding the molecular mechanisms by which TCAP-1 regulates hippocampal cytoskeletal dynamics and cell survival. In cultured mouse hippocampal cells, TCAP-1 co-localizes with the dystroglycan complex at the plasma membrane and stimulates extracellular signal-regulated kinases (ERK1/2)-dependent phosphorylation of the microtubule regulatory protein stathmin at serine-25 and a corresponding dephosphorylation at serine-16 and -63. TCAP-1 also stimulated an ERK-dependent phosphorylation of ribosomal S6 kinase at serine-380, actin cross-linking protein filamin A at serine-2152 and the Bcl-2-associated death promoter protein at serine-112. TCAP-1 treatment did not induce any significant changes in focal adhesion kinase phosphorylation. Furthermore, TCAP-1 treated hippocampal cells, showed a reorganization of actin- and tubulin-based cytoskeletal elements, and a corresponding increase in filopodia formation. The TCAP-dystroglycan system represents a novel mechanism associated with the integration of cytoskeletal dynamics and cell survival in the hippocampus.

1-E-75 The corticotropin-releasing factor (crf) inhibiting peptide, tcap-1, acts independently of the hypothalamic pituitary adrenal (hpa) axis to modulate stress.

Reuben De Almeida[1], Tanya Nock,[1] Dhan Chand[1], Dalia Barsyte-Lovejoy[1], David A Lovejoy[1]

[1]University of Toronto

Teneurin C-terminal associated peptide (TCAP-1), a 41-residue peptide with about 20% sequence identity with CRF, is widely expressed in the central nervous system. TCAP-1 has been previously shown to ablate a number of CRF-induced behaviours and block CRF-induced cocaine-seeking behaviour following cocaine withdrawal. Moreover, acute intracerebroventricular administration of TCAP-1 blocks CRF-mediated cfos labelling in the prefrontal cortex, septum, hippocampus, amygdala and dorsal raphe nucleus of rats and stimulates dendritic arborisation and increases spine density. However, the mechanism by which this occurs is not known. Therefore, the interaction of TCAP-1 with elements of the HPA system, were investigated in a number of in vitro and in vivo models. Although in vitro, TCAP-1 induces cAMP accumulation, it does not activate the cAMP response element in CRF1 or CRF2 receptor transfected cells or the glucocorticoid responsive element in glucocorticoid expressing cells. TCAP does not modulate total protein or cellular localization of CRF and glucocorticoid receptors. In mice, basal HPA activity was not modulated by repeated TCAP-1 administration. These studies indicate that TCAP-1 acts independently of either CRF-, or glucocorticoid- mediated signal transduction and transcription, and confers a number of regulatory changes on neurons, which consequently impact on the function of HPA elements. (This work was supported by grants from NSERC)

1-E-76 Acute REM sleep deprivation has differentiating effects on reward circuitry and related behavioural outcomes

Megan Dunbar[1], Idu Azogu[1], Patricia Tremblaye[1], Marianne LeBreton[1], Nathalie LeMarec[1], Hélène Plamondon[1]

[1]University of Ottawa

Chronic sleep deprivation can have detrimental effects on physiological processes leading to impairments in cognition, mood, and cardiovascular health. Less is known, however, about the impact of acute sleep deprivation on brain activity and function. Recent research has suggested that acute sleep deprivation can exert short-term beneficial effects, including improvement in mood. Of particular interest is the role of the diencephalic dopamine (A11) neurons, located in the hypothalamus and project to the Ventral Tegmental Area (VTA), which represent key structures involved in both sleep and reward. In the present study we analysed the effects of acute REM sleep deprivation (ARSD), a single day of 4 hour SD, and chronic sleep deprivation (CRSD), 4 hour SD imposed for 5 consecutive days. Control rats were either kept in their home cage for the duration of the experiment or exposed to a similar sleep deprivation milieu that allowed REM sleep to occur. Our findings revealed significant group differences in Tyrosine Hydroxylase immunoreactivity (THir) within the area of A11 dopamine projections with ARSD rats showing significantly increased THir compared to all other groups [$F(3,13)=20.78$, $p<0.01$]. When tested in the Morris Water Maze (MWM), ARSD rats showed reduced swim distance and latency to find the hidden platform in a non-matching to sample task ($p<0.05$), indicating enhanced spatial memory. Chronic sleep deprivation failed to significantly alter THir in these brain regions or MWM performance. Further examination of other areas, including the Nucleus Accumbens are ongoing.

1-E-77 Cellular mechanisms underlying enhanced catecholamine secretion during sepsis

Mark Lukewich[1], Alan E Lomax[1]

[1]Queen's University

Sepsis is associated with a rapid and sustained activation of the sympathetic nervous system, which is thought to

result from a combination of increased preganglionic activity, and altered adrenal chromaffin cell (ACC) and postganglionic sympathetic neuron (PGSN) function. We hypothesized that enhanced Ca2+ signaling in ACCs and PGSNs contributes to the increased catecholamine secretion that is observed during sepsis. Systemic inflammation was induced in C57Bl/6 mice using the endotoxemia and cecal ligation and puncture (CLP) models of sepsis. Mice were euthanized at various time points following the induction of sepsis, and ACCs and PGSNs were dissociated from adrenal medullae and superior mesenteric ganglia, respectively. A two-fold increase in high-K+-stimulated epinephrine secretion was observed in ACCs isolated from endotoxemic mice compared to controls. Ratiometric Ca2+ imaging with Fura-2 revealed a 50% increase in high-K+-induced Ca2+ transients in ACCs and PGSNs from 1-12 hour endotoxemic mice and 12 hour CLP mice. Although sepsis did not affect voltage-gated Ca2+ currents or cellular excitability, the amplitude of caffeine-stimulated Ca2+ transients was almost doubled during sepsis. Our results suggest that enhanced Ca2+ release from intracellular stores contributes to the elevated catecholamine secretion that occurs during sepsis. It is likely that circulating factors play a role in this response, as incubation of ACCs from naïve mice in media containing serum from endotoxemic or CLP mice recapitulated the effects of sepsis on high-K+-stimulated Ca2+ transients.

1-E-78 Nesfatin-1 exerts depolarizing actions on NPY and Nucb2 expressing neurons in the nucleus of the solitary tract

Andrea Mimee[1], Alastair V Ferguson[1]

[1]Queen's University

Nesfatin-1 (nes-1), a product of the nucleobindin-2 gene (NUCB2), is a neuropeptide with potent anorexigenic and cardiovascular effects. Immunohistochemical studies have shown the expression of nes-1 throughout the brain and, in particular, in the medullary autonomic gateway known as the nucleus of the solitary tract (NTS). To date, there is minimal data describing the specific functional roles of nes-1, and what is known has been focused on hypothalamic actions. Thus in order to elucidate the mechanisms by which nes-1 exerts its autonomic effects, the present study was undertaken to explore the cellular correlates of nes-1 actions in the NTS. We combined current-clamp electrophysiology with post-hoc single cell reverse transcription polymerase chain reaction to correlate the depolarizing and hyperpolarizing effects of nes-1 with the molecular phenotype of affected cells. Cytoplasm from 56 neurons was analyzed using primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), pro-opiomelanocortin (POMC), neuropeptide Y (NPY), glutamate decarboxylase (GAD67), NUCB2, and the melanocortin 4 receptor (MC4R). We observed a depolarizing effect of nes-1 on NPY neurons (n=11), and all neurons expressing Nucb2 depolarized in response to nes-1(n=3). We found heterogeneous effects of nes-1 on MC4R (n=11, depolarize=6, hyperpolarize=1) and GAD67 neurons (n=6, depolarize=4, hyperpolarize=2). Our results highlight the NTS as a key structure mediating the autonomic effects of nes-1, and provide insight into the neuronal circuitries influenced by this peptide. Funding: NSERC, FQRNT, HSFO.

1-E-79 Sympathetic neuroanatomical remodelling following endotoxemia in mice

Derek Moynes[1], Mark K Lukewich[1], Alan E Lomax[1]

[1]Queen's University

Sepsis is a severe systemic inflammatory disorder that produces chronic abnormalities following the resolution of inflammation, including increased sympathetic nervous system (SNS) activity. We hypothesized that altered SNS function following sepsis is associated with neuroanatomical remodelling of postganglionic sympathetic neurons (PGSNs). Endotoxemia was induced in C57Bl/6 mice through intraperitoneal injection of lipopolysaccharide (5 mg/kg). This causes a systemic inflammatory response that resembles septic shock and lasts approximately 24 hours. Colonic and liver sections obtained 2, 7 and 28 days following the induction of endotoxemia were immunohistochemically labeled for tyrosine hydroxylase (TH), a marker of axons of PGSNs. At each of these time points, TH-immunoreactivity was approximately two-fold greater within the colon and liver when compared to saline-injected controls. Changes in SNS activity have previously been linked to increased TH-immunoreactivity. To verify that axonal sprouting was the cause of increased TH-immunoreactivity following sepsis, we compared neurite outgrowth from PGSNs of superior mesenteric ganglia obtained from 12, 24 and 48 hour endotoxemic mice, and saline-injected controls. PGSNs isolated from endotoxemic mice at all three time points exhibited enhanced neurite outgrowth in Campenot chambers. These results suggest that adult PGSNs develop an enhanced ability to extend neurites within the first few days of sepsis, resulting in an increased sympathetic innervation of visceral organs that persists beyond the resolution of inflammation.

F – Cognition and Behaviour

1-F-61 Acute stress-induced impairment in set-shifting is dependent on circadian rhythm

Kelly Butts[1], Stan B Floresco[1], Anthony G Phillips[1]

[1]University of British Columbia

The ability to update and modify previously learned behavioural responses in a changing environment is essential for successful adaptation to promising opportunities and for coping with adverse events. Two main forms of cognitive flexibility are referred to as set-shifting and reversal learning. The medial prefrontal cortex (mPFC)

is required for set-shifting but not for reversal learning (Floresco et al., 2008). We have previously shown that the immediate effect of acute stress selectively impairs performance on a task mediated by the mPFC but not the hippocampus (Butts et al., 2011). The aim of this study was to determine whether exposure to a similar stressor (15 min of tail-pinch stress) given immediately before testing on set-shifting and reversal learning tasks would selectively impair performance. We also compared the influence of relative time of testing (during light or dark phases) to determine if this might influence the effects of acute stress on measures of cognitive flexibility. Exposure to acute stress significantly disrupted set-shifting when testing occurred during the light phase, but had no effect on reversal learning. In contrast, exposure to stress failed to impair set-shifting when rats were tested during the dark phase. These results suggest that the effects of acute stress on cognitive flexibility are only evident when testing takes place during times when animals are normally quiescent thereby raising the distinct possibility that disturbance in circadian rhythm is a critical factor in vulnerability to stress in mPFC-dependent cognitive tasks.

1-F-62 Regulation of MAPK/ERK signaling and photic entrainment of the SCN circadian clock by Raf kinase inhibitor protein

Hai-Ying Cheng[1], Ghadi Antoun[2], Pascale Bouchard Cannon[1]

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

Activation of the MAPK/ERK signaling cascade in the suprachiasmatic nucleus (SCN) is a key event that couples light to circadian clock entrainment. However, we do not fully understand the mechanisms that shape the properties of MAPK/ERK signaling in the SCN, and how these mechanisms may influence overt circadian rhythms. Here we show that Raf kinase inhibitor protein (RKIP) controls the kinetics of light-induced MAPK/ERK activity in the SCN and photic entrainment of behavioural rhythms. Light triggers robust phosphorylation of RKIP in the murine SCN and dissociation of RKIP and c-Raf. Overexpression of a non-phosphorylatable form of RKIP in the SCN of transgenic mice blocks light-induced ERK1/2 activation in the SCN and severely dampens light-induced phase delays in behavioral rhythms. Conversely, in RKIP knockout (RKIP-/-) mice, light-induced ERK1/2 activity in the SCN is prolonged in the early and late subjective night, resulting in augmentation of the phase-delaying and -advancing effects of light. Re-entrainment to an advancing light cycle was also accelerated in RKIP-/- mice. In relation to the molecular clockwork, genetic deletion of RKIP potentiated light-evoked PER1 and PER2 protein expression in the SCN in the early night. Additionally, RKIP-/- mice displayed enhanced transcriptional activation of mPeriod1 and the immediate early gene c-Fos in the SCN in response to a phase-delaying light pulse. Collectively, our data reveal an important role of RKIP in the regulation of MAPK/ERK signaling in the SCN and photic entrainment of the SCN clock.

1-F-63 Involvement of the α5 and β2 nAChR subunits in signaling the aversive and rewarding motivational effects of acute nicotine

Taryn Grieder[1], Taryn E Grieder, Morgane Besson, Christie Fowler, Paul J Kenny, Uwe Maskos, Derek van der Kooy[1]

[1]University of Toronto

Various nicotinic acetylcholine receptors (nAChRs) in the brain, and specifically the ventral tegmental area (VTA), have been implicated in the processing of nicotine's acute motivational effects. The β2 nAChR subunit is necessary for nicotine motivation, and is found on both DA and GABA neurons in the VTA. The α5 nAChR subunit is suggested to be important for signaling the aversive motivational effects of self-administered nicotine. We hypothesized that β2 nAChRs on VTA DA and GABA neurons respectively mediate acute nicotine aversions and reward. We also hypothesized that knockout (KO) of α5 nAChRs would prevent acute nicotine aversions but not reward in a place conditioning paradigm. We selectively re-expressed the β2 nAChR subunit on GABA or DA neurons in the VTA of β2 KO mice, obtained α5 KO mice, and subjected these mice and their controls to place conditioning after injection of a low rewarding (0.35 mg/kg) or high aversive (1.75 mg/kg) dose of acute nicotine. Our results show that selective re-expression of the β2 nAChR on VTA DA neurons rescued the aversive but not the rewarding motivational response to acute nicotine, while re-expression of the β2 nAChR on VTA GABA neurons restored the rewarding but not the aversive motivational response. α5 KO mice will not demonstrate acute nicotine aversions, but will show a rewarding response to acute nicotine. These results doubly dissociate the role of β2 nAChRs on DA and GABA VTA neurons in the motivational response to acute nicotine, and suggest that the α5 subunit is involved in acute nicotine aversion but not reward.

1-F-64 Common brain activations for painful and non-painful aversive stimuli

Dave Hayes[1], Georg Northoff[1]

[1]University of Ottawa, Institute of Mental Health Research

Identification of potentially harmful stimuli is necessary for the well-being and self-preservation of all organisms. However, the neural substrates involved in the processing of aversive stimuli are not well understood. For instance, painful and non-painful aversive stimuli are largely thought to activate different neural networks. However, it is presently unclear whether there is a common aversion-related network of brain regions responsible for the basic processing of aversive stimuli. To help clarify this issue, this report used a cross-species translational approach in

humans (i.e. meta-analysis) and rodents (i.e. systematic review of functional neuroanatomy). Animal and human data converged in showing a core aversion-related network, consisting of similar cortical (e.g. mid cingulate, anterior insula, supplementary motor cortex) and subcortical (e.g. dorsal striatum, thalamus, midbrain) regions. In addition, a number of regions appeared to be pain-specific (e.g. sensory cortex) or non-pain-specific (e.g. amygdala). This investigation suggests that aversive processing, at the most basic level, relies on similar neural substrates, and that differential responses may be due, in part, to the recruitment of additional structures as well as the dynamic activity of the network.

1-F-65 Representation of operant-action sequences by prefrontal cortex ensembles

Liya Ma[1], James M Hyman[1], Daniel Durstewitz[2], Anthony G Phillips[1], Jeremy K Seamans[1]

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

Many everyday tasks require us to chain together multiple actions to achieve a single goal. While numerous studies indicate that prefrontal cortex (PFC) neurons accurately monitor and track most actions or stimuli in an animal's experience, we are interested in whether PFC neurons also encoded the sequence in which actions are completed. Rats were trained to respond in the order: nose-poke-> lever-press->wheel-turn, to receive food reward. Successful completion of this training stage was followed by acquisition of the reversed order: wheel-turn->lever-press->nose-poke. On the subsequent sequence-switch sessions, rats first performed the newly established reversed sequence, then shifted back to the original sequence. At the ensemble level, statistical methods evaluating the distance between the behavior-related clusters in the multiple single-unit activity (MSUA) spaces revealed that identical actions, if approached from different directions, or occupying different serial positions, were represented as unique activity state patterns. On average, 12.3% of all neurons recorded contributed to this separation significantly. These neurons responded differentially to the actions depending on the sequence, and more often than not they were responsive to multiple actions rather than a single one. We conclude that PFC cells possess a variety of functional properties, including sensitivity to action type, to direction of action approach, and to serial order of actions. Together these properties contributed to observed separate representations of sequences at the ensemble level.

1-F-66 Exercise frequency predicts better cognitive inhibitory control and brain blood flow regulation in healthy young adults

Liana Machado[1], Hayley Guiney[1], Samuel Lucas[1], James D Cotter[1]

[1]University of Otago

Recent evidence indicates that regular exercise could be linked to better executive functioning in young adults, despite presumed 'optimal' brain health in that population. We sought to understand which cognitive functions are linked to regular exercise in young adults, and to gain insight into the underlying mechanisms. To this end, we examined performance on executive function tasks in relation to aerobic fitness, habitual physical activity, and cerebrovascular function. Multiple regression analyses revealed that more frequent physical activity, but not necessarily higher aerobic fitness, predicted better cerebrovascular function and superior inhibitory control. Cerebrovascular function also predicted better inhibitory control. Finally, mediation analyses indicated that participation in frequent physical activity may bring about improvements in inhibitory control through improved brain blood flow regulation. These results provide novel insight into the cognitive and cerebrovascular benefits that may be gained with regular exercise in high-functioning populations.

1-F-67 Evaluation of memory capacities and anxiety level in two models of successful aging: the Lou/C/Jall and calorie-restricted Sprague Dawley rats

Caroline Menard[1], Rémi Quirion[1], Guylaine Ferland[2], Pierrette Gaudreau[2]

[1]McGill University (DMHUI), [2]Université de Montréal

Long-term caloric restriction results in increased longevity in several species including the Lou/C/Jall (Lou) rat, a model of successful and healthy aging characterized by the maintenance of a low and stable adipose tissue mass throughout life and a low incidence of common age-related diseases. However, it is still unknown if memory and anxiety are affected during aging in this model. Memory performances of 6 to 42 month-old male and female Lou rats and 3 to 20 month-old male ad-libitum-fed and calorie-restricted (CR) Sprague Dawley (SD) rats were compared using object recognition for reference memory and Morris Water Maze (MWM) for spatial learning. All animals had free access to chow and water except at 8 months, SD rats were divided into control and 40% caloric restriction groups. MWM performance of Lou rats was slightly affected by aging whereas strong individual variations were seen among both aged SD groups. Lou rats spent significantly more time in the elevated plus maze open arms and center area of the open field, suggesting low anxiety and high exploratory behavior despite aging. Only the CR-SD rats exhibited similar behavior. Object recognition analysis and biochemical studies are currently in progress. Spatial memory capacities and low anxiety level are maintained in Lou rat during aging while calorie restriction seems to be more effective in reducing anxiety than preserving spatial memory. Identification of molecular targets involved in the healthy aging phenotype of Lou rat may open new avenues to better understand the underlying mechanisms of aging.

1-F-68 Hyperlocomotion upon activation of the ventral hippocampus in mice

Robin Nguyen[1], Vivek Mahadevan[1], Michael McPhail[1], Melanie Woodin[1], John Yeomans[1], Junchul Kim[1]

[1]University of Toronto

Stimulation of the ventral hippocampal formation (vHPC) has been found to both activate dopamine release in the nucleus accumbens and increase locomotion in rats. These methods include electrical stimulation and local microinfusion of NMDA and carbachol. To extend these findings in mice, we investigated the locomotor effects of pharmacological and pharmacogenetic stimulation of the vHPC. Retrograde tracing from the nucleus accumbens shell using Alexa Fluor-cholera toxin subunit B resulted in dense cell body labeling in the ventral CA1 and subiculum. Microinfusions of carbachol and bicuculline into this region caused a significant increase in locomotion. In order to gain cell specific targeting and visualization of the manipulated region, we used designer receptors exclusively activated by designer drugs (DREADD). We delivered AAV-FLEX-hM3D-mcherry into the ventral hippocampus of mice broadly expressing cre recombinase. Activation of the hM3D receptor with clozapine-N-oxide (CNO) increased the firing rate of pyramidal neurons recorded in the CA1. Furthermore, systemically injected CNO significantly increased spontaneous locomotion. Our findings suggest that enhancing vHPC activity can induce hyperlocomotion in mice. We propose the use of genetic models to better understand the functional role of the ventral hippocampus in regulating dopamine system activity.

1-F-69 Important role of BK potassium channels in sensory gating and cognitive function

Susanne Schmid[1], Magdalena Mirkowski[1], Marei Typlt[1], Erin Azzopardi[1], [2]Peter Ruth

[1]University of Western Ontario, [2]University of Tuebingen

Habituation and prepulse inhibition (PPI) of startle are two operational measures of sensory gating. Habituation is also a very basic form of learning. Habituation has been studied in many animal models, but the underlying molecular mechanism has never been resolved. Studies in Aplysia and rodents have indicated a presynaptic, calcium dependent mechanism at the sensorimotor synapses of the reflex pathway. Furthermore, mutations in C. elegans and Drosophila have indicated that functional potassium channels are required for short-term habituation. We here tested mice with a genetic disruption of the large conductance calcium activated potassium channel (BK channel) for short-term and long-term habituation of startle as well as for PPI and other cognitive function. Knock-out (KO) mice show obvious motor impairments, but the average startle amplitude of KO-mice did not differ from those of wild-type (WT) animals. WT-mice habituated to 70% of their initial startle response, while no significant short-term habituation was observed in KO-animals. Habituation levels in heterozygous littermates were intermediate. None of the mice seemed to show long-term habituation. PPI was significantly attenuated in KO-mice and PPI in heterozygous mice was intermediate. The data suggests that the activation of BK potassium channels in the brainstem is crucial for short-term habituation of startle, confirming invertebrate findings. Additionally, BK channels seem to play an important role in PPI, indicating a more generally involvement of BK channels in sensory gating processes.

1-F-71 Chronic administration of estradiol, but not estrone, increases hippocampal neurogenesis and activation of new neurons in response to spatial memory

Robyn Stewart[1], Cindy K Barha[1], Liisa A Galea[1]

[1]University of British Columbia

Estrogens fluctuate, with estradiol being higher pre-menopause and estrone being higher post-menopause. Estrone is a common component of hormone replacement therapies (HRTs), but estradiol may have a greater positive impact on cognition. Estrone and estradiol impact hippocampus-dependent learning and cell proliferation in the dentate gyrus in a dose-dependent manner. The current study explores how chronic high doses of estrone and 17β-estradiol differentially influence spatial learning, neurogenesis and activation of new neurons in response to spatial memory. Female rats received daily injections of vehicle (sesame oil), or a 10g dose of either 17β-estradiol or estrone for 20 days. One day following the first hormone injection all rats were injected with the DNA synthesis marker, bromodeoxyuridine. On days 11-15 of hormone treatment rats were trained on the Morris water maze (MWM), and five days later (day 20 of estrogens treatment) were given a probe trial to assess memory retention. There were no significant differences between groups in relation to the MWM. However, the 17β-estradiol group had significantly higher, while the estrone group had lower, levels of BrdU-ir cells in the dentate gyrus compared to controls. Furthermore, rats injected with 17β-estradiol showed significantly higher levels of activation of new neurons in response to spatial memory compared to controls. These results provide insight into how estrogens influence the brain and behaviour, while impacting the development of HRTs for postmenopausal women.

1-F-72 Calcium/Calmodulin-dependent protein kinase 1 is required for short-term habituation

Tiffany Timbers[1], Evan L Ardiel[1], Catharine H Rankin[1]

[1]University of British Columbia

The Calcium/Calmodulin-dependent protein kinases CaMKI and CaMKIV are abundant in the brain and are activated in response to elevated intracellular Ca2 levels by Calmodulin and phosphorylation by the upstream kinase CaMKK. We tested whether the Caenorhabditis elegans CaMKI homologue, CMK-1, was necessary for short-term mechanosensory habituation. Worms were habituated to mechanical stimuli (taps to the side of the Petri dish) using different stimulation protocols known to induce short-term habituation. Worms with mutations in cmk-1 habituated normally when stimuli are presented at a 10s interstimulus interval (ISI), but did not habituate as deeply as wild-type animals when stimuli were presented at a 60s ISI. No deficits in short-term habituation were found in animals with a mutation in the putative upstream kinase ckk-1 (homologous to CaMKK). A habituation screen of 46 predicted CMK-1 targets revealed that worms with a mutations in ogt-1 (homologous to mammalian O-GlcNAc transferase) phenocopied cmk-1 mutants to various extents, suggesting OGT-1 could be functioning downstream of CMK-1 in habituation. Currently we are using cell-specific rescues to investigate which neurons require functional CMK-1 for wild-type habituation at a 60s ISI. This work was supported by operating grants from NSERC to CHR and by Graduate Fellowships from NSERC to TAT.

G – Novel Methods and Technology Development

1-G-80 Development of novel dopaminergic D2 allosteric modulators into improved antipsychotic drugs for treatment of schizophrenia: Studies on mechanism of drug action

Dipa Basu[1], Jian R Jiang[1], Michael G Beyeart[1], Rodney L Johnson[2], Ram K Mishra[1]

[1]McMaster University, [2]University of Manitoba

Schizophrenia (SZ) remains a poorly treated mental disorder, with current drugs targeting the dopamine D2 receptor (D2R) orthosteric site, and causing very serious side-effects. Our current study helps develop novel allosteric D2R ligands into antipsychotic drugs, by investigating the mechanistic action of tripeptide L-prolyl-L-leucyl-glycinamide (PLG), and its potent analogue, PAOPA ((3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide)). Preclinical data have shown PAOPA to attenuate SZ-like behaviours and the molecular mechanism of this therapeutic effect was explored in this study. It was hypothesized that PLG and PAOPA, by potentiating the effects of increased striatal dopamine levels present in SZ, caused agonist-induced D2R internalization, thereby attenuating the hyperdopaminergic disease state. In vitro studies used HEK293 cells transfected with D2R/ YFP, GRK2 and arrestin-3, to visualize receptor internalization using microscopy, and quantified using [3H]-sulpiride to radioactively tag membrane D2Rs. Results showed that 1.5 hrs treatment with PLG and PAOPA caused a 25% and 35% increase in D2R internalization respectively. Additionally, in vivo studies in rats showed, using western blot analysis, a 20% increase in GRK2 expression with chronic PLG treatment. Future studies will investigate time course of D2R internalization, investigating possible receptor recycling/ degradation. Additionally, expression of more proteins relevant to receptor internalization, such as arrestin 3, will also be studied in treated rats.

1-G-81 Competitive tractography for extracting brain connectivity from diffusion MRI

Brian Booth[1], Ghassan Hamarneh[1]

[1]Simon Fraser University

Diffusion magnetic resonance imaging (dMRI) provides us with the ability to non-invasively infer the directions of coherently aligned axons in the brain's white matter. This innovation has led to the computational problem of tractography: the delineation of neural connections in the brain. We introduce the notion of competition into tractography algorithms and use it as a mechanism to reduce the likelihood of discovering erroneous connections. For every location x (green in figure a) in the brain, we wish to compute the probability of an axonal connection (blue) between x and a set of target regions (red). Virtual particles are inserted at location x and their diffusion is simulated according to the dMRI data until they reach one of the target regions. The likelihood of an axonal connection to a target region becomes the fraction of particles that reached that target region *before* any other target region. This temporal aspect of the simulation is what introduces the competition into our algorithm. The resulting algorithm is an example of the random walker technique and allows us to solve the problem without simulation, but instead through solving a system of equations [1]. Results on the analysis of the Corpus Callosum in figures (b) and (c) shows the connection probabilities from our algorithm are more robust to noise (see blue arrow) than [2] while reducing erroneous connectivity outside the targeted tracts (see green arrows). References: [1] L Grady, IEEE Trans. Patt. Anal. Mach. Intel.:28 (2006) 1768-1783. [2] A. Zalesky, IEEE Trans. Med. Imag.:27 (2008) 1458-1571.

1-G-82 The characterization of a long term co-culture system for the study of mature neuromuscular synaptic function

Peter Chipman[1], Victor F Rafuse[1]

[1]Dalhousie University

The neuromuscular junction (NMJ) has long been a model system for the study of synaptic function due to its large size and accessibility. Recent evidence suggests that

synaptic dysfunction at the NMJ is a primary hallmark of motoneuron disease (MND). These studies, however, are limited to genetically-defined models and omit the study of sporadic cases of MND. Recent advances in the directed differentiation of somatic cells allows for the first time the detailed study of sporadic MND, as patent-specific cells can be isolated non-invasively and directed to differentiate into motoneurons in vitro. However, traditional in vitro model systems of neuromuscular synaptic function have been restricted to the study of immature synapses and have not been used to model mature NMJs. Here, we describe the characterization of a long-term motoneuron/myotube co-culture system which allows the detailed analysis of synaptic function at mature NMJs grown in vitro. To assess whether we could effectively identify synaptic pathology using this system, we generated embryonic stem cell- derived motoneurons from wild-type mice or from NCAM-/- mice which exhibit normal developmental synaptogenesis, but exhibit progressive synaptic dysfunction and muscle weakness during ageing. We find that although both genotypes readily form NMJs in vitro, NCAM-/- NMJs exhibit specific deficits in synaptic vesicle cycling and synaptic stabilization. Finally, we validate this model by demonstrating in vivo that presynaptic NCAM is required for regenerative synaptogenesis associated with MND.

1-G-83 Switching LAser Mode (SLAM) microscopy; a new, flexible microwatt super-resolution approach

Harold Dehez[1], Michel Piché[2], Yves De Koninck[1]

[1]CRIUSMQ (Université Laval), [2]COPL (Université Laval)

For biomedical applications, high resolution is needed and a large variety of techniques have been developed to overcome the diffraction barrier. Most of them are probe dependent (PALM/STORM) or require a complete modification of the microscope (STED). We present here a procedure independent of the probe, laser, or objective being used, that can easily be retrofitted in conventional laser scanning microscopes to improve the transverse resolution by a factor of two. PARA We proceed by subtracting two specific images: a positive image recorded with a laser beam having a maximum of intensity at its center and a negative image taken with a laser beam having null intensity at its center. By doing so the dimension of the PSF is strongly reduced, and resolution is enhanced. With an appropriate choice of the positive and negative beams, a circular PSF without side lobes is obtained. The beam switching device is composed of achromatic components to preserve versatility. Switching can be done faster than the line scanning dwell-time, allowing the user to record the two images line-by-line to avoid artefacts due to motion of the specimen. PARA Results on fixed neuronal structures in confocal and two-photon microscopy will be presented where finer details are revealed using the subtraction method. In confocal imaging, structures were revealed by m-ruby transfection or immunohistochemistery labeling of tubulin (alexa 546). In two-photon imaging, single cells of mice brain slices are labeled by microinjection of Lucifer-Yellow.

1-G-84 Modeling chloride fluctuations in the synaptic cleft with the method of finite elements

Nicolas Doyon[1]

[1]Université Laval

Dynamic fluctuations of ionic concentrations (Cl-, K) in nanidomains are difficult to measure in vivo with good spatiotemporal. However these fluctuations have important functional impacts for example in pathological brain conditions. To better understand these impacts, we resort to modeling with the finite element methods. We first show how the method of finite elements is the ideal tool to perform such simulations, allowing to rapidly generate 3D movies with a spatial resolution that cannot be achieved through the NEURON environment. To apply the method to specific functional situations, we built a realistic model of a synaptic cleft and the surrounding extracellular space. We found that, under physiological conditions, the onset of a GABAA receptor-mediated synaptic event can trigger a drop in Cl- concentration in the cleft between 5 mM and 15 mM. Under conditions of sustained GABAA activity, this depletion adds up to a 10-20 mM drop in the surrounding extracellular space. Though not enough to cause a large shift in chloride reversal potential, the drop in extracellular [Cl-]i may impact the activity of ion sensitive membrane proteins such as receptors. Chloride concentration is also known to exhibit large fluctuations in distal dendrites where the small volume restrains diffusion. Modeling several synapses within a small diameter dendritic section, we found that local chloride fluctuations create a 'regional' plasticity affecting signal integration. Indeed since dendritic GABAA activity impacts [Cl-]i concentration in a 30-50 microns wide zone.

1-G-85 Application and evaluation of automated methods to extract connectivity statements from neuroscience literature

Leon French[1], Suzanne Lane[1], Lydia Xu[1], Paul Pavlidis[1]

[1]University of British Columbia

Automated annotation of connectivity statements from the neuroscience literature would enable accessible and large scale connectivity resources. We report progress on a system for extracting connectivity statements from neuroscience abstracts. We have extended our earlier work on identifying brain region mentions by evaluating methods for normalizing brain regions to existing atlas identifiers. The evaluations reveal that over 63% of mentions can be resolved to a region at 97% precision (37% mapped to higher level enclosing regions). As an application of our tuned system, we analyzed 12,557 abstracts from the

Journal of Comparative Neurology, yielding 7,923 brain region concepts from 95,895 normalized mentions. Using a manually annotated set of reported brain region connections we tested several methods for their ability to extract reported connectivity relationships between the brain region mentions. We observe that about a quarter of connectivity relationships span sentences and limit the ability of sentence based extraction. We tested several baseline measures based on co-occurrence and lexical rules. We compare results from several advanced kernel methods adapted from the protein interaction extraction domain. Our results show that syntax and dependency tree based methods do not provide optimal performance.

1-G-86 Small molecule KCC2 activators as novel therapeutic agents for neuropathic pain

Martin Gagnon[1], Marc J Bergeron[1], Robert P Bonin[1], Karine Bachand[1], Karen Vandal[1], Mariève Jacob-Wagner[1], Paul Isenring[1], Giorgio Attardo[2], Jeffrey A Coull[2], Yves De Koninck[1]

[1]Centre de recherche de l'Institut universitaire en santé mentale de Québec, [2]Chlorion Pharma Inc

The cation-chloride cotransporter KCC2 is responsible for maintaining low intracellular chloride concentration in central nervous system neurons, which is essential for enabling postsynaptic inhibition through GABAA and glycine receptor/channels. Loss of KCC2 activity in spinal dorsal horn neurons following injury or peripheral inflammation causes disinhibition which appears to be a critical substrate of central hypersensitivity in several models of chronic pain. We therefore sought to develop compounds that would target this mechanism as novel analgesics. We designed a simple fluorometric assay allowing real-time quantification of intracellular chloride in a cell line endogenously expressing low levels of KCC2. High throughput screening of a 92,500 compound library with this assay led to the identification of small molecule KCC2 activators that can reduce intracellular chloride concentration. Optimization of a hit compound resulted in an analog (CLP257) having an EC50 in the sub-micromolar range, while showing selectivity towards KCC2 versus other cation-chloride cotransporters and classical pharmacological targets. Spinal cord slices showed increased surface expression of KCC2 following CLP257 exposure. Intraperitoneal administration of CLP257 yielded significant analgesia in a rat model of neuropathic pain, while avoiding unwanted motor effects commonly seen in neuropathic pain drugs such as gabapentin. These findings validated KCC2 as a druggable target for the treatment of neuropathic pain.

1-G-87 Effects of MRI field strength on functional connectivity data

Stanislau Hrybouski[1], Corey Baron[1], Peter Seres[1], Carter Rawle[1], Fraser Olsen[1], Nikolai Malykhin[1]

[1]University of Alberta

Magnetic resonance imaging (MRI) is the most widely used method to study structural and functional connectivity in the human brain. In this project we looked at effects of field strength on detectable functional connectivity in functional MRI (fMRI) datasets. Resting-state scans were acquired from 15 healthy volunteers on a 1.5T Siemens system with 1-channel head coil (EPI sequence, 35 axial slices, TE = 40ms, TR = 2000ms, 240 volumes, resolution 4.0×4.0×4.0mm³, 2 sessions) and on a 4.7T Varian Inova system with a 4-channel head coil (EPI sequence with GRAPPA acceleration factor = 2, 45 axial slices, TE = 19ms, TR = 3000ms, 200 volumes, resolution 3.0×3.0×3.0mm³, 1 session). PARA All functional volumes were corrected for geometric distortion, slice acquisition delay and in-scanner head motion using SPM8. Images were warped to MNI space and smoothed with 8mm Gaussian Kernel. Network analyses were done in GIFT software, and only voxels with $z \geq 3$, were labeled as active. PARA GIFT's component detection algorithm identified 32 independent components in our 4.7T data and 15 independent components in our 1.5T data. Close inspection revealed that this difference is due to the higher number of small sub-networks detected in the 4.7T data. This observation is in contrast to larger, well-documented networks, which are almost identical in 1.5T and 4.7T datasets. In conclusion, higher field strength is required for studies looking at network sub-systems, but is not essential for studies of relatively large networks.

1-G-88 Cellular response to electrode implantation in 2D and 3D cultures

Andrea Jeffery[1], Alexandra Chow[1], Anthony DeCesare[1], Anastasia Elias[1], Kathryn Todd[1]

[1]University of Alberta

For spinal cord injury patients, regaining even a portion of movement positively impacts quality of life. Intraspinal microstimulation (ISMS) is the surgical implantation of electrodes into the spinal ventral horn to restore lower limb movement. For ISMS to be used in long-term treatment, electrodes must be designed to minimize foreign body response and optimize functionality. Proliferation of nearby immune cells and formation of the glial scar are both indicators of foreign body response. This study investigates two potential models for high throughput in vivo analysis of immune cell responses to different ISMS electrodes. Two-dimensional mixed microglia and astrocyte cultures with stabilized ISMS electrodes were studied over 8 days. Microglia and astrocytes proliferated and encapsulated ISMS electrodes, mimicking the in vivo foreign body

response. However, electrode movement within the culture introduced artifacts into results. To improve functionality of the model, a new 3D hyaluronic acid (HA) hydrogel culture method was also investigated. Instead of using UV light to initiate polymerization, a 3-component visible light photoinitiating system capable of photopolymerizing hydrogels from modified HA was developed. Preliminary results demonstrated short-term biocompatibility of the HA hydrogels with astrocytes and microglia and improved functionality of the model. These results indicate that using a new three-dimensional HA hydrogel for culture will allow accurate and efficient in vitro testing of ISMS electrodes with varying physical properties and chemical coatings.

IBRO - International Brain Development Association

1-IBRO-58 Ultrastructure and gene expression in the posterodorsal medial amygdala

Janaina Brusco[1]

[1]Ribeirao Preto

The posterodorsal medial amygdala (MePD) is implicated in sexual behavior. The density of dendritic spines is different between male and female rats along the estrus cycle. The MePD should be different between the two hemispheres in male and female rats. The aim of this work was to evaluate the modifications caused by gonadal hormones on the MePD. We studied the tridimensional (3D) ultrastructure of the MePD neuropil; and the mRNA and protein expression of NR1, NR2B, Narp, GAD65 and GAD67 in male and female rats along the estrus cycle in both hemispheres. Dendritic spine density of adult male rats was (mean ± SD) 1.15 ± 0.67 spines/µm. Spines were classified as thin (53%), mushroom (22%), stubby/wide (22%), ramified and filopodium (3%). Around 8% of synapses on dendritic spines were symmetric and GABAergic. NR1 mRNA was more expressed in estrus females than in males or proestrus females. Narp mRNA was higher in estrus than in diestrus females. Males had higher expression of GAD65 mRNA than females, and females in estrus had higher levels than females in diestrus or proestrus. GAD67 mRNA was higher in males than in females in diestrus or proestrus. Estrus females had higher GAD65 protein expression in the left MePD than diestrus females. This is the first report of inhibitory terminals and multisynaptic dendritic spines in the MePD of adult rats. This work indicates that gene expression might be modulated by sex hormones and is different between both hemispheres. These results provide basic morphological and molecular data for MePD under the influence of sex hormones.

1-IBRO-59 Control of synaptic morphology and function by the Smaug1-Nanos1 translation regulation pathway

Dario Maschi[1]

[1]Ciudad autonoma de Buenos Aires

Local translation at the synapse is an important mechanism for synaptic plasticity. Smaug is a translational repressor initially identified in Drosophila. Fly Smaug regulates the stability and/or translation of hundreds of maternal mRNAs that contain specific motifs termed SRE. We have previously shown that mammalian Smaug1 represses the translation of reporter mRNAs with SRE motifs (Baez and Boccaccio, JBC 2005). Smaug1 forms granules containing silenced mRNAs located at the post-synapse (Baez*, Luchelli*, Maschi* y col. JCB 2011. *equal contribution). Here, we show that mammalian Smaug1 has an important effect on synapse morphology. Smaug1-depleted neurons provokes smaller and more numerous PSD95 synaptic clusters. We found less mushroom-shaped and more thin spines upon Smaug1 knockdown. In addition, Smaug1-depleted neurons respond defectively to a repetitive depolarizing stimulus, as indicated by a reduced induction of ARC, an early gene marker of activity. The mRNA encoding the translational repressor Nanos 1 has SREs and we found that Smaug1 knockdown increased Nanos1 protein level. Moreover, a Smaug1/Nanos1 double KD partially revert the Smaug1-KD phenotype. Our results suggest that the Smaug1-Nanos1 pathway is an important mechanism for local mRNA regulation that affects synapse morphology and plasticity. Work supported by ANPCyT, CONICET, UBA, Argentina and NIH-USA.

1-IBRO-60 EFFECTS OF KHAT (Catha edulis Forsk) ON LEARNING AND MEMORY USING THE MORRIS WATER MAZE (MWM)

Nchafatso Gikenyi Obonyo[1]

[1]Nairobi

Introduction:The first scientific description of Catha edulis was in Flora Aegyptiaco-Arabica by the Swedish botanist Peter Forskal (1768). Khat refers to the young leaves and shoots of the plant Catha edulis (a species of the Celestracecae family), a flowering evergreen tree or shrub, native to regions of Eastern Africa and Southern Arabia. It has various local names such as qat in Yemen, eschat in Ethiopia and miraa in Kenya. Khat is chewed and the juice is swallowed, which induces a stimulatory and euphorogenic effect in the user. Objective:To determine the effects of khat treatment on spatial learning and memory of CBA mice in the Morris water maze task. Materials and Methods: Twenty five male CBA mice, 5 - 6 months old and weighing 20-35 grams were used in the experiments. They were divided into 5 groups (n=5);no injection, saline, khat extract groups 40mls/kg,120mls/kg,360mls/kg.Khat plant extract was obtained by methanol extraction. Results:

Results showed that the escape latency and swim-distance of CBA mice in the Morris water maze task decreased over repeated trials and also after injection of khat plant extract, with the mice taking a shorter time and distance to locate the escape platform. However, there was no significant difference between the groups. A MANOVA test on spatial acquisition performance measures yielded no significant difference between the groups: $F(3, 76) = 0.99$; $p < 0.960$ (escape latency), $F(3, 76) = 0.105$; $p < 0.957$ (swim distance), and $F(3, 76) = 0.916$; $p < 0.437$ (swim velocity).All group E mice died. Keywords: Khat (Catha edulis forsk)

1-IBRO-98 Comparative immunocytochemical analysis of the pangolin and hedgehog retina

Joan David[1]

[1]University of Ilorin,Nigeria

Comparative anatomy yields information useful in analyzing the range of variable possibilities in a wide range of species with different habitats and lifestyle. The basic cellular organization of the mammalian retina has been investigated extensively in a lot of species with rodents and primates ranking high in the list and species such as the pangolin being rarely studied. In the current study, different molecular markers are applied to sections of the pangolin and hedgehog retina to study their cellular and synaptic localization by using confocal microscopy. The markers used were from four categories namely calcium binding proteins such as calbindin,antibodies that recognize specific transmitter systems such as γ-aminobutyric acid or actylcholine, antibodies that recognize transmitter receptors and show their aggregation at specific synapses such as Glyt1, markers that recognize transcription factors (proteins which bind to DNA to regulate gene transcription) based on their vital role in cellular processes. The result is presented to generate interest in these rarely studied species and serve as a background to broader range of study approaches in physiology and behavior

Poster Session 2: Tuesday May 22 2012

A - Development

2-A-82 In vivo requirement for p600 in spindle orientation of neuronal progenitors and mammalian neurogenesis

Camille Belzil[1], Naoyuki Asada[2], Kei-ichiro Ishiguro[3], Takeo Nakaya[3], Gernot Neumayer[1], Valentina Pendolino[4], Marina Mapelli[4], Kamon Sanada[2], Yoshihiro Nakatani[3], Minh Dang Nguyen[1]

[1]Hotchkiss Brain Institute University of Calgary, [2]Graduate School of Medicine, Osaka University, [3]Dana Farber Cancer Institute, Harvard Medical School, [4]European Institute of Oncology, IFOM Campus

The positioning of the spindle apparatus determines the axis of cleavage during cell division. In the developing neocortex, the orientation of the mitotic spindle controls the symmetry of neuronal progenitor (NP) division, defining the fate of the daughter cells. Perturbations of spindle orientation promote apoptosis of neuroepithelial stem cells (NSCs) and precocious differentiation of radial glia (RG) into neurons. Both effects deplete the pool of neuronal progenitors and ultimately impact neurogenesis. Using knockout animals and in utero electroporation, we now report a key role for the microtubule-associated protein p600 in the precise spindle orientation of NPs during mammalian neurogenesis. Mice with a targeted disruption of p600 in pluripotent epiblast cells (p600sc-/-) die around embryonic day (E)13.5 with severe microcephaly. p600sc-/- animals display fewer histone H3-positive NPs, increased cortical apoptosis, and tilting of the spindle apparatus in ventricular NPs (predominantly NSCs). NPs (mainly RG) depleted of p600 by in utero electroporation of RNAi at E13 also exhibit randomized spindle orientation but, predictably, no significant apoptosis. In both p600sc-/- animals and ex vivo NP cultures depleted of p600, the production of Tuj1-positive neurons is significantly impaired. In sum, our study identifies p600 as a novel player in mammalian neurogenesis. Our data suggest a proximate mechanism in which p600 orients the mitotic spindle in NPs, allowing for normal symmetry of cell division, timely differentiation into neurons, and ultimately normal neurogenesis.

2-A-83 Role of mitochondria in GABAergic synapse development

Bidisha Chattopadhyaya[1], Josianne N Carriço[1], Martin Berryer[1], Graziella Di Cristo[1]

[1]CHU Ste. Justine Research Centre, University of Montreal

GABAergic interneurons are a diverse population of neurons that exhibit distinct physiological properties, express diverse neuropeptides and preferentially synapse onto distinct subcellular compartments of their postsynaptic targets. Basket cells are a subtype of GABAergic interneurons that form perisomatic synapses essential for regulating neural networks, and their alterations are linked to various cognitive dysfunctions. Maturation of basket synapses in postnatal cortex is activity dependent, and recent studies have implicated different molecular pathways involved in the process. Here we look at whether mitochondrial dynamics are important for GABAergic perisomatic synapse development. Recent studies have implicated both mitochondrial fission and the calcium dependent transport of mitochondria in excitatory synapse maturation, however, whether these aspects of mitochondrial dynamics are involved in inhibitory synapse development is unknown. Using single cell genetics to perturb different aspects of mitochondrial dynamics such as fission/fusion capabilities, we find that mitochondrial fission may not be important for GABAergic synapse maturation but for its maintenance. Further, deficits in calcium dependent mitochondrial transport in individual basket interneurons, at specific developmental time periods, affect both perisomatic synapse maturation and maintenance. Therefore, our studies so far show that different aspects of mitochondria function are important in distinct stages of GABAergic synapse development.

2-A-84 Postnatal development of Homer 1a in the hippocampus

Corinne Montes-Rodriguez[1], Valerie Lapointe[1], Bruce l McNaughton[1]

[1]University of Lethbridge

Homer 1a (H1a) is an immediately early gene involved in multiple forms of synaptic plasticity that exhibits a postnatal increase in the rat forebrain. H1a reduces the density and size of dendritic spines in cultured hippocampal neurons; and it is necessary for homeostatic scaling. Therefore, H1a is involved in an activity-dependent negative feedback regulation of the synaptic structure and function. In this context, we evaluated the hippocampal expression of H1a at different postnatal ages. Brown Norway rats were sacrificed at different postnatal ages (P3, P5, P7, P9, P15, P19, P23, P35, and adult). Maximal electroconvulsive shock (MECS) rats were studied at each stage. Fluorescence in situ hybridization (FISH) for H1a was performed (Vazdarjanova et al., 2002). Z-stacks from CA1, CA3, dentate gyrus (DG) and dorsal subiculum (DS) were acquired with either 40X or 60X oil immersion objective lenses on a confocal microscope. Manual quantification was performed by two experimenters blind to the conditions. Our results reveal that from P3 H1a expression is found in a significant proportion of cells (around 50%) in DS, CA1 and CA3. H1a+ cells reach a plateau in the second week of life for CA1, CA3 and DS. Laminar maturation of the DG was observed; H1a+ cells appear in the outer layer of the granular cells at the beginning of postnatal development moving towards the inner layer later on. In conclusion, in CA1, CA3 and DS the maximum amount of cells capable to express H1a reaches near adult levels by P7-P9.

2-A-85 Temporal modulation of visual activity regulates axon branch dynamics in the developing visual system

Martin Munz[1], Jessie Poquérusse[2], Edward Ruthazer[2]

[1]McGill University, [2]Montreal Neurological Institute

In Xenopus laevis the optic tectum is innervated by retinal ganglion cell (RGC) axons from the contralateral eye. However, in some cases a few RGC axons (<1%) are mistargeted to the ipsilateral tectum. These axons innervate tectal neurons mainly driven by the contralateral eye; consequently they fire out of synchrony with their synaptic partners. To determine how correlation in patterned vision regulates the developmental remodelling of RGC axons, GFP-expressing RGC axons were imaged in vivo by 2-photon microscopy every 10 min for >5h while presenting visual stimuli. Optical fibers delivered light directly to the eyes to control their firing independently. After imaging axon dynamics for 90 min in darkness, visual stimuli designed to either decrease or increase correlation between the two eyes were applied. We show rapid changes in the rates of branch motility in response to these stimuli. To determine the effects of correlated activity on RGC axon morphology over longer periods, behaving tadpoles were reared under visual stimulation designed to alter correlation of firing between the two eyes. We imaged individual RGC axon terminals daily for up to ten days. Tadpoles reared with moving dots to asynchronously activate the two eyes developed larger, more complex and dynamic ipsilateral RGC axon arbors than animals strobe-reared to correlate firing across both eyes. We show that specific patterns of sensory input differentially modulate the rapid dynamics and morphology of developing axonal arbors resulting over many days in dramatic changes in arbor size and structure.

2-A-86 The role of Notch signaling during cell fate determination in the postnatal mouse retina

Kara Ronellenfitch[1], Robert L Chow[1]

[1]University of Victoria

The mammalian retina is easily accessible for experimental manipulation and provides an excellent model for studying cell fate determination. Its layered structure and highly stereotyped organization facilitates the analysis of phenotypic differences caused by experimental manipulation. This study focuses on how cell-cell signaling regulates fate determination during retinal development. Specifically we are exploring the role of Notch signaling during the cell fate decisions of late-born neurons, which differentiate into either photoreceptors or bipolar interneurons. Notch signaling has been shown to promote bipolar interneuron specification at the expense of photoreceptor specification. We investigated whether cell fate specification remains plastic at certain times during development and predicted that Notch inhibition will reverse photoreceptor specification and re-direct cells towards a bipolar cell fate. Late born cell types were tracked and quantified throughout development via in vitro electroporation of plasmid DNAs, which mark late born progenitors early in differentiation, into live retinal tissue. Pharmacological treatments of a Notch inhibitor were applied at various points in postnatal development to target Notch signaling specifically in newly born bipolar and photoreceptor cells. By quantifying the final cell fate of these early progenitor cells we can determine the temporal effect of Notch signaling on the fate determination of these late born retinal cell types.

2-A-87 Regulation of interneuronal subtype identity by the Iroquois homeobox gene, Irx6

Erin Star[1], Erin N Star, Minyan Zhu, Zhiwei Shi, Haiquan Liu, Mohammad Pashmforoush, Yves Sauve, Benoit G Bruneau, Robert L Chow

[1]University of Victoria

Interneuronal subtype diversity lies at the heart of the distinct molecular properties and synaptic connections that shape the formation of neuronal circuits necessary for the complex spatial and temporal processing of sensory information. Here we investigate the role of Irx6, a member of the Iroquois homeodomain transcription factor family, in regulating the development of retinal bipolar interneurons. Using a knock-in reporter approach, we show that in the mouse retina, Irx6 is expressed in Types 2 and 3a OFF bipolar interneurons and is required for the expression of cell type specific markers in these cells. In Irx6 mutant mice, presumptive Type 3a bipolar cells exhibit an expansion of their axonal projection domain to the entire OFF region of the inner plexiform layer, and adopt molecular features of both Types 2 and 3a bipolar cells, highlighted by the ectopic upregulation of neurokinin 3 receptor (NK3R) and Vsx1. These findings reveal Irx6 as a key regulator of Type 3a bipolar cell identity that prevents these cells from adopting characteristic features of Type 2 bipolar cells. Genetic interaction experiments suggest that the terminal differentiation of Type 2 bipolar cells is dependent on the combined expression of the transcription factors Irx6 and Vsx1, but also point to the existence of Irx6/Vsx1-independent mechanisms in regulating OFF bipolar subtype-specific gene expression. This work provides insight into the generation of neuronal subtypes by revealing a mechanism in which opposing, yet interdependent transcription factors regulate subtype identity.

2-A-88 Regulation of activated Notch2 nuclear export by Vsx2

Minyan Zhu[1], Robert L Chow[1]

[1]University of Victoria

The mouse Vsx2 ocular retardation (orJ) mutant exhibits a microphthalmic retina with prolonged G1 phase, reminiscent of the persistent Notch/Hes1 upregulation phenotype in neural progenitor cells. Here, we show that the activated form of Notch2 (N2ICD) is present in the developing retina and forms a complex with Vsx2. N2ICD and the Vsx2(19) splice variant undergo Crm1-mediated nuclear co-export in vitro. We identified regions in N2ICD and Vsx2(19) that when mutated abolish nuclear co-export while maintaining complex formation, suggesting the two proteins are exported as a complex. The Vsx2(19) nuclear export mutant also increased N2ICD-mediated Notch reporter activity compared to intact Vsx2(19). Consistent with these findings, N2ICD and its downstream target Hes1 were elevated in the orJ retina. Furthermore, inhibiting Crm1 nuclear export in situ, resulted in a Vsx2-dependent increase in N2ICD levels. Additional inhibition of proteasomal degradation in wild type retinas did not further increase N2ICD although inhibition of proteasomal degradation, alone, increased N2ICD levels. These results suggest that N2ICD is degraded via the proteasome immediately after Crm1-mediated nuclear export, in vivo. Our findings reveal transcription factor-mediated nuclear export as a mechanism for regulating Notch signal strength.

B – Neural Excitiability, Synapses, and Glia: Cellular Mechanisms

2-B-1 Characterization of spinal microglia

Sam Joshva Baskar Jesudasan[1], Kathryn G Todd[1], Ian R Winship[1]

[1]University of Alberta

Microglia, the resident immune cells of the central nervous system (CNS), have a ramified morphology with long processes that constantly survey the brain and spinal parenchyma. Numerous studies have shown that after injury to the CNS microglia are capable of synthesizing and releasing neurotrophic factors, pro-inflammatory factors, and reactive oxygen species. However, it remains unclear whether microglia derived from the spinal cord respond similar to brain microglia (BM), and how the pro- and anti-inflammatory responses will affect the spinal cord after CNS injury. Our aim was to develop a highly reproducible system to study spinal cord microglia and compare them to brain-derived microglia. We adapted the protocol of Saura et al. (2003) using the mild trypsinization method to isolate spinal microglia from one-day old Sprague Dawley rat pups. Isolated spinal microglia (SCM) were treated with lipopolysaccharide (LPS) or ATP, agents known to induce inflammatory responses in cultured microglia. Notably, LPS-mediated NO release was significantly greater in SCM than in BM. Interestingly, basal release of NO by SCM was also significantly greater than BM. A similar trend was observed in response to ATP. Additionally, there was a decreasing trend in LPS mediated brain derived neurotrophic factor and TNFα release by SCM compared to BM. Thus our data suggest that SCM cultures respond to LPS mediated injury differently than that of BM.

2-B-2 TRPM2 is Implicated in Cellular Senescence and Amyloid-Mediated Toxicity in Hippocampal Neurons

Jillian Belrose[1], Matthew L Johnston[1], Megan M Chen[1], Hongbin Li[1], Fabiana A Caetano[1], Amanda C Martyn[1], Marco A Prado[1], Vania F Prado[1], Michael F Jackson[1], John F MacDonald[1]

[1]Robarts Research Institute, University of Western Ontario

The Transient Receptor Potential Melastatin 2 (TRPM2) channel plays a role in cell death due to oxidative stress, and has been implicated in neurotoxicity. Alzheimer's disease has been attributed to aging, oxidative stress, beta-amyloid (1-42) oligomers (Abeta), and a triad between hyperphosphorylated tau, Fyn kinase, and GluN2B containing NMDA receptors. We report here that TRPM2 currents are enhanced by glutathione (GSH) depletion, treatment with Abeta, and Fyn kinase. Primary cultured hippocampal neurons or HEK293 cells expressing TRPM2 were employed. As hippocampal neurons aged in vitro, TRPM2 currents increased due to a reduction in intracellular GSH. GSH inhibited TRPM2 currents in HEK293 cells, producing a 3.5-fold shift in activation by ADP-ribose, the intracellular ligand for TRPM2. We also demonstrate that Fyn kinase, recently implicated in Alzheimer's disease, co-immunoprecipitated with and phosphorylated TRPM2, and augmented TRPM2 currents. Furthermore, in cultured hippocampal neurons, treatment with Abeta enhanced TRPM2 responses. This data suggests that TRPM2 may play a role in Alzheimer's disease. Indeed, neurons cultured from TRPM2-/- mice were protected from Abeta induced cell death. Preliminary evidence also suggests that knock-out of TRPM2 in an Alzheimer's mouse model may improve performance in a spatial learning task. Our results implicate TRPM2 in the calcium dysregulation and neurodegeneration associated with Alzheimer's disease, and suggest that pharmacological manipulation of these channels may have therapeutic potential.

2-B-3 Loss of Ca2+ permeable ampa receptor function in inhibitory neurons of the substantia gelatinosa after sciatic chronic constriction injury and axotomy

Yishen Chen[1], Victor A Derkach[2], Peter A Smith[1]

[1]University of Alberta, [2]Oregon Health and Sciences University

Nerve injury promotes neuropathic pain. Chronic constriction injury (CCI) of rat sciatic nerve increases excitatory synaptic drive to excitatory neurons yet decreases that to inhibitory, tonic firing neurons in the substantia gelatinosa (SG; Balasubramanyan et al., J.Neurophysiol., 96: 579, 2006). Similar, but weaker, effects are seen with axotomy (Chen et al., J. Neurophysiol.,102: 3202, 2009). To probe underlying mechanisms, we examined AMPA receptors (AMPAR) on inhibitory neurons in rat SG after CCI and axotomy. Sprague Dawley rats (18d) were subjected CCI or axotomy for 14-24d and whole-cell recordings made from SG neurons in spinal cord slices. In sham operated animals, IEM1460 (50ìM), a blocker of Ca2 permeable AMPA receptors, significantly reduced the amplitude of evoked EPSC's, spontaneous EPSC's (sEPSC's) and miniature EPSC's (mEPSC's) whilst in axotomized animals, IEM1460 only reduced the amplitude of mEPSC's. By contrast, IEM1460 was ineffective after CCI. Single channel conductance (ã) of AMPARs was 15.9±2.1pS for sham tonic neurons, 9.2±1.0 pS for CCI tonic neurons and 13.2±1.4 pS for axotomy tonic neurons. This is consistent with the presence of Ca2 permeable (GluR2 lacking) AMPAR on inhibitory neurons but these appear to be downregulated or internalized after CCI but not after axotomy. This contrasts with internalization of Ca2 impermeable receptors in chronic inflammatory pain (Park et al., J. Neurosci., 29:3206, 2009). Distinct molecular mechanisms of AMPAR modification in different neuron types may thus underlie different forms of chronic pain.

2-B-4 Fluoxetine reduces microglial release of neurotoxic mediators and promotes microglial apoptotic cell death

Kam Dhami[1], Kathryn G Todd[1]

[1]University of Alberta

A body of evidence suggests that antidepressants, including fluoxetine, affect brain inflammation mediated by microglial cells (Ha et al., 2006; Lim et al., 2009; Chung et al., 2011). We expanded on these studies and, using in vitro methods, showed that microglia treated with fluoxetine were less toxic to injured neurons than non-treated microglia. Therefore, we hypothesized that fluoxetine attenuated toxic microglial responses by reducing microglial pro-inflammatory responses and by decreasing microglia density by promoting microglial apoptotic death. To prove our hypothesis, we incubated isolated primary microglial cultures in various concentrations of fluoxetine (0 - 40 µM). After 24 hours, we found that activated microglia treated with fluoxetine showed an attenuated release of nitric oxide, tumor necrosis factor alpha, and glutamate compared to non-treated microglia. Protein determination showed that fluoxetine decreased overall protein levels in the microglial cultures in a dose-related manner. MTT assays corroborated the observed decrease in microglia density suggesting cellular dysfunction or death induced by fluoxetine. Fluoxetine-treated cells appeared shrunken with blebbed morphology. Western blot analyses and double immunohistochemical analysis revealed increased levels of the apoptotic effector cleaved-caspase-3 in fluoxetine-treated microglial cells. Thus, our results show that fluoxetine reduces microglial release of pro-inflammatory cytokines and promotes apoptotic cell death in activated microglia.

2-B-5 Spinal Cord Injury Induces Membrane Expression of PIAS3

Karsten Gehrig[1,2], Daniel R Marsh[1,2], Jason G Meisner[1,2]

[1]Department of Anatomy and Neurobiology, Dalhousie University, [2]The Atlantic Mobility Action Project (AMAP), Faculty of Medicine

*Dalhousie University*Treatment strategies to ameliorate, or abrogate, the development of spinal cord injury (SCI)-induced neuropathic pain (NP) are in demand since NP after SCI is often refractory to standard treatment. In mice, we have shown that administration of the gabapentinoid drug, pregabalin (PGB), within 2 hours after SCI is efficacious in blocking the development of NP. If PGB treatment is delayed to 1 week post SCI, then the effect is lost and NP develops as if treated with saline. Binding of PGB to the calcium channel subunit α2δ-1 is essential for its therapeutic activity, yet its subcellular mechanisms remain unclear. To specifically address the effects of PGB on neuroplasticity during the first week after injury, SCI mice were administered PGB or saline within 2 hours of injury, and twice daily for 1 week. The lumbar expression of the pre-synaptic protein synaptophysin was not altered by SCI or PGB treatment. In contrast, the post-synaptic protein, PSD-95, demonstrated increased lumbar expression in whole cell homogenates and crude membrane fractions 3 days post SCI. Immunoprecipitation of PSD-95 from 3 day SCI tissue followed by mass spectroscopy analysis resulted in identification of a transcriptional regulator and E3 SUMO ligase, PIAS3. Concomitantly, PIAS3 expression was found to be significantly elevated in the crude membrane fractions after SCI and decreased in PGB-treated samples. These findings suggest that pre-emptive PGB treatment may serve to alleviate the severity of SCI-induced development of below-lesion NP by PIAS3-dependent neuroplasticity.

2-B-6 Mechanisms controlling GABAA receptor diffusion

Kimberly Gerrow[1], Antoine Triller[2]

[1]University of British Columbia, [2]Ecole Normale Superieure

Modulating inhibitory synaptic strength is important in learning and memory, but also, its dysfunction is thought to underlie numerous pathologies - including but not limited to - epilepsy, autism and addiction. Fast inhibitory neuronal transmission in the brain is mainly mediated through a-aminobutryric acid type A receptors (GABAARs). GABAARs mediate both phasic (synaptic) and tonic (extrasynaptic) inhibition. Little is known about the regulatory mechanisms governing the lateral diffusion and exchange of GABAARs at synaptic versus extrasynaptic membrane. In the hippocampus, the majority of GABAARs contain either α1- and α2-subunits, both of which contain bind to gephyrin and are mostly synaptic, whereas the α5 is mainly found extrasynaptically where it is bound to radixin. Using a quantum dot (QD) based single particle tracking (SPT) approach we studied mechanisms that regulate GABAAR diffusion and exchange between synaptic and extrasynaptic membrane. Interestingly, these mechanisms appear to function in a subunit specific manner. The identification and functional characterization of extrasynaptic receptor reservoirs and understanding the regulation of synaptic versus extrasynaptic shuttling provides new insights into synaptic plasticity and neuronal disease.

2-B-7 A role for mitochondrial Ca2+ release in regulating membrane excitability and secretion in neuroendocrine cells

Chris Groten[1], Neil S Magoski[1]

[1]Queen's University

As well as controlling metabolism in neurons, mitochondria regulate several other fundamental processes. These organelles also influence Ca2 dynamics, neurotransmission, and apoptosis. The bag cell neurons of the mollusc, Aplysia californica, are used to examine the regulation of secretion and excitability. Upon stimulation, these neuroendocrine cells undergo an afterdischarge during which hormones are released to initiate reproduction. To sustain the afterdischarge, bag cell neurons have several adaptations to maintain excitability and hormone release. We tested the contribution of mitochondrial Ca2 uptake and release to these components of the afterdischarge. Cultured neurons were loaded with fura under whole-cell recording, allowing for monitoring of intracellular Ca2 in voltage-clamp. Secretion was determined by measuring changes in membrane capacitance. Mitochondrial motility and membrane potential were quantified using MitoTracker and JC-1 respectively. A train of depolarizing stimuli caused a rise in cytosolic Ca2 , which accumulated in the mitochondria and was subsequently released into the cytosol. In response to mitochondrial Ca2 liberation with FCCP, voltage-gated Ca2 currents were reduced. Furthermore, mitochondrial Ca2 caused secretion as evidenced by a rise in membrane capacitance. Consistent with this was the observation that mitochondria were localized to regions of hormone release in the growth cones. These findings implicate mitochondrial Ca2 release as a primary regulator of prolonged excitability and secretion in the nervous system.

2-B-8 A SNARE sensitive pathway in astrocytes mediates damage following stroke

Dustin Hines[1], Philip G Haydon[1]

[1]Tufts University School of Medicine

A strong body of research has defined the role of excitotoxic glutamate in animal models of brain ischemia and stroke. We propose that astrocyte-neuron signaling represents an important modulatory target that may be useful in mediating damage following stroke. To assess the impact of astrocyte signaling on damage following stroke we have used the astrocyte specific dominant-negative SNARE mouse model (dnSNARE) where expression of the SNARE domain of the vesicle protein VAMP2 reduces extracellular adenosine. Recent findings in our lab have shown that the astrocytic SNARE signaling pathway can affect neuronal excitability on a long time scale by regulating the surface expression of NMDA receptors. We have used both focal photothrombosis and excitotoxic NMDA lesions and targeted the motor and somatosensory cortex to impact readily observable behavioral outcomes in mice. dnSNARE animals that had undergone photothrombosis or NMDA lesion showed a sparing of damaged tissue quantified via histology and immunohistochemistry. Animals were also tested in behavioral tasks corresponding to stroke damaged motor and somatosensory areas. Corresponding to tissue sparing, we found that dnSNARE mice performed significantly better than stroke litter mate controls on cylinder, rung walk and adhesive dot removal tasks. Taken together, our results demonstrate the important contribution of astrocytic signaling in neuronal damage under ischemic conditions. Drugs targeting astrocyte signaling have potential benefit for the outcome of stroke in human patients by limiting the spread of damage.

2-B-9 Anoxia induced GABA receptor-mediated electrical suppression is mimicked by ROS scavenging in turtle cortex

David Hogg[1], Matthew E Pamenter[2], Leslie T Buck[1]

[1]University of Toronto, [2]University of California San Diego

The western painted turtle, Chrysemys picta bellii, is extremely anoxia tolerant surviving months of complete anoxia. In anoxic mammalian brain, ATP dependent ion

pumping is compromised and the consequent loss of membrane ion gradients elicits hyper-excitability and cell death. Adaptations responsible for anoxia tolerance in C. picta brain include an 80-fold increase in [γ-aminobutyric acid] (GABA) resulting in a 75-95% decrease in electrical activity. Electrical suppression results from increased GABA receptor (GABAR)-mediated postsynaptic currents (PSC) that shunt excitatory current, thereby avoiding excessive action potential (AP) firing. The signaling pathway responsible for initiating GABAergic neuroprotection is unknown but could involve decreases in the [reactive oxygen species] (ROS) that occurs following onset of anoxia. Using whole-cell and perforated patch clamp techniques we determined that, similar to anoxia, ROS scavenging caused: GABA-mediated increase in whole cell conductance from normoxic values of 4.5 ±0.4 to 6.6 ±1.1 nS; AP threshold to depolarize from -45 ± 4.2 to -24 ± 2.2 mV; and GABAR-mediated currents to double in amplitude from -237.8 ± 81.6 to -449.8 ± 136.9 pA. We conclude that decreased ROS production is a signal that activates electrical suppression in anoxic turtle cortex.

2-B-10 Noradrenaline is a stress-associated metaplastic signal at GABA synapses

Wataru Inoue[1], Dinara V Baimoukhametova[1], Tamas Fuzesi[1], Quentin J Pittman[1], Jaideep S Bains[1]

[1]Hotchkiss Brain Institute, University of Calgary

During stress, noradrenaline (NA) enhances the capacity of glutamate synapses to exhibit plasticity (metaplasticity). The impact of NA on metaplasticity at GABA synapses, however, remains unexamined. Here, we studied GABA synapses onto parvocellular neurosecretory cells in the paraventricular nucleus of the hypothalamus (PVN) that control the hypothalamic-adrenal-pituitary axis. We found that prior in vivo stress experience is obligatory for these GABA synapses to undergo long-term potentiation (LTPGABA) in response to high frequency afferent stimulation. This stress-dependent gating of LTPGABA requires activation of β-adrenergic receptors (β-ARs) during stress. Using optogenetics, we found that a brief stimulation of brainstem-derived catecholaminergic terminals in the PVN, which mimics stress-induced local NA release, is sufficient to unmask the capacity of GABA synapses to exhibit LTP in naïve unstressed animals. Mechanistically, β-ARs activation causes a functional upregulation of metabotropic glutamate receptor1 (mGluR1). This allows glutamate spillover, during high frequency stimulation, to induce mGluR1-dependent LTPGABA that manifests as unmasking of silent GABA synapses. Our findings provide the first demonstration that NA release during an in vivo challenge alters the information storage capacity of GABA synapses. These changes may be the building blocks of learning and memory that contribute to neuroendocrine adaptations to stress.

2-B-11 A deletion mutation of the C. elegans CaMKII gene unc-43 inhibits associative conditioning and modulates non-associative conditioning

Sara Knauft[1], Kevin Hope[1], Robert M Bragg[1], Jade R Brusseau[1], Jacqueline K Rose[1]

[1]Western Washington University

Calcium-calmodulin protein kinase II (CaMKII) is widely expressed throughout the mammalian brain and has long been known to play a role in learning and memory. In C. elegans, the unc-43 gene is an ortholog to mammalian CaMKII and although the role of unc-43 has been well-characterized in basic worm function (e.g., egg-laying, defecation, etc.), studies investigating the role of unc-43 in C. elegans learning has been limited due to the uncoordinated motor phenotype unc-43 mutant strains exhibit. The current study utilized the unc-43(gk452) mutant strain (generated and identified by the C. elegans Knockout Consortium) consisting of an insertion/deletion modification affecting exon 5 of the UNC-43T isoform. As this strain is superficially wild-type, displaying no obvious motor defects, this allows for its use in behavioral paradigms. Associative learning was tested by employing both an associative chemotaxis assay and an associative chemoavoidance test. Results from both associative protocols reveal that unlike wild-type, unc-43(gk452) worms continue to show a preference for NaCl after a one-hour "no food" associative pairing. As well, unc-43(gk452) worms show a slower rate of response decrement compared to wild-type during habituation training (non-associative conditioning). Current studies seek to determine if expressing the UNC-43T isoform is sufficient to rescue these learning deficits.

2-B-12 Endogenous D-serine and NMDA receptor activation mediate astrocyte-induced cerebral vasodilation

Jillian LeMaistre[1], Lingling Lu[1], Hope Anderson[1], Christopher Anderson[1]

[1]University of Manitoba

Neuronal energy demand is met with increased blood flow in a process known as functional hyperemia. Astrocytes are central to this mechanism, since cytoplasmic Ca2+ elevations in response to neurotransmission trigger release of arachidonic acid (AA) metabolites and gliotransmitters, like D-serine. D-Serine is a co-agonist of NMDA-type glutamate receptors, which are expressed by brain vascular endothelial cells. Thus we hypothesized that astrocyte activation leads to perivascular D-serine release and NMDA receptor-mediated vasodilation in brain in situ. To test this we used two-photon imaging of mouse cortical slices aerated with 20% O2 to assess astrocyte Ca2+ (rhod-2/AM) and monitor arteriolar diameter. Arteriolar vasodilation occurred after astrocyte Ca2+ transients in

response to mGluR agonist, 1-aminocyclopentane-trans-1,3-dicarboxylic acid (tACPD) or photolysis of caged Ca2+ compound, o-nitrophenyl EGTA/AM in perivascular astrocytes. Vasodilation was reduced dramatically by the D-serine degrading enzyme, D-amino acid oxidase (DAAO), indicating that D-serine is involved in vasodilatory signalling induced by both tACPD and photolysis. NMDA receptor antagonists significantly blocked dilation after tACPD and photolysis, indicating a role for NMDA receptors. Both prostaglandin E2 and nitric oxide (NO) mediated dilation after Ca2+ uncaging. NO appeared to cause dilation by suppressing baseline constriction caused by the AA metabolite, 20-HETE. Overall, our results provide evidence of a novel functional hyperemia mechanism involving astrocyte D-serine and NMDA receptors.

2-B-13 Calcium-calmodulin dependent regulation of TRPM2 currents

Brian Lockhart[1], Oies Hussein[1], John F MacDonald[1], Michael F Jackson[1]

[1]Universityof Western Ontario/Robarts Research Institute

TRPM2 (1507AA), a non-selective cation channel with substantial permeability for Ca2 , is responsive to oxidative stress, and is a mediator of cell death in several cell types. The channel is gated through binding of ADP-ribose to a site within its intracellular C-term (1047-1507AA) as well as through binding of Ca2 -calmodulin to the intracellular N-term (1-750AA) domain. In addition to promoting channel activation, Ca2 has also been shown to promote channel inactivation, however the mechanisms are not fully understood. Identifying candidate CaM binding sites using in silico screening, we hypothesized that the Ca2 -dependent inactivation of TRPM2 is mediated by additional intracellular CaM binding domains. TRPM2 currents were recorded from transfected HEK293 cells using 2mM Ca2 extracellular fluid exposures and ADPR in the patch pipette. We determined TRPM2 inactivation is time-, ADPR-, Ca2 - and CaM-dependent by altering time between Ca2 exposures or altering molecular concentrations of the intra- or extracellular solutions. We systematically determined the minimum binding domains for three CaM candidate sites on TRPM2's intracellular domains using truncated fragments and subsequent CaM-Sepharose pull-downs. TRPM2 with substitution mutations to candidate sites were transfected into HEK293 and used for recordings. Currents were completely abolished by a mutated 172-187AA region, amplitudes reduced by mutated 1087-1101AA region and finally they unaltered by 1352-1365AA. These two sites may establish a potential molecular link to Ca2 -dependent inactivation of TRPM2.

2-B-14 Activation of innate immune cells in the choroid plexus by ATP

Valentyna Maslieieva[1], Roger J Thompson[1]

[1]University of Calgary; Hotchkiss Brain Institute

Epiplexus cells (also referred to as Kolmer cells) represent a population of monocyte-derived native immune cells on the surface of the choroid plexus in the brain ventricles. These cells are thought to perform protective functions by reacting to brain injury, infection, foreign bodies and toxins. For epiplexus cells it is unknown whether they are activated by the inflammatory mediator adenosine triphosphate (ATP), and if they are, it is unclear what this reaction is. Possibilities include the protraction of cellular processes or cell movement. A new technique for the isolation of live and intact rat choroid plexus has been developed. It allowed us for the first time to observe the behaviour of epiplexus cells in situ over several hours. Alexa Fluor 488 isolectin B4 conjugate from Griffonia simplicifolia was used as an epiplexus cells marker. Under control conditions, epiplexus cells seem to be actively monitoring the state of epithelial cells and this does not require movement of the epiplexus cell body. Bath application of ATP caused undirected movements of epiplexus cells, which we think was a consequence of their inability to sense the exact source of ATP. Brilliant blue G (BBG) did not have an effect, while pyridoxalphosphate-6-azophenyl-2´,4´-disulfonic acid (PPADS) significantly ($P<0.05$) decreased the distance travelled by epiplexus cells by approximately half. These data suggest that P2X1, P2X2, P2X3, P2X5 or P2Y1 receptors (all PPADS sensitive), but not P2X7 receptors (BBG sensitive) may contribute to the activation of epiplexus cells by exogenous ATP.

2-B-15 Role of the Synaptotagmin-Dynamin interaction in synaptic vesicle recycling

Robyn McAdam[1], Fiona Young[1], Vanessa Blandford[1], Sebastien Thomas[1], Peter McPherson[1], Wayne Sossin[1]

[1]McGill University

Synaptic vesicle protein Synaptotagmin I (Syt I) is a well-studied calcium sensor critical for synchronized neurotransmission. However, the role of its highly conserved juxtamembrane domain has yet to be investigated. We show that the Syt I juxtamembrane region interacts with the endocytic protein Dynamin. We hypothesize a role for this interaction in mediating activity-dependent retrieval of synaptic vesicles. Using pulldown assays with GST-Syt I fusion proteins, we have shown that this interaction is modified by phosphorylation, and have localized the interaction to the membrane-binding pleckstrin homology domain of Dynamin 1. To determine if this interaction mediates vesicle recycling, we are using pulse-chase FM dye assays in mature hippocampal cultures in which the Syt I-Dynamin interaction has been disrupted.

2-B-16 Modulation of retinal ganglion cell excitability by chloride pumping: role of extrasynaptic glycine receptor

Lois Miraucourt[1], Jennifer F Tsui[1], Jean-François Desjardins[1], Annie Castonguay[2], Yves De Koninck [2], Paul W Wiseman[1], Edward S Ruthazer[1]

[1]McGill University, [2]Université Laval

Type I cannabinoid receptor (CB1R) is present throughout the retina of all vertebrates, from human to fish, however no clear functional role has yet been shown for the endocannabinoid system (ECS) on vision. Using a behavioral assay based on their innate ability to avoid dark moving dots, we found that Xenopus tadpoles treated with CB1R agonist WIN performed better than control animals under low light conditions. WIN enhanced the tectal neurons EPSCs evoked by electrical stimulation of retinal ganglion cells (RGCs). The CB1R antagonist AM-251, the glycine receptor antagonist strychnine or the NKCC1 chloride transporter blocker bumetanide blocked this effect. Responses evoked by stimulation of the optic tract in an isolated brain preparation were unaffected by bath application of WIN, implicating the retina as the site of relevant CB1R activation. WIN increases the spike frequency of RGCs to visual stimulation, an effect that was also prevented by pretreatment with AM-251, strychnine or bumetanide. However, ERGs, which measure responses in the outer retina, showed no effect of WIN on light-evoked response. To investigate the role of chloride in the excitability change of RGCs we performed 2-photon imaging of retinal neurons expressing the fluorescent chloride indicator clomeleon. WIN rapidly decreased the intracellular chloride concentration of RGCs, but induced no change in bipolar, amacrine or Muller glia cell types. These results present a model where the ECS improves vision at low light conditions by modulation of chloride homeostasis in RGCs to increase excitability.

2-B-17 Mobility impairment following acute toluene exposure is associated with increased GABA transporter expression in C. elegans motor neurons.

Samuel Moss[1], Katrina Mar[1], Nicole Stankowicz[1], Jacqueline Rose[1]

[1]Western Washington University

The effects of the commonly abused solvent toluene were investigated using the model organism Caenorhabditis elegans. After 10 minutes of ambient exposure resulting from either 0, 2.5, or 5µL toluene application, mobility of worms was determined in liquid media and quantified as number and magnitude of body bends. A dose-dependent decrease in the number of body bends and a subsequent increase in body bend magnitude resulted. In C. elegans mobility involves activation by both acetylcholine, for muscle activation, and GABA for relaxation of opposing muscles. To begin to determine the possible role of GABA a transgenic reporter strain (UNC-47::GFP) was employed to visualize GABA transporter expression following 10-minute ambient exposure resulting from either 0 or 5 µL toluene. Imaging and analysis was restricted to motor neuron terminals. Worms exposed to toluene showed a significant increase in UNC-47::GFP expression at the anterior dorsal nerve cord. To determine if the change in UNC-47::GFP fluorescence was due to an upregulation of expression, heat shock was delivered immediately following toluene exposure to arrest de novo protein synthesis. Preliminary results indicate that after heat shock, toluene-exposed C. elegans showed significantly less UNC-47::GFP. Additional tests employing qRT-PCR are underway to determine if these changes in expression involve transcriptional modifications.

2-B-18 Sumatriptan inhibition of N-type calcium channel signaling in dural CGRP terminal fibres

Sean Mulligan[1], Landon D Baillie[1], Andrew H Ahn[2]

[1]University of Saskatchewan, [2]University of Florida

Sumatriptan is an effective therapeutic for the treatment of migraine pain yet the antimigraine mechanisms of action remain controversial. 5HT1 receptors in the dura colocalize with calcitonin gene-related peptide (CGRP) containing fibres in high density and thus provide a possible peripheral site of action for sumatriptan. We used high-resolution optical imaging selectively within individual mouse dural CGRP nociceptive fibre terminations and found that application of sumatriptan caused a rapid, reversible dose-dependent inhibition in the amplitude of single action potential evoked Ca2 transients. Pre-application of the 5-HT1 antagonist GR127935 or the selective 5-HT1D antagonist BRL 15572 prevented inhibition while the selective 5-HT1B antagonist SB 224289 did not, suggesting this effect was mediated selectively through the 5-HT1D receptor subtype. Sumatriptan inhibition of the action potential evoked Ca2 signaling was mediated selectively through N-type Ca2 channels. Although the T-type Ca2 channel accounted for a greater proportion of the Ca2 signal it did not mediate any of the sumatriptan inhibition. Our findings support a peripheral site of action for sumatriptan in inhibiting the activity of dural pain fibres selectively through a single Ca2 channel subtype. This finding adds to our understanding of the mechanisms that underlie the clinical effectiveness of 5HT1 receptor agonists such as sumatriptan and may provide insight for the development of novel peripherally targeted therapeutics for mitigating the pain of migraine.

2-B-19 Activity-dependent secretion of progranulin

Eugenia Petoukhov[1], Sarah Fernando[2], Farhan Shivji[1], Diana Hunter[1], Michael A Silverman[2], Shernaz X Bamji[1]

[1]University of British Columbia, [2]Simon Fraser University

Frontotemporal dementia (FTD) has been linked to mutations in the progranulin gene (GRN) that lead to progranulin (PGRN) haploinsufficiency. Previous work from our lab has demonstrated that knocking down PGRN levels in rat primary hippocampal cultures reduces the density of synapses, but enhances the size and efficacy of remaining synapses. In the current study we hypothesized that this secreted glycoprotein may be secreted in an activity-dependent manner to regulate synapse formation and function. We first demonstrate that PGRN is preferentially recruited to synapses in cultures treated with 4-aminopyridine (4-AP), a compound that enhances activity by blocking potassium channels and activating Ca2 channels. 4-AP treatment also results in a 6-fold increase in PGRN secretion from axons and a 3-fold increase from dendrites. Interestingly, this secretion is observed to occur from both synaptic and non-synaptic sites. Treatment of neurons with recombinant PGRN enhances synapse density, suggesting that activity-mediated secretion of PGRN from synapses may enhances the formation of new synapses in a cell-autonomous manner.

2-B-20 Modulation of GABA(A) receptor trafficking by tumor necrosis factor-alpha

Horia Pribiag[1], David Stellwagen[1]

[1]McGill University

Brain function is subject to immune modulation under both normal and pathological states; however the underlying mechanisms remain largely unexplored. Tumor necrosis factor-alpha (TNFa) is a pro-inflammatory cytokine released by various cell types, including microglia and astrocytes in the central nervous system. TNFa has recently been implicated in the regulation of neurotransmission through various mechanisms including regulation of receptor trafficking, homeostatic plasticity and gliotransmission. Here we characterize the effects of TNFa on GABA(A) receptor trafficking in primary hippocampal neuron cultures. GABA(A)Rs mediate the majority of fast inhibitory neurotransmission in the brain. These receptors are heteropentameric ligand-gated ion channels of varying subunit composition, localized in clusters at inhibitory post-synaptic specializations. We observe a rapid down-regulation of alpha, beta, and gamma GABA(A)R subunits in response to TNFa exposure. Functionally, this is confirmed by decreased mIPSC amplitude and frequency. To investigate the mechanisms underlying these effects we are characterizing the effects of TNFa on GABA(A)R endocytosis and on clustering of inhibitory synapse scaffold proteins, as well as identifying signaling components downstream of TNFa receptors that impact GABA(A)R trafficking. Our findings describe a plausible mechanism for the progression brain diseases associated with high levels of TNFa production (such as neurodegeneration and epilepsy), through post-synaptic down-regulation of inhibitory neurotransmission.

2-B-21 Synaptic and extrasynaptic NMDA receptor signaling in cortical-striatal co-culture.

Marja Sepers[1], Alexandra M Kaufman[1], Austen J Milnerwood[1], Ainsley Coquinco[1], Kevin She[1], Ann Marie Craig[1], Max Cynader[1], Lynn A Raymond[1]

[1]University of British Columbia

NMDA receptors exhibit a dichotomous role in subcellular signaling. NMDAR activation at the synapse increases phosphorylated cAMP response element binding protein (pCREB) and promotes neuroprotection. Overactivation of NMDAR at extrasynaptic sites reduces pCREB and leads to toxicity and cell death. The divergence of synaptic and extrasynaptic NMDAR signaling has been shown in cortical and hippocampal cultures, but has not been studied in the GABAergic striatal medium spiny neurons (MSN) that are most susceptible to neurotoxicity in Huntington Disease. To that end we generated co-cultures with both MSN and the cortical cells that provide their main glutamatergic input. In comparison to monocultures, MSN in rat primary cortical-striatal cultures show a more typical morphology with a profusion of dendrites. NMDA-evoked current density and synaptic signaling is robust in cocultured MSN. Stimulating synaptic NMDAR increased pCREB in MSN, while extrasynaptic GluN2B-containing NMDAR reduced pCREB. Using cortical-striatal co-culture from mice, we were able to compare synaptic and extrasynaptic NMDAR signaling in WT and HD transgenic YAC128 MSN. Although synaptic NMDAR current was similar in both genotypes, YAC128 MSN had elevated extrasynaptic NMDAR current density and GluN2B surface expression. These results support the role of extrasynaptic NMDAR in the pathophysiology of MSN in Huntington Disease. Cortical-striatal co-cultures provide a model system of striatal excitatory transmission amenable to genetic and pharmacological intervention.

2-B-22 NMR analysis of the role of conserved Tryptophan residues within the linker-region of neuronal-synaptobrevin

Marzena Serwin[1], Colin DeMill[1], Sameer Al-Abdul-Wahid[1], Scott Prosser[1], Bryan Stewart[1]

[1]University of Toronto

Synaptobrevin (Syb) is a single-pass transmembrane protein found on synaptic vesicles critical for synaptic transmission by virtue of its participation in the SNARE complex. neuronal-Syb (n-Syb) from the model organism Drosophila melanogaster varies only by three residues in

the linker region (between the SNARE and transmembrane domain, residues 85-96) when compared with Syb found in vertebrates. One of these differences is the presence of two tryptophan residues in Syb, compared to the single tryptophan found in n-Syb. Recent studies have suggested the n-Syb/Syb linker region may play a role in vesicular fusion. It has been speculated that the aromatic residues anchor the protein and introduce a rigid conformation thus increasing the stability of this region. To address the importance of the tryptophan residue to synaptic transmission we have created site-directed mutants within the linker region using a truncated version of n-Syb that included the linker region and transmembrane domain (residues 75-121). In the current study HSQC-NMR analysis of 15N-labelled Drosophila melanogaster n-Syb samples was performed on wild type and three mutant n-Syb constructs. Spectra of all three constructs contain characteristic signatures expected for a single-pass transmembrane protein lacking any tertiary structure. We determined that reducing the number of tryptophan residues increases the immersion depth of the linker region within the membrane and reduces water exposure of adjacent residues. This research sets the stage for future characterization of n-Syb by NMR spectroscopy.

2-B-23 Muscarinic receptor activation and the modulation of disinhibition-mediated LTP in the CA1 of the ventral hippocampus

Petri Takkala[1], Melanie A Woodin[1]

[1]University of Toronto

Muscarinic receptor (mAChR) activation has a profound effect on GABAergic interneurons in the CA1, including depolarization of the resting membrane potential, increasing spiking frequency, and increasing inhibitory postsynaptic potential (IPSP) frequency. The objective of the current study is to examine the mAChR regulation of GABAergic synaptic efficacy and disinhibition-mediated LTP (dmLTP), as mAChR dependent modulation has previously been shown to be an important regulator of LTP at glutamatergic synapses in the dorsal hippocampus. All experiments utilized C57/Bl6 mice, from which acute brain slices were prepared by horizontal sectioning. Whole-cell patch clamp recordings were made in the ventral hippocampus from putatively identified CA1 pyramidal neurons. Postsynaptic potential (PSP) amplitude and PSP reversal potential were recorded in pyramidal neurons by extracellular stimulation of Schaffer collaterals. A spike-timing dependent protocol was used to induce dmLTP (at 5Hz for 60 seconds). We found that mAChR activation (using 1 μM carbachol) does not alter the IPSP reversal potential, nor does it prevent the induction of dmLTP, despite a significant postsynaptic membrane potential depolarization and increased spike frequency. We are currently determining the effect of mAChR activation on the paired pulse ratio, and further examining differences in the neuromodulatory effects of mAChR activation during plasticity induction between the ventral and dorsal hippocampus.

2-B-24 A novel form of anticipation in the retina mediated by electrical synapses

Stuart Trenholm[1], Gautam B Awatramani[1]

[1]Dalhousie University/University of Victoria

Introduction: In visual processing, phototransduction introduces a temporal delay such that it takes ~50 ms before signals leave the retina. This delay is problematic for encoding moving objects, which cover distance during the delay. In compensation, the visual system appears able to anticipate moving stimuli, though mechanisms remain unclear. Here, we examined whether lateral gap junction (GJ) coupling of retinal ganglion cells might contribute to anticipatory signals. PARA Methods: We recorded responses from directionally selective ganglion cells (DSGCs) in mouse retina. We included the GJ tracer Neurobiotin in the intracellular solution. PARA Results: We found anterior coding DSGCs (Hb9::eGFP) to be the only strongly electrically coupled DSGC subtype. Paired recordings revealed reciprocal coupling between anterior coding DSGCs. GJs acted as low-pass filters, preventing action potentials in one cell from triggering suprathreshold activity in its neighbours. Coupled and uncoupled DSGCs possessed similar sized classic (suprathreshold) receptive fields (RFs). However, only coupled DSGCs exhibited extensive subthreshold RFs well beyond their classic RFs. Interestingly, coupled DSGCs responded with suprathreshold responses to moving edges far outside their classic RFs. PARA Conclusions: Electrically coupled DSGCs exhibit a novel anticipatory behaviour. This anticipation appears to arise from non-linear summation of coincident lateral gap junction signals and vertical (bipolar cell) chemical synaptic inputs.

2-B-25 Src Family Kinases Open Pannexin-1 Channels During Anoxia

Nicholas Weilinger[1], Peter L Tang[1], Roger J Thompson[1]

[1]Hotchkiss Brain Institute, Univeristy of Calgary

Vascular diseases can cause acute and chronic oxygen deprivation (hypoxia), and subsequently lead to central nervous system dysfunction. Pyramidal neurons in the CA1 region of the hippocampus are particularly susceptible to hypoxia, and respond initially with an "anoxic depolarization" (AD), characterized by the activation of multiphasic inward currents that are strongly correlated with neuronal death and dysfunction. Anoxic depolarization of pyramidal neurons results from a large inward current that is activated both by excessive presynatpic release and reversed uptake of glutamate during exposure to anoxia / ischemia. Panx1 channels can then be activated directly by ischemia, as well as by NMDA receptors (NMDAR), but the upstream mechanisms of Panx1 opening are unknown. We

used whole-cell recordings to show that pharmacological inhibition or conditional genetic deletion of Panx1 strongly attenuates the anoxic depolarization of CA1 pyramidal neurons in acute brain slices. Anoxia activated Src family kinases (SFKs), as measured by increased phosphorylation of SFKs at Y416 and the SFK inhibitor, PP2, prevented Src activation and Panx1 opening during anoxia. Importantly, the NMDAR antagonists, D-APV and R-CPP, attenuated AD currents carried by Panx1 and the combined application of D-APV and 10panx (a Panx1 blocker) inhibited AD currents to the same extent as either blocker alone. We conclude that, in addition to activation of NMDARs, anoxia / ischemia recruits SFKs to open Panx1, leading to sustained neuronal depolarizations.

2-B-26 Chronic unpredictable stress attenuates synaptic plasticity in the dentate gyrus and increases the incidence of depressive-like behaviours

Emily White[1], Patricia S Brocardo[1], Darby F Hawley[1], J. Leigh Leasure[1], Brian R Christie[1]

[1]University of Victoria

Chronic stress is an epidemic in today's society; approximately 6.5 million adult Canadians experience high or extreme stress most days of their lives. One region of the brain particularly affected by chronic unpredictable stress (CUS) is the hippocampus. The hippocampus is not a uniform structure, as dorsal regions may be more involved in spatial function with ventral more involved in affect. Previous studies have found decreased survival of progenitor cells in the ventral, but not the dorsal, hippocampus. The purpose of this study was to determine if CUS preferentially reduced synaptic plasticity in the ventral dentate gyrus (DG). Twenty-four adult male Long-Evans rats were divided into control or stressed groups. For 14 days, stressed animals were subjected daily to an unpredictable schedule of ethologically relevant stressors, such as altered light cycle, predator sounds, and damp bedding. Twenty-four hours following the last stressor, sucrose preference and splash tests were performed. Both tests indicated that animals were exhibiting signs of stress. CUS animals exhibited a reduction in sucrose preference, and increased latency to start grooming coupled with diminished time spent grooming when compared to controls during the splash test. Immediately after behavioural testing, animals were sacrificed for in vitro electrophysiology field recordings in which long-term potentiation (LTP) was elicited in the dorsal and ventral DG. In contrast to a region specific effect for neurogenesis, synaptic plasticity was attenuated in both ventral and dorsal slices following CUS.

2-B-27 Relative changes of intrinsic excitability are sufficient for preferential recruitment of neurons into a fear memory trace

Adelaide Yiu[1], Valentina Mercaldo[1], Blake Richards[1], Matthew Florczynski[2], Qui Jing Wu[2], Steven A Kushner[3], Melanie A Woodin[2], Paul W Frankland[1], Sheena A Josselyn[1]

[1]Hospital for Sick Children, [2]University of Toronto, [3]Erasmus MC: University Medical Centre

A fundamental goal of neuroscience is to elucidate mechanisms memories are encoded and stored. Identifying the physical representation of memory within the brain (memory trace) is a long-standing challenge since Lashley's "search for the engram". We've shown that lateral amygdala (LA) neurons with increased CREB are competitively advantaged for recruitment into a fear memory trace. Here, we examined the mechanism underlying this competitive advantage. Given that CREB activity robustly increases neuronal excitability, we examined whether directly manipulating intrinsic excitability alone is sufficient to select neurons for inclusion in the memory trace. To directly manipulate intrinsic excitability in a subset of LA neurons, we infused viral vectors expressing a dominant-negative KCNQ2 channel. Intrinsic excitability in ~20% LA neurons enhanced memory in wild-type mice, analogous to previous findings of overexpressing CREB. Further, increasing excitability in LA neurons was sufficient to rescue memory deficit in genetically-engineered mice with targeted disruption of CREB. Our results suggest that eligible neurons are selected in a memory trace as a function of their relative excitability around the time of learning.

C – Disorders of the Nervous System

2-C-29 New insights into the role of diabetes and the secondary somatosensory cortex in recovery of somatosensory function after ischemic stroke

Craig Brown[1], Danielle Sweetnam[1], Andrew Holmes[1], Kelly Tennant[1], Akram Zamani[1]

[1]University of Victoria

Diabetics are at greater risk of having a stroke and are less likely to recover from it. In order to understand this clinically relevant problem, we induced an ischemic stroke in the primary forelimb somatosensory (FLS1) cortex of diabetic mice and then examined sensory-evoked changes in cortical membrane potentials and behavioural recovery of forelimb sensory-motor function. Consistent with previous studies, focal stroke in non-diabetic mice was associated with acute deficits in forelimb sensori-motor function and a loss of forelimb-evoked cortical depolarizations in peri-

infarct cortex that gradually recovered over several weeks time. In addition, we discovered that damage to FLS1 cortex led to an enhancement of forelimb-evoked depolarizations in secondary forelimb somatosensory (FLS2) cortex. Enhanced FLS2 cortical responses appeared to play a role in stroke recovery given that silencing this region was sufficient to re-instate forelimb impairments. By contrast, the functional re-organization of FLS1 and FLS2 cortex was largely absent in diabetic mice and could not be explained by more severe cortical infarctions. Diabetic mice also showed persistent behavioural deficits in sensori-motor function of the forepaw, which could not be rescued by chronic insulin therapy after stroke. Collectively, these results indicate that diabetes has a profound effect on brain plasticity, especially when challenged by an ischemic event. Further, our data suggest that secondary cortical regions play an important role in the restoration of sensori-motor function.

2-C-30 Neurodevelopmental consequences of diabetes during pregnancy for the offspring: A role for RAGE signaling?

Andrew Chandna[1], Chester Thai[1], Quinten Greba[1], Naila Kuhlman[1], Courtney Bryce[1], John Howland[1], Veronica Campanucci[1]

[1]University of Saskatchewan

Inflammatory responses to infection during pregnancy increase the risk of neurodevelopmental psychiatric disorders such as schizophrenia and autism in the offspring. However, whether maternal diabetes leads to a similar inflammatory state with long-term consequences for the offspring is not known. A major source of inflammatory signaling in diabetes is activation of the receptor for advanced glycation end products (RAGE). Interestingly, several studies have shown increased RAGE activation in psychiatric disorders. Thus, our hypothesis is that maternal diabetes creates a pro-inflammatory state, triggered largely by RAGE signaling, that alters normal neural development of the offspring. We tested this hypothesis in rats using the streptozotocin (STZ; 50 mg/kg; i.p.) model of maternal diabetes during the last week of pregnancy. Following STZ treatment, we observed a large increase in protein expression of RAGE and the pro-inflammatory nuclear factor NF-êB in the forebrain of the offspring (postnatal day 0). Electrophysiological characterization of hippocampal cultures grown from the offspring of STZ-treated dams also showed a striking increase in excitability. When tested in a battery of cognitive tasks in early adulthood, the offspring of STZ-treated dams had significantly lower prepulse inhibition than control offspring without changes in anxiety or recognition memory. Collectively, these results suggest that RAGE-mediated inflammatory responses to maternal diabetes alter normal brain development of the offspring ultimately leading to behavioural changes in early adulthood.

2-C-31 Activity of neural stem cell in Alzheimer's disease

Athena Vai Lam Chou[1], Jane Roskams[1]

[1]University of British Columbia

Despite the neurogenic capacity of neural stem cells (NSCs) in adult mammalian brain, adult neurogenesis does not appear to alleviate the neuronal loss in the cerebral cortex and hippocampus, which contributes to cognitive decline and memory loss in Alzheimer's disease (AD). The combination of amyloid plaques and neurofibrillary tangles could impose an increased progenitor demand to compensate neuronal loss, resulting in the disruption of NSC niche over time. As Presenilin mutations are implicated in familial AD, the intrinsic NSC regulation modulated by Notch signaling pathway could be disrupted during disease progression and further contribute to defects in neurogenesis in AD. We used a transgenic mouse model of AD (APPswe/PS1dE9) in which the APP mutation predisposes the animals to develop amyloid pathology and the PS1 variant accelerates the appearance of amyloid pathology. We studied the cellular activity of NSC niche in these AD mice when amyloid deposition begins to appear using thymidine analog and immunofluorescence. Also, we have developed techniques to detect human NSCs and progenitor markers in formalin-fixed frozen sections of post-mortem human brain. Changes in proliferation activity are most abundant in the ependymal layer of the SVZ. Our data strongly suggest that NSCs are present in the neurogenic niche of human patients with advanced AD and we are testing if the same repertoire of progenitors is retained or compromised in the neurogenic niche of normal aged brain.

2-C-32 The influence of HspB1 phosphorylation on its interaction(s) with actin

Joseph Clarke[1], Firoozeh Nafar[1], Karen M Mearow[1]

[1]Memorial University

The small heat shock protein (sHSP) HspB1 is implicated in cellular survival following stress. One potential mechanism is through its stabilizing interactions with different cytoskeletal elements, such as microfilaments. It has been suggested that the phosphorylation state of HspB1 influences these interactions, and may be important in HspB1's role in neurite growth. In this study, we wished to assess whether: 1) there is a direct interaction between HspB1 and filamentous actin (F-actin); 2) the phosphorylation state of HspB1 influences this interaction. We focused upon two cell models. We used HEK293A cells transfected with wild-type hamster HspB1, or its phosphorylation mutants, and PC12 cells, in which we inhibited HspB1 phosphorylation pharmacologically. F-actin-HspB1 interactions were analyzed using two pull-down techniques: either biotinylated phalloidin (selective for

F-actin) and streptavidin bound Dynabeads®, or an anti-HspB1 antibody (selective for HspB1) and A/G coated Dynabeads ®. The results suggest that non-phosphorylated HspB1 specifically precipitated with F-actin, while phospho-HspB1 (Ser 15 and 86) interacted with F-actin only after cellular stress. Immunocytochemical (ICC) analyses further confirm these results. These data demonstrate that non-phosphorylated HspB1 directly interacts with F-actin, while phospho-HspB1 (Ser 15 and 86) may have a different modulatory effect on F-actin depending on the state of the cell. This further demonstrates that the non-phosphorylated state of HspB1 may be the key form that interacts with F-actin. Supported by NSERC

2-C-33 Altered extrasynaptic NMDA receptor localization on aberrant signalling in Huntington Disease

Alejandro Dau[1], Clare M Gladding[1], Jian Xu[2], Paul J Lombroso[2], Lynn A Raymond[1]

[1]University of British Columbia, [2]Yale University Medical School

In Huntington disease (HD), activation of STriatal-Enriched tyrosine Phosphatase (STEP) and the protease calpain may contribute to apoptotic signalling. Additionally, elevated extrasynaptic NMDA receptor (Ex-NMDAR) expression, which predisposes neurons to dysfunction and death, has been reported in the YAC128 mouse model that expresses mutant huntingtin (mHtt) with 128 CAG repeats. By mediating post-translational modifications of NMDARs, increased STEP and calpain activity in HD could alter NMDAR synaptic/extrasynaptic localization; on the other hand, enhanced Ex-NMDAR expression is known to stimulate STEP and calpain activity. To determine whether increased Ex-NMDAR is a cause or consequence of altered signalling in HD, we treated 2-month old wildtype and YAC128 mice with memantine (1 or 10mg/kg/day), a selective Ex-NMDAR inhibitor, for 2 months. Following treatment, we quantified localization and calpain cleavage of NMDARs by western analysis. Untreated YAC128 mice exhibited enhanced calpain-cleaved and full-length GluN2B expression extrasynaptically compared to wildtype. This effect was reversed by memantine at both doses, suggesting a role of enhanced Ex-NMDAR currents on receptor mislocalization. We are currently testing whether memantine can restore other HD-induced signalling changes, including an extrasynaptic shift of Post-Synaptic Density-95, activation as well as calpain-cleavage and inactivation of STEP, and p38 MAP kinase expression. Understanding the role of NMDAR mislocalization on HD-induced signalling changes could provide novel therapies for this disease.

2-C-34 PTEN deletion promotes regenerative sprouting in the aged rubrospinal tract

Brett Hilton[1], Wolfram Tetzlaff[1]

[1]University of British Columbia

The devastating paralysis that occurs following spinal cord injury is the result of central nervous system (CNS) regenerative failure. A major contributor to this regenerative failur is a diminished intrinsic capacity of adult CNS axons to grow, based largely on inactivity in the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway which is negatively regulated by phosphatase and tensin homolog (PTEN). The rubrospinal tract (RST) is a model of CNS axon regenerative failure that functions in skilled limb movement. Here, we assessed whether PTEN deletion would promote axon regeneration in the RST of aged (7-8 month old) mice. Floxed PTEN mice were injected with adeno-associated virus serotype 2 expressing Cre and GFP (AAV2-Cre) or GFP alone for control (AAV2-GFP) into the right red nucleus. Four weeks later, mice underwent a left dorsolateral crush at cervical level C4/C5. Six weeks later, mice were injected with biotinylated dextran amine (BDA) into the right red nucleus to trace the RST. Two weeks later, mice were sacrificed. AAV2-Cre injected animals showed significantly decreased dieback and increased regenerative sprouting of rubrospinal axons through the lesion site in comparison to AAV2-GFP injected animals, for up to 100µm caudal measured from the middle of the lesion site. Our findings suggest that PI3K/Akt/mTOR activity is a significant determinant of rubrospinal regenerative potential, yet advanced age may play an important role in decreasing the ability of RST axons to regenerate long distances.

2-C-35 Investigating the role of the GABAA alpha2 subunit in models of addiction

Rochelle Hines[1], Dustin J Hines[1], Stephen J Moss[1]

[1]Tufts University School of Medicine

Addiction is a chronic disease involving multiple brain circuits including reward, motivation, and memory pathways. Although common pathways and mechanisms have been discovered, addiction comprises a complex group of disorders, and has been related to both genetic and environmental risk factors. Genetic studies conducted in families with multiple addicts have revealed critical targets for alcoholism and addiction. In particular, linkage and association studies have identified single-nucleotide polymorphisms in the gene encoding the GABAA α2 subunit (GABRA2) to be significantly associated with alcoholism and addiction to other drugs of abuse. We have developed a mouse model with a mutation in the intracellular loop of GABRA2 (Gabra2-1) to study the mechanisms by which α2 contributes to addiction. Gabra2-1 mice show increases in the expression of α2 in the nucleus accumbens and frontal cortex. In contrast to litter

mate controls, heterozygous and homozygous Gabra2-1 mutants do not show increases in voluntary drinking of alcohol using the intermittent access paradigm. Gabra2-1 mice also do not develop conditioned place preference to alcohol. These data demonstrate that GABRA2 plays a critical role in the rewarding effects of alcohol. The rewarding effects of other drugs of abuse are currently being examined to determine if α2 signaling contributes to addiction in a general fashion. These studies will provide novel insights into the pathways mediating alcoholism and clarify the contribution of GABAergic signaling to the addiction circuitry.

2-C-36 Modeling the cyclicity of depression episodes in rats

Katherina Lebedeva[1], Rudy Bowen[1], Hector Caruncho[2], Lisa Kalynchuk[1]

[1]University of Saskatchewan, [2]University of Santiago de Compostela

Animal models are essential tools for studying the pathogenesis of major depression. However, the complex structure of human depression as well as the subjective nature of its symptoms has made the development of animal models very challenging. Almost without exception, animal models of depression focus on reproducing single incidences of depression endophenotypes (e.g., anhedonia), and therefore, they do not provide any means to understand the typical cycling of mood seen in most patients. In our recent work, we attempted to recapitulate the cyclical disease course of depression in the animal model. In the experiment described here, we assessed depression-like behavior through 3 cycles of treatment with and recovery from the stress hormone corticosterone (CORT). Each cycle of CORT treatment comprised 21 days of CORT injections (10, 20, or 40 mg/kg) followed by 21 days of recovery. We found that CORT produced increasingly greater effects on depression-like behavior through each cycle. In the 1st cycle, CORT increased depression-like behaviour after 21 days of treatment, which then normalized after the recovery. In the 2nd and 3rd cycles however, we observed an early manifestation of depression-like behavior after only 10 days of CORT injections. This stress model of depression provides stronger face validity as it mimics several aspects of human depression, including the phenomenon of episode recurrence seen in more than 70% of depressed patients. These results present a promising model for better understanding the mechanisms of depression and novel therapeutic strategies.

2-C-37 Neurobehavioral deficits caused by post-embryonic ethanol exposure versus nutritional deficiency in C. elegans: similarities and differences

Conny Lin[1], Sasha Sa[1], Ricardo Bortolon[1], Denny Soe[1], Catharine Rankin[1]

[1]University of British Columbia

Research on neurobehavioral defects in fetal alcohol spectrum disorder is complicated by the fact that alcohol consumption often, directly or indirectly, causes nutritional deficiency. In addition, alcohol has been shown to affect a wide range of molecular targets and to interfere with nervous system development. To fast track progress in this field, we use a powerful genetic, development, and nervous system model, C. elegans, to compare the effects resulting from ethanol exposure versus nutritional deficiency. C. elegans were starved or exposed to 3 days of 0.5M ethanol (w/w in growth medium, ~0.12ºC) during post-embryonic development, and allowed to recover for 2 days before testing. We found that ethanol exposure but not starvation caused abnormal neurites morphology in mechanosensory neurons similar to those seen in C. elegans lacking an E3 ubiqutin ligase (rpm-1) important for axon termination. These mechanosensory neurons are required for habituation learning in C. elegans. Interestingly, we found that rpm-1 mutants had similar learning deficits as seen in ethanol exposed animals; both groups failed to develop an age-dependent change in the pattern of habituation learning as seen in the wildtype unexposed control. However, starved animals also exhibited the same learning defects. These results suggest that ethanol exposure and starvation may cause both convergent (learning deficit) and divergent (neurite abnormality) defects. Future direction will focus on investigating whether the convergent phenotype was produced through the same or different molecular pathways.

2-C-38 Epigenetic regulation of methyl CpG binding protein-2 in neural stem cells

Vichitra Liyanage[1], Robby M Zachariah[1], Carl O Olson[1], Mojgan Rastegar[1]

[1]University of Manitoba

Methyl CpG binding protein-2 (MeCP2) is a nuclear protein which specifically binds to methylated DNA. MeCP2 is highly expressed in brain and has been detected in both neurons and glia. MeCP2 isoforms (MeCP2E1 and MeCP2E2) are suggested to have crucial non-redundant functions raising the importance of proper regulation of both isoforms. Altered expression of MeCP2 leads to a range of neurodevelopmental disorders including the autism spectrum disorder Rett Syndrome (RTT). Although expression of MeCP2 in neurons is well studied, comprehensive studies on the expression, function and regulation of MeCP2 in both neurons and glia have been

impeded by the lack of an appropriate in vitro model system. In this study we investigate the expression and epigenetic regulation of MeCP2 in embryonic forebrain derived neural stem cells (NSCs) which differentiate into neurons and glia. Expression of Mecp2 is known to be regulated by epigenetic mechanisms, epigenetics being referred to the inheritable modifications that regulate gene expression without involving the underlying DNA sequences. Here we study the role of DNA methylation in regulation of Mecp2 during NSC differentiation, by treating differentiating NSCs with global DNA demethylating agents. We believe that our findings will establish differentiating NSCs as an ideal in vitro model system to understand the function and regulation of MeCP2. Our results will give insights to understand the potential role of MeCP2 in neurodevelopmental disorders.

2-C-39 NMDA receptor expression is reduced in the dentate gyrus of Fmr1 knockout Mice

Namat Majaess[1], Kristin Morch[1], Brian R Christie[1]

[1]University of Victoria

Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability affecting about 1 in 4000 males and 1 in 8000 females. FXS is caused by a trinucleotide expansion that silences the Fragile X Mental Retardation 1 (Fmr1) gene resulting in the loss of its protein product, the Fragile X Mental Retardation Protein (FMRP). PARA FMRP is highly expressed in the hippocampus, a region of the brain involved in learning and memory processes. In the present study we focus on the dentate gyrus (DG) subfield of the hippocampus, a region that we have just shown to have impaired N-methyl-D-aspartate receptor (NMDAR) functioning in Fmr1 knockout (KO) mice. This impairment also resulted in reduction in both long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy, two biological models of learning and memory. We hypothesize that the reduced LTP and LTD we have observed in Fmr1 KO mice are due to either reduced amounts of total NMDARs (GluN1) or due to changes in NMDAR subunit composition (GluN2A and GluN2B). Using Western blotting, we found that there is a decrease in the GluN1 subunit as well as both the GluN2A and GluN2B subunits in the DG of young adult Fmr1 KO mice, indicating that these mice have significantly lower amounts of total NMDARs. These results could explain the altered LTP and LTD seen in Fmr1 KO mice at the molecular level and might contribute to the intellectual impairments seen in these KO mice.

2-C-40 Synchronizing effects of entopeduncular nucleus deep brain stimulation in anesthetized rats

Clinton McCracken[1], Zelma H Kiss[1]

[1]University of Calgary

High-frequency electrical stimulation of specific brain structures, commonly known as deep-brain stimulation (DBS), is an effective treatment for intractable neurological disorders, but mechanisms of action remain unclear. Here, we examine the circuit-level effects of DBS applied to the entopeduncular nucleus (EP), the rat homolog of the internal globus pallidus and a target for treatment of dystonia and Parkinson's disease. We used simultaneous multi-site local field potential (LFP) recordings in urethane-anesthetized rats to assess the effects of high-frequency (HF, 130 Hz; clinically effective), low-frequency (LF, 15 Hz; clinically ineffective) and sham DBS delivered to EP. LFP activity was recorded from dorsal striatum, ventroanterior thalamus, primary motor cortex, and the stimulation site in EP. Spontaneous and acute stimulation-induced LFP oscillation power and coherence were assessed at baseline, and after 30, 60, and 90 minutes of stimulation. HF DBS produced a significant, time-dependent increase in slow (0.5-4 Hz) oscillation power in all regions, and enhanced coherence in this frequency band between all regions. Furthermore, compared to LF and sham, HF DBS enhanced coherence in the high beta (20-30 Hz) and gamma (30-85 Hz) bands between a number of regions along the circuit. Similarly, only HF DBS elevated coherent high beta activity induced by acute electrical stimulation. These data suggest that EP DBS may produce therapeutic effects by enhancing coherent activity along a neural circuit implicated in dystonia and Parkinson's disease.

2-C-41 A bioinformatic approach to link clinical phenotype to genotype in Intellectual Disability

Eloi Mercier[1], Paul Pavlidis[1], Jesse Gillis[1], Evica Separovic[1], Ying Qiao[1]

[1]Center for High-Throughput Biology, University of British Columbia

Intellectual Disability (ID) is a congenital disorder with a prevalence of 2~3% in the general population. Subjects with ID show limitations in mental function, communication and social skills. It is believed that genetic abnormalities, specifically copy number variant (CNV), are a major cause of ID and CNV detection using array CGH is increasingly being used routinely in patients with ID. In this study, we characterize CNVs found in ID individuals and aim to prioritize genes by disease-specificity within CNV regions. Our cohort is composed of 180 individuals with ID who are described by a set of 60 clinical features and have been screened for CNVs, resulting in a set of 1188 variants. Our preliminary results show that non-common CNVs are

overrepresented in subjects with ID compared to normal individuals. Moreover, we found that non-inherited CNVs are correlated with other major impairments such as dysmorphysm, neurological and muscular defects. We also found that non-common CNVs are preferentially located in telomeric regions and are disrupting more genes than common CNVs. In order to identify culprit genes in pathogenic CNVs, methods based on the "guilt by association" principle are often employed. Our group recently showed that these algorithms tend to prioritize genes according to the number of annotated functions they have. Although multifunctional genes might be important, we believe that genes with more specific functions are more likely better candidates for ID-causing genes. We are currently developing new methods controlling for the effect of multifunctionality.

2-C-42 Does an animal's environment affect outcome from experimental stroke? A systematic review

John Mielke[1], Christine Hawkes[2]

[1]University of Waterloo, [2]McMaster University

Background: Psychosocial environment can have a profound effect on health, and may influence recovery from disease and injury. The present study sought to determine whether the nature of an animal's environment after experimental stroke can affect outcome measures such as mortality, infarct size, and task performance. Methods: We completed a systematic review of studies wherein living conditions post-stroke were manipulated in a positive (i.e., environmental enrichment; EE), or negative (i.e., stress exposure) fashion. Medline and Scopus were searched and combined with manual searching through reference lists. Inclusion criteria involved: primary research studies using animal models of ischemic stroke, manipulation of post-insult environment, and publication in the English language. Collected studies were screened for methodological quality using modified STAIR criteria prior to data extraction. Results: 32 studies were examined during the initial screen, and outcome measures were grouped into behaviour and histology. 19 studies examined neurobehavioural performance: 13 of these showed enhanced performance with EE and 4 studies showed inferior performance with applied stress. 7 studies examined infarct size: 2 reported smaller infarct size after EE and 4 studies concluded infarct size was larger with applied stress. Conclusion: The data suggest EE after stroke is associated with greater functional recovery, but the effect on infarct size is unclear. In contrast, applied stress is associated with reduced functional recovery and increased infarct size.

2-C-43 The pathophysiology of Parkinson disease: LRRK2, neurotransmission and synaptic maintenance

Austen Milnerwood[1], Lucia Tapia[1], Mattia Volta[1], Patrick Chou[1], Carles Vilarino-Guell[1], Heather Melrose[2], Lynn A Raymond[1], Matthew J Farrer[1]

[1]University of British Columbia, [2]Mayo Clinic

Point mutations in Leucine-Rich Repeat kinase 2 (LRRK2) are the most common cause of inherited Parkinson's disease (PD). Despite many recent advances, our current understanding of the physiological function of LRRK2, and the perturbations to LRRK2 function produced by PD mutations, is disappointingly inadequate. A greater understanding of LRRK2 biology and pathophysiology are essential to the development of therapeutic interventions aiming to prevent or delay progression of this devastating disease. Our group, and others, have developed a number of transgenic mouse models to investigate LRRK2. Although all models lack substantial neuropathology, they do exhibit commonalities with each other and PD, including alterations to dopamine transmission and cognitive/motor dysfunction. In order to further investigate LRRK2 we have taken a multi-modal approach to LRRK2 biology. Ongoing comparative electrophysiological investigation in acute brain slices from LRRK2 overexpressing, knock-out and mutant LRRK2 mice has revealed early synaptic dysfunction in medium spiny neurones (MSNs) of the corpus striatum (the target structure of nigral dopamine projections) prior to detection of a dopamine phenotype. Whereas LRRK2 overexpressing mice exhibit decreased spontaneous excitatory post-synaptic current (EPSC) frequencies with respect to their littermates, LRRK2 KO mice appear to display the opposite phenomenon, suggesting a role for LRRK2 in synaptic maintenance and function.

2-C-44 Selective overexpression of androgen receptor within motoneurons produces motor deficits in transgenic mice

Ashley Monks[1], Katherine Halievski[1], Michael McPhail[1], Firyal Ramzan[1], Pengcheng Rao[1]

[1]University of Toronto

Androgens contribute to health and disease of neuromuscular systems, but the cellular bases of androgen action on muscle and motoneuron remain poorly defined. Kennedy's Disease/Spinobulbar muscular atrophy (KD/SBMA) is an example of an androgen related neuromuscular disease. KD/SBMA is caused by trinucleotide expansion within the androgen receptor (AR) gene, resulting in an enlarged polyglutamine tract. KD/SBMA pathology is believed to result from primary pathology in motoneurons. In order to better understand the pathophysiology of KD/SBMA, we created several strains of transgenic mice which allow us to selectively

overexpress polyglutamine AR (AR113Q) in either myocytes or motoneurons. In addition to polyglutamine expanded Ar, we are also able to overexpress AR with a wild type number of glutamine repeats (AR24Q). AR113Q but not AR24Q mice exhibit motor deficits typical of mouse models of KD/SBMA when expressed globally and selective overexpression of AR113Q in motoneurons reproduces some of these gross motor deficits. Interestingly, motor tasks which are sensitive to muscular endurance (hang test, constant speed rotorod) are affected but grip strength, which is sensitive to peak force generation, is not. These results are consistent with primary pathology of AR in producing some features of KD/SBMA pathology, although it is unclear that polyglutamine expansions per se is responsible.

2-C-45 Connexin43 in reactive astrocytes promotes glioma invasion

Christian Naus[1], Wun-Chey Sin[1], Qurratulain Aftab[1], John Bechberger[1], Justin Leung[1], Helen Chen[1]

[1]University of British Columbia

The interaction of tumor cells with its microenvironment has a pivotal role in determining the extent of glioma invasion into brain parenchyma. In this study, we examined whether intercellular interaction mediated by the connexin family of proteins is critical in this process. We focused on the role of connexin43 (Cx43) that is widely expressed in astrocytes encountered by the invading glioma cells. In order to alter Cx43 levels in glioma and host cells in an immune-competent animal, we employed syngenic intracranial implantation of fluorescently-labeled GL261 glioma cells into C57BL/6 mice that were either wild type or conditional knockout. We observed the colocalization of the astrocyte maker GFAP and Cx43 protein directly surrounding the tumor mass. Single or small clusters of GL261 cells that broke away from the tumor core contributed to the uneven tumor-host interface, evidence of an invasive front. Such invasion was reduced when Cx43 was eliminated in Nestin-Cre:Cx43fl/fl mice where Cx43 was deleted from neural progenitors and astrocytes, and when glioma cells with knockdown Cx43 expression were introduced into wild type brains. Glioma invasion was not attenuated by expression of a channel-defective Cx43 mutant (T154A) in glioma cells. Our results demonstrate that glioma invasion into brain is supported by Cx43-mediated interactions between glioma and astrocytes not requiring intercellular communication. Supported by grants from CIHR to WCS and CCN. CCN holds a Canada Research Chair.

2-C-46 Age-dependent impairments in motor ability, but not in olfactory memory in the 5xFAD mouse model of Alzheimer's disease.

Timothy O'Leary[1], Richard E Brown[1]

[1]Dalhousie University

The double-transgenic 5xFAD mouse which carries mutant APP and PS-1 transgenes was developed as an animal model of Alzheimer's disease. However, this mouse also develops motor impairments which confound performance on many learning and memory tests. Therefore, this experiment examined age-related changes in locomotor activity (open-field), motor coordination and motor learning (rota-rod and balance beam), as well as learning and memory on a conditioned odor preference paradigm which was not confounded by impaired locomotion and motor ability. Male and female 5xFAD and wild-type control (C57xSJL) mice were tested at 3, 6, 9, 12 and 15 months of age in a cross-sectional experimental design. 5xFAD mice showed decreased rearing in the open-field at 9, 12 and 15 months of age and travelled shorter distances in the open-field at 12 and 15 months of age. 5xFAD mice fell from the rota-rod and balance beam sooner than wild-type mice at 9, 12 and 15 months of age and motor learning was impaired in 12 and 15-month-old 5xFAD mice on the rota-rod. No differences were found in olfactory learning and memory at any age tested. These results show that age-related changes in motor ability in the 5xFAD mice begin at 9 months of age and can be reliably detected with small sample sizes using different tests of motor ability. Therefore, it is important to dissociate motor and memory dysfunctions in the 5xFAD mice, and measures of motor ability may be useful therapeutic end-points for the assessment of disease modifying therapies in pre-clinical research using the 5xFAD mouse.

2-C-47 Expression analysis of spinal cord radial glia in the neonatal and adult reveals a neural progenitor cell character

Audrey Petit[1], Ashley D Sanders[1], Timothy E Kennedy[2], Wolfram Tetzlaff[3], Katie J Glattfelder[4], Rachel A Dalley[4], Ralph B Puchalski[4], Allan R Jones[4], Jane A Roskams[3]

[1]University of British Columbia, [2]Montreal Neurological Institute, McGill University, [3]ICORD University of British Columbia, [4]Allen Institute for Brain Science

The Allen Spinal Cord Atlas reports the expression of > 17,000 genes in both the juvenile and adult mouse spinal cord (SC) and serves as a resource to enhance our understanding of the SC's cellular heterogeneity. Using BLBP (FABP7) as a prototypical marker to demarcate the anatomical distribution of spinal cord radial glia (SCRG), we identified a subset of 122 genes expressed by cells anchored laterally at the SC margin and arrayed radially toward the central canal (CC) with a distinct radial glial morphology. Gene ontology analyses reveal that both

developing and adult SCRG express genes from pathways that regulate proliferation, differentiation and migration in neural stem cells. The unique genetic profile identified suggests these cells represent a discrete white matter radial glial population with expression coincident with a neural progenitor identity. This is supported by immunofluorescence and confocal analysis for radial glial proteins in the Blbp-GFP transgenic mice, confirming the existence of a progenitor population in adult SC white matter. Adult SCRG respond to both contusion and demyelination lesions by becoming highly proliferative and serving as a structural scaffold for migrating cells. Our results suggest that, in addition to the established progenitors of the CC, a population of non-ventricular radial glia persist in the adult SC and retain a progenitor phenotype. This analysis enhances our understanding of the in vivo potential of SCRG and reveals important pathways that may be critical for driving them to participate in repair.

2-C-48 The gap junction protein Connexin43 is a biomarker for astrogliosis in response to a stab wound lesion

Wun Sin[1], Wun Sin, Nicolas Theodoric, John Bechberger, Christian C Naus, Wun Chey Sin

The gap junction protein connexin43 (Cx43) is widely expressed in adult astrocytes and has been a key candidate in neuroprotection because of its enhanced expression in peri-lesion region. Reactive astrocytes are major component in astrogliosis and formation of the glial scar that is believed to isolate inflamed regions, preventing leakage of cytotoxic chemicals secreted by reactive microglia to surrounding healthy neurons. By allowing passage of small molecules between adjacent cells, the gap junction protein connexin43 (Cx43) has been proposed to facilitate removal of such cytotoxic substances from inflamed regions and therefore further promoting neuronal survival.To determine the temporal and spatial dynamics of glial-specific gap junction protein Cx43 in response to brain injury, we correlated the expression of Cx43 to astrocytic and microglial activation in a simple needle stab wound. To elucidate whether Cx43 plays a role in the observed glial response, we also analyzed the distribution of major glial cell types during injury in Cx43 conditional knockout (Cx43cKO) mice. A redistribution of Cx43 to the region of the needle site, corresponding to the increased presence of GFAP-positive reactive astrocytes in the lesion was only apparent from 6 dpi. This is in sharp contrast to the observed abscence of any increase in Cx30, another major astrocytic gap junction, at 6 dpi. Our results showed that the kinetics of Cx43 expression followed the temporal and spatial distribution of GFAP intermediate filament.

2-C-49 Gabapentinoid permeation of neuronal TRPV1 channels increases their effectiveness both in vitro and in vivo.

Peter Smith[1], James E Biggs[1], Patrick L Stemkowski[1], Nataliya Bukhanova[1], Klaus Ballanyi[1], Naresh Kumar[1]

[1]University of Alberta

The use of gabapentin (GBP) and pregabalin (PGB) in management of neuropathic pain is occasionally associated with unwanted somnolence. It was recently shown that local anaesthetics can pass through TRPV1 channels following their activation by capsaicin. Our unpublished data suggest that this may also be a viable route for facilitating the access of gabapentinoids to their target protein; the á2ä subunit of voltage-gated Ca2 channels. To test whether capsaicin can increase gabapentinoid effectiveness, we exposed organotypic cultures of rat spinal cord to GBP or PGB for 5 days and examined changes in stimulation-evoked Ca2 signals as an index of overall dorsal horn excitability. Whereas 100ìM GBP or 1ìM PGB (5-6 days) significantly reduced amplitude or area under the Ca2 signal following electrical stimulation, 10ìM GBP was only effective in the presence of 100nM capsaicin (1h exposure every 2 days). Application of BCH, an amino acid transport inhibitor that blocks GBP entry into neurons, did not reverse effects of GBP in the presence of capsaicin, as would be expected if GBP enters via TRPV1 channels. Preliminary in vivo experiments showed that GBP (30mg/kg, IP, every 12h for 5d) increased paw withdrawal threshold following sciatic chronic constriction injury and its effectiveness was increased by intraplantar capsaicin injections (0.1ìg every 48h for 5d). Potential improvements in the delivery of gabapentinoids to TRPV1 expressing neurons may lead to improved pharmacological management of neuropathic pain and limitation of their side effect profile.

2-C-50 Electrophysiological properties of dystrophin-deficient Purkinje neurons in the mouse cerebellum

Wanda Snow[1], Judy E Anderson[1], Mark Fry[1]

[1]University of Manitoba

Dystrophin protein, abundant in muscle, also resides in specific neurons, including cerebellar Purkinje neurons. Its absence causes Duchenne muscular dystrophy (DMD) in humans, characterized by muscle wasting and cognitive deficits. At present, the neurophysiological effects of dystrophin deficiency are poorly understood. Evidence suggests dystrophin and its complex of associated proteins play a role in ion channel and receptor maintenance. Specifically, GABA receptor clustering is reduced in Purkinje neurons of the genetic homologue of DMD, the mdx mouse, as is GABA-mediated current. As dystrophin deficiency results in altered ligand-gated ion channel function, and basal electrophysiological characteristics of

neurons are dictated by ion channel function, this research examined intrinsic electrophysiological properties of mdx Purkinje neurons. In mdx and wild-type mice, Purkinje neurons from the vermal and lateral cerebellum were acutely dissociated, removing the dendritic tree. Purkinje neuron somata were subjected to whole-cell patch clamping to examine action potential threshold, resting membrane potential, firing frequency, input resistance, and amplitude. Preliminary data reveal similar firing thresholds (p= 0.85) between vermal mdx (mean membrane potential = -49.85 ± 1.25 mV; n=13) and wild-type (mean membrane potential= -49.43 mV ± 1.74; n=15) Purkinje neurons. Collectively, this research is expected to offer insights into the degree of neuronal dysregulation associated with DMD and may have broader implications for understanding the cerebellum's role in cognition.

2-C-51 Epigenetics of depression: MeCP2 as a novel mechanism for decreased reelin expression in an animal model of depression

Erin Sterner[1], Hector J Caruncho[2], Lisa E Kalynchuk[1]

[1]University of Saskatchewan, [2]University of Santiago de Compostela

Although little is known about the neurobiological causes of depression, there is a strong link between stress and the emergence of depressive symptomatology. Repeated stress is characterized by dendritic atrophy, cell death, and a reduction of molecules important for facilitating synaptic growth. Reelin is a glycoprotein that plays a key role in cell survival, and is implicated in a number of neuropsychiatric disorders. Importantly, reduced reelin expression is associated with increased depressive-like behavior and decreased neuronal maturation. Although the mechanisms responsible for decreased reelin expression are not well understood, recent evidence suggests that epigenetic regulation of the reelin gene may play a role. Specifically, the methyl binding protein MeCP2 is known to form a repressor complex with methylation enzymes at the reelin gene promoter, silencing its expression. To evaluate whether this mechanism is at play in our chronic stress model, we administered vehicle or the stress hormone corticosterone (CORT; 40mg/kg) for 21 days to naïve male rats. Immunohistochemistry revealed that animals treated with CORT had significantly fewer reelin positive cells in the hilus and subgranular zone of the hippocampus, while MeCP2 was elevated in these regions. Although more work is needed to determine the methylation status of the reelin gene promoter, the fact that we find increased MeCP2 in the same places we see decreases in reelin suggests that chronic stress recruits methyl donors to silence the expression of genes important for regulating synaptic plasticity.

2-C-52 HIP14-like deficient mice develop some neuroanatomical and motor deficits associated with Huntington disease

Liza Sutton[1], Roshni Singaraja[1], Shaun Sanders[1], Stefanie Butland[1], Sonia Franciosi[1], Fiona Young[1], Amanda Spreeuw[1], Crystal Doty[1], Michael Hayden[1]

[1]University of British Columbia

Huntingtin-interacting protein 14-like (HIP14L) belongs to the mammalian DHHC-containing palmitoyl acyltransferase (PAT) family, which catalyses palmitoylation: the dynamic post-translational addition of the lipid palmitate to proteins, to alter their function and localisation. Dysregulated palmitoylation is linked to a number of neuropsychiatric disorders, including mental retardation, schizophrenia, infantile and adult-onset forms of neuronal ceroid lipofuscinosis and Huntington disease (HD). HIP14L is structurally and functionally similar to the neuronal PAT, HIP14, which has been implicated in HD, a disease caused by a polyglutamine expansion in the huntingtin protein. Here we show that HIP14L interacts less with the mutant huntingtin protein and that mice deficient in Hip14l develop some features of HD, namely, adult-onset neuroanatomical deficits, hypoactivity and neuronal protein palmitoylation deficits. Thus HIP14L has an important role in the central nervous system, and in addition to HIP14, may also play a role in the pathogenesis of HD.

2-C-53 Expression of purinergic receptors during acute retinal degeneration induced by mechanical trauma.

Vivian Weber[1], Vera Paschon[1], Otavio A Casado[1], Guilherme S Higa[1], Alexandre H Kihara[1]

[1]Universidade Federal do ABC

ATP is a signaling molecule that can be released into the synaptic cleft, usually with other neurotransmitters or as a result of injury. The purinergic receptors that can be activated in the presence of ATP are divided into two groups: P2X (ionotropic receptors) and P2Y(metabotropic receptors). There is growing interest in studying the effects of these receptors in the central nervous system (CNS), in both development-related events and also in neurodegeneration. The aim of this study was to access the spatio- temporal expression of P2Y1 and P2X7 after mechanical and located trauma in the retina, a model that allows visualization of the focus, penumbra and adjacent areas. For this purpose, we used combined techniques such as TUNEL, real-time PCR and immunohistochemistry. Using TUNEL technique, cell death was characterized by apoptosis in the degeneration model in different post-lesion time points. We observed a particularly large number of cells exhibiting TUNEL-positive nucleiin 1-day lesioned retinas. In PCR analysis, we observed a significant up regulation of P2X7 gene expression after 2 hours elapsed from injury (1,84-fold, p<0,05). P2X7 e P2Y1

immunoreactivity was observed in outer and inner plexiform layers, in both lesioned and control retinas, revealing the presence of these receptors in the lesion focus and penumbra areas. With this study we intend to elucidate the participation of purinergic receptors in neurodegeneration and to contribute of new strategies for neuroprotection.

2-C-54 Functional analysis of MeCP2 isoforms in chromatin organization

Robby Zachariah[1], Mojgan Rastegar[1]

[1]University of Manitoba

Methyl CpG Binding Protein 2 (MECP2) is a transcriptional regulator capable of recognizing and binding to methylated DNA. MeCP2 is known to be involved in a variety of cellular functions including chromatin compaction, RNA splicing, chromatin loop formation etc. Mutations in the MECP2 gene are most frequently associated with Rett Syndrome (RTT) patients and have also been detected in patients with other neuronal disorders. RTT is a progressive neurological disease affecting young females and occurs with an incidence of 1 in 10,000 live births. Alternative splicing of MECP2 leads to the formation of two isoforms, MECP2E1 and MECP2E2. The distinct expression pattern of the two isoforms in the developing brain as well as the cellular dysfunctions associated with known mutations in Mecp2 strongly suggests that the two isoforms have non-redundant functions. In the present study, by overexpressing individual MeCP2 isoforms, we have undertaken a comparative functional analysis of both isoforms in heterochromatic organization and subcellular compartmentalization. It is believed that the identification of molecular functions of the MeCP2 isoforms will further our knowledge on the contribution of MECP2 mutations towards the pathology of Rett Syndrome and other neuronal disorders.

2-C-55 Lentiviral-mediated upregulation of progranulin in ameliorating targeted neuronal loss in a genetic model of amyotrophic lateral sclerosis

Pierre Zwiegers[1], Grace Lee[1], Chris A Shaw[1]

[1]University of British Columbia

Progranulin (PGRN) actions in the context of neurodegenerative disease have become an area of intense interest as the growth factor has been implicated to be crucially important in the long-term survival of neuronal cells. PGRN is derived from 13 exons and consists of 7.5 tandem repeats of a conserved 12-cysteinyl granulin motif. Secreted as a 90 kDa protein, post-transcriptional cleavage yields several 6-kDa peptides which exert a multitude of effects on cellular physiology. PARA In vitro studies have shown that PGRN can act to prevent neuronal cell death following the exposure to toxins or other stressors. In vivo studies using animal models of Parkinson's- and Alzheimer's disease have demonstrated a reduction in neuronal loss or other indices of neurodegeneration (e.g. Van Kampen & Kay, 2011). These outcomes have led to speculation that PGRN upregulation might also serve to protect motor neurons in animal models of amyotrophic lateral sclerosis (ALS). PARA Preliminary work done by our group posits that a putative neuroprotective effect stemming from exogenously upregulated PGRN gene expression may exist. Bilateral administration of a lentiviral vector containing PGRN cDNA into the gastrocnemius muscles of the G37R mSOD variant prior to phenotypic onset showed a marked increase in the number of surviving lumbar motor neurons. Current work is assessing the efficacy of PGRN upregulation at an earlier intervention timepoint. PARA Successful outcomes to these experiments would have major implications for ALS therapy by providing a minimally invasive means for neuroprotection

D – Sensory and Motor Systems

2-D-89 Requirement of neuronal connexin36 in processes mediating presynaptic inhibition of low threshold afferents in functionally mature motor systems of mouse spinal cord

Wendy Bautista[1], James I Nagy[1], David A McCrea[1]

[1]University of Manitoba

Electrical synapses formed by gap junctions containing connexin36 (Cx36) promote synchronous activity of interneurones in many regions ofmammalian brain, however, there is limited information on the role of electrical synapses in spinal neuronal networks. Here we show that Cx36 is widely distributed in the spinal cord and is involved in processes that govern presynaptic inhibition of primary afferent terminals. Electrophysiological recordings were made in spinal cord preparations from 8-11 day oldwild-type and Cx36 knockout mice. Several features of presynaptic inhibition evoked by conditioning stimulation of low threshold hindlimb afferents were severely compromised in Cx36 knockout mice. Dorsal root potentials (DRPs) evoked by low intensity stimulation of sensory afferents were reduced in amplitude by 79% and in duration by 67% in cords from Cx36 knockouts. DRPs were similarly affected in wild-types by bath application of gap junction blockers. Consistent with presynaptic inhibition of group Ia muscle spindle afferent terminals onmotoneurones described in adult cats, conditioning stimulation of an adjacent dorsal root evoked a long duration inhibition of monosynaptic ventral root reflexes in wild-type mice, and this inhibition was antagonized by bicuculline. The same conditioning stimulation failed to inhibit monosynaptic reflexes in Cx36 knockout mice. In laminae V-VI, where interneurones involved in presynaptic inhibition of large diameter muscle afferents are located,interneurones

showed to be extensively dye-coupled and display Cx36-positive puncta.

2-D-90 Topographic distribution and ultrastructural features of the serotonin innervation of primate globus pallidus

Lara Eid[1], Sarah Petryszyn[1], André Parent[1], Martin Parent[1]

[1]Laval University

The primate basal ganglia display a highly heterogeneous serotonin (5-HT) innervation arising essentially from the dorsal raphe nucleus located in the brainstem. The main purpose of this light and electron microscopic study was to characterize and compare the 5-HT innervation of the internal (GPi) and external (GPe) pallidal segments in the squirrel monkey (Saimiri sciureus) following labeling with an antibody against the 5-HT transporter (SERT). Unbiased counts of SERT+ axon varicosities revealed similar density of innervation in the GPi (0.57 million of varicosities/cubic mm) and the GPe (0.60 million), the anterior half of both segments being more densely innervated than their posterior half. At the electron microscopic level, SERT+ varicosities appeared comparable in size and vesicular content. Synaptic incidence was relatively low for the GPi (29%) and the GPe (21%), indicating that synaptic and diffuse 5-HT transmission might occur in the primate pallidum. Symmetrical and asymmetrical synaptic junctions were observed in almost equivalent proportion and these synapses were exclusively found on dendrites. Altogether, our morphological findings indicate that the dorsal raphe nucleus may exert strong influences through both synaptic delivery and diffusion of serotonin in the GPi, the main output structure of the basal ganglia, as well as in the GPe, a key integrative component of the basal ganglia. This 5-HT midbrain input may play an important role in the pathogenesis of motor disorders that result from a malfunction of the basal ganglia microcircuitry.

2-D-91 Molecular determinants of TRPV1 channel assembly and trafficking

Robyn Flynn[1], Tamia K Lapointe[1], Christophe Altier[1]

[1]University of Calgary

Transient receptor potential vanilloid 1 channels (TRPV1) are non-selective cation channels that detect and integrate thermal and chemical stimuli and thus play a key role in initiating and maintaining inflammatory pain. TRPV1 channels are composed of four subunits surrounding a central pore; each subunit contains six transmembrane domains flanked by long intracellular N- and C-terminal extensions. To function as pain receptors, TRPV1 subunits must assemble into tetramers, a process dependent on the C-terminal extensions. The C-terminal region contains a highly conserved TRP domain, comprised of a 25-residue coiled-coil, as well as an area of helices and beta strands containing the heat sensor, a calmodulin-binding motif, and a lipid-binding domain. Previous studies have not agreed on the specific region of the C-terminus involved in TRPV1 tetramer assembly; both the TRP domain and a downstream helix (the Tetrameric Assembly Domain, TAD) have been implicated. To narrow down the specific motif of subunit assembly, we created a series of C-terminally truncated TRPV1 proteins. We used a bimolecular fluorescence complementation (BiFC) assay coupled to a bioluminescence resonance energy transfer (BRET) method to analyze the assembly of these shortened TRPV1 mutants. Our results indicate that a region of 18 residues between the coiled-coil domain and the TAD is responsible for TRPV1 subunit assembly. Identification of the TRPV1 subunit interaction domain will lead to the ability to disrupt subunit assembly and thus attenuate channel function.

2-D-92 The dorsal raphe nucleus ascending projections: A dual and widely distributed neuronal system that target independently motor and limbic forebrain structures

Dave Gagnon[1], Maria Gabriela Sanchez[1], Martin Parent[1]

[1]Laval University

This morphological study aimed at providing the first detailed description of single-axon projections arising from the dorsal raphe nucleus (DRN) in rats. We used electrophysiological guidance to microiontophoretically label DRN neurons with biotinylated dextran amine. These neurons were easily recognizable by their highly regular (2-3 Hz) spontaneous firing patterns. Somatodendritic domains and axon projections of labeled neurons were reconstructed individually in three dimensions from serial sagittal sections using a computerized image analysis system. Typically, DRN neurons have a medium size cell body (15-20 µm) from which emerge 3-5 primary dendrites, mainly oriented along the anteroposterior axis. Multiple axonal branching patterns were observed and DRN neurons could be classified into motor or limbic type, based on their axonal target sites. Motor neurons innervate the substantia nigra, the dorsal striatum, the subthalamic nucleus, the frontal cortex and the pallidum whereas limbic neurons arborize in the nucleus accumbens, the lateral hypothalamic area, the habenula, the lateral septum, the amygdala, the bed of the stria terminalis and the prefrontal cortex. Both neuronal types display different axon morphology depending on their target sites. Our results provide the first evidence that the DRN ascending projections are composed of two parallel and widely distributed neuronal systems that target independently motor or limbic structures. These novel findings should be taken into account to reach a better understanding of the functional organization of the DRN.

2-D-93 Modification of tibial nerve evoked cutaneous reflexes on the basis of vision during walking

Dan Marigold[1], Casey R Ruff[1], Mona L Delva[1]

[1]Simon Fraser University

Vision contributes to our ability to adapt to upcoming changes in ground terrain that may affect stability during walking. Thus, the nervous system may utilize visual information to predicatively modify other sensory input, such as cutaneous feedback from the foot, depending on the nature of the terrain. In this study, cutaneous reflexes in six university-aged adults were evoked by electrical stimulation of the tibial nerve (supplying the plantar foot surface) in different phases of the gait cycle while walking on uniform ground and across a horizontal ladder. The ladder required subjects to step precisely on a series of 4, 9cm wide rungs. We recorded muscle activity from tibialis anterior (TA) and medial gastrocnemius (MG). Reflex responses were separated into 10 equal bins; average amplitude within each bin was compared across walking conditions using a Wilcoxon Signed Rank test. We found phase-dependent modulation of cutaneous reflexes across the gait cycle in both muscles and conditions. In ipsilateral TA we noted significantly increased amplitude while on the ladder in mid-swing ($p<0.03$), and a trend towards decreased amplitude on the ladder at heel strike and in early stance ($p=0.06$-0.10). In contrast, reflex amplitude in ipsilateral MG was significantly decreased while on the ladder in mid swing ($p<0.03$), and there was a trend towards increased amplitude in early swing and stance ($p=0.09$). These results suggest that cutaneous input from the foot is modified on the basis of visual information in a phase-dependent manner. This work was supported by NSERC.

2-D-94 Functional clustering drives encoding improvement in the awake brain

Kaspar Podgorski[1], Derek Dunfield[2], Kurt Haas[1]

[1]University of British Columbia, [2]Massachusetts Institute of Technology

Sensory experience drives dramatic structural and functional plasticity in developing neurons. However, for single-neuron plasticity to optimally improve whole-network encoding of sensory information, changes must be coordinated between neurons to ensure a full range of stimuli is efficiently represented. Using two-photon calcium imaging to monitor evoked activity in over 100 neurons simultaneously, we investigate network-level changes in the developing Xenopus laevis tectum during visual training with motion stimuli. Training causes stimulus-specific changes in neuronal responses and interactions, resulting in improved population encoding. This plasticity is spatially structured, increasing tuning curve similarity and interactions among nearby neurons, and decreasing interactions among distant neurons. Training does not improve encoding by single clusters of similarly responding neurons, but improves encoding across clusters, indicating coordinated plasticity across the network. NMDA receptor blockade prevents coordinated plasticity, reduces clustering, and abolishes whole-network encoding improvement. We conclude that NMDA receptors support experience-dependent network self-organization, allowing efficient population coding of a diverse range of stimuli.

2-D-95 Reference frames for visual and motor responses in the Frontal Eye Fields

Amirsaman Sajad[1], Xiaogang Yan[1], Gerald P Keith[1], Hongying Wang[1], J. Douglas Crawford[1]

[1]York University

It is known that neurons in the Frontal Eye Fields (FEF) show both visual and saccade-related responses. However, the activity of FEF neurons during head-unrestrained gaze shifts has not been systematically explored. Here, we recorded from FEF neurons in two head-unrestrained monkeys while performing a visually-guided memory delayed-saccade task (400-800ms delay), serving to dissociate visual and movement activities. Initial gaze positions were randomized within a central window to increase eye and head position variability, and relatively inaccurate gaze shifts were tolerated in order to spatially dissociate the movement goal (i.e., visual target) from the gaze movement. To date, the response field of 35 neurons with visual, movement, or both activity types have been analyzed by statistically comparing the coherence of their response fields in different spatial models (Keith et al. J. Neurosci. Meth. 2009). Most neurons had contralateral response fields with optimal coherence of activity in an eye-centered frame. Population analysis revealed that the visual activity of both visual (n=8) and visuomovement (n=16) neurons codes for the location of the visual target in eye-centered coordinates, whereas the movement activity of visuomovement and movement (n= 11) neurons codes for the metrics of the impending gaze shift in the eye-frame. Collectively, these results suggest that neurons within the FEF participate in the transformation of visual signals into commands for eye head gaze shifts in eye-centered coordinates.

2-D-96 Spatiotemporal profile of spinal plasticity following photothrombotic stroke of the sensorimotor cortex

Bernice Sist[1], Karim Fouad[1], Ian Winship[1]

[1]University of Alberta

Plasticity in areas distant to the stroke, such as the corticospinal tract (CST) of the spinal cord, is not well understood. This project aims to (i) define the effect of lesion size on spinal plasticity after focal stroke, (ii) examine expression profiles of trophic factors and

cytokines in the spinal cord, and (iii) examine contributions of contralesional and ipsilesional CST sprouting to functional recovery. Rats were trained on the Montoya Staircase task prior to partial or complete lesion of the forelimb sensorimotor cortex (FL-SMC) via photothrombotic stroke. Reaching was assessed 3, 7, 14, 21 and 28d after stroke, anterograde tracers were injected into ipsilesional and/or contralesional FL-SMC 6 wks after injury to evaluate collateral sprouting from the CST into spinal grey matter. In other animals quantitative immunoassays were used to evaluate cortical and spinal levels of BDNF, inflammatory cytokines, and growth-associated protein 43 (GAP-43, index of structural plasticity) at 3, 7, 14 and 28d post-stroke. Results showed distinct spatiotemporal profiles of axonal sprouting that were lesion-size dependent. Partial injury to FL-SMC significantly elevated GAP-43 expression in cervical spinal cord (CSC) peaking at 7d. After complete FL-SMC lesion, significantly higher GAP-43 levels were found in CSC and lumbar spinal cord peaking at 14d. CSC BDNF levels showed a significant but transient increase at 3d. Preliminary tracer data suggests that partial injury of the FL-SMC induced moderate sprouting of spared ipsilesional fibres that correlated with functional recovery.

2-D-97 Modified areal cartography in auditory cortex following early and late-onset deafness

Carmen Wong[1], Nicole Chabot[2], Melanie Kok[2], Stephen G Lomber[2]

[1]Cerebral Systems Laboratory, University of Western Ontario, [2]Brain and Mind Institute, University of Western Ontario

Cross modal plasticity following deafness enables deaf auditory cortex to provide enhanced abilities to remaining sensory systems. This reorganization has been shown in cat auditory cortex following early onset deafness in recording and psychophysical studies. However, little information is available concerning structural compensations underlying these functional adaptations. We hypothesized that areal cartography in auditory cortex is modified following early onset deafness (ED, n=5). As a control, we also examined mature hearing cats (n=5) and cats with late onset (LD, >6M, n=5) deafness. Cats were deafened postnatally before hearing onset or in adulthood. Cerebral cytoarchitecture was revealed using SMI-32, a monoclonal antibody that stains neurofilaments and is used to distinguish auditory and visual areas in many species. Areas of auditory cortex were delineated in coronal sections and their volumes measured. The data show: 1) Staining profiles observed in hearing cats are conserved in both ED and LD cats. 2) In both ED and LD cats there was a reduction in primary auditory cortex (A1) volume. 3) In ED cats, the anterior auditory field (AAF) and ventral auditory field volumes were greater than hearing controls. 4) In LD cats, the second auditory cortex showed vast volume expansion. Overall, borders among dorsal areas (AAF, dorsal zone) and adjacent visual areas were shifted ventrally in ED cats. This may reflect a reduction in A1 volume and/or expanded visual cortex. These results show that cortical plasticity results in different areal cartography following deafness.

E – Homeostatic and Neuroendoctrine Systems

2-E-75 GnRH and GnIH immunoreactivity within the rat hippocampus

Jennifer Ferris[1], Maric T Tse[1], Dwayne K Hamson[1], Nicolette L McGuire[1], Liisa A Galea[1], Stan B Floresco[1], Kiran K Soma[1]

[1]University of British Columbia

Gonadotropin-Releasing Hormone (GnRH) is a neuropeptide secreted by the hypothalamus that controls reproductive behaviour via the hypothalamic-pituitary-gonadal (HPG) axis. GnRH receptors are abundant in the pituitary of male rats, but are also expressed in the hippocampus, suggesting that GnRH regulates hippocampal function. In addition, the recently discovered Gonadotropin-Inhibitory Hormone (GnIH), which inhibits pituitary gonadotropin secretion, was detected in the hippocampus of birds. Here, we examined GnRH and GnIH immunoreactivity in the adult rat hippocampus. Immunohistochemistry revealed GnRH1 and GnRH2 fibers within CA1 and CA3 of the hippocampus, as well as near cerebral ventricles in both male and female rats. GnIH immunoreactivity was also present in the hippocampus, with particularly dense distribution within the dentate gyrus. Possible sex differences in GnRH and GnIH immunoreactivity will be examined. Future studies will examine the possible roles of GnRH and GnIH in hippocampal function.

2-E-76 Insulin modulates the electrical activity of subfornical organ neurons

Mark Fry[1], Suman Lakhi, Wanda Snow

[1]University of Manitoba

Hypothalamic nuclei are well-known targets of insulin in the regulation of energy balance, however insulin must be transported across the blood brain barrier (BBB) to access these areas. In contrast, neurons of the subfornical organ (SFO), a sensory circumventricular organ, are not protected by the blood brain barrier and are in direct contact with circulating insulin. SFO neurons are well-known to detect and respond to circulating satiety signals such as leptin, ghrelin and amylin with changes in electrical activity, but whether they demonstrate the same sensitivity to insulin is unknown. Using whole-cell current clamp techniques, we examined the electrophysiological effects of 100nM insulin on rat SFO neurons maintained in culture. Application of insulin to SFO neurons hyperpolarized 33% of neurons tested (n=9/27; mean change in membrane

potential of -8.67 ± 1.7 mV) and depolarized 37% of neurons tested (n=10/27; mean change of membrane potential of 10.5 ± 2.8 mV). Insulin modulated at least two ion currents in SFO neurons, an inward rectifier K+ current and a non-selective cation current. The hyperpolarization arose from the opening of KATP channels, whereas depolarization was caused by the opening of Ih channels. These data indicate that the SFO is a key location for detection of circulating insulin.

2-E-77 Luman/CREB3 recruitment factor (LRF or CREBRF) is a repressor of the glucocorticoid receptor and a key attenuator of the hypothalamic-pituitary-adrenal axis

Ray Lu[1], Amanda C Martyn[1], Elena Choleris[1], Daniel J Gillis[1], John N Armstrong[1], Patricia V Turner[1], Genqing Liang[1], Kimberly Cai[1]

[1]University of Guelph

LRF/CREBRF was identified as an inhibitor of Luman/CREB3, is a transcription factor involved in the unfolded protein response. LRF sequesters Luman to distinctive subnuclear domains and promotes Luman protein degradation. We established the first LRF gene knockout mouse model and found a severe maternal behavioral defect in LRF-/- females -- 80% of the litters died within 24 hrs due to neglect or infanticide while most pups survived if cross-fostered. The hypothalamic-pituitary-adrenal (HPA) axis is a key animal stress system. The activation of HPA axis by stress leads to the release of glucocorticoids, which in turn represses the HPA through a negative feedback. The HPA axis is known to be attenuated at parturition to prevent detrimental effects (such as inhibition of lactation and maternal responsiveness) that may be caused by the glucocorticoid-meditated stress responses. The glucocorticoid receptor (GR) signaling was markedly augmented in these mice while prolactin levels were significantly repressed in lactating LRF-/- dams. We found that LRF colocalizes with the ligand-activated GR and the known GR repressor, NRIP1/RIP140, and that LRF inhibits transcriptional activity of GR and promotes degradation of the GR protein. Furthermore, supplementation of prolactin or administration of GR antagonist RU486 restored maternal responses in the LRF-/- females. Based on these results, we postulate that LRF is essential for the development of maternal behaviour, and plays a critical role in the attenuation of HPA axis and repression of glucocorticoid signaling.

2-E-78 Reduced synaptic melanocortin effects in the PVN of obese rats may underlie resistance to weight loss

Dirk Luchtman[1], Nina Pronchuk[1], Vickie E Baracos[1], William F Colmers[1]

[1]University of Alberta

Obese individuals can rarely maintain stable reductions in body weight, for poorly-understood reasons. We have previously shown that anorexigenic melanocortin peptides and orexigenic neuropeptide Y (NPY) may regulate energy intake by modulating synaptic GABA release at parvocellular neuroendocrine (NE) cells in the paraventricular nucleus (PVN) of the hypothalamus. Here, we tested whether the response of this GABA synapse to feeding related peptides is altered in obese rats. PARA Weight-matched, male littermates of outbred Sprague-Dawley (S-D) rats and a S-D substrain inbred for vulnerability to diet-induced obesity (DIO) to prolonged (10 weeks) exposure to a high-energy diet (HED) or chow for 10 weeks; all were then fed chow for 3 more weeks. HED-fed animals that gained excess body weight compared to chow-fed littermates and maintained high body weight during chow challenge were considered DIO. Rats were then decapitated and coronal slices of the PVN prepared for electrophysiological recordings from NE cells. PARA The melanocortin agonist MTII potentiated evoked GABA responses in NE cells, but this effect was significantly reduced in both inbred and outbred DIO littermates. By contrast, NPY inhibited evoked IPSCs but this response was similar between groups. Interestingly, loss of melanocortin responsiveness was highly and significantly correlated to excess weight gain in HED-fed rats. In conclusion, plasticity in the melanocortin-mediated enhancement of GABA synaptic responses in the PVN may underlie resistance to weight loss in obese individuals.

2-E-79 Hydrogen sulfide acts in the paraventricular nucleus to increase blood pressure

Pauline Smith[1], Alastair V Ferguson[1]

[1]Queen's University

Hydrogen sulfide acts as an endogenous gaseous transmitter that has been shown to act in the central nervous system (CNS) to influence cardiovascular function. Lateral ventricular administration of hydrogen sulfide has been shown to cause increases in blood pressure although the specific nucleus/nuclei responsible for mediating these effects have not been fully elucidated. The paraventricular nucleus (PVN) is an important autonomic region that has been shown to have a significant role in cardiovascular regulation. The present study was undertaken to examine the effects of microinjection of the hydrogen sulfide donor, sodium hydrogen sulfide (NaHS), into the PVN of urethane anesthetized male Sprague Dawley rats on blood pressure (BP) and heart rate (HR). Microinjection (1.0 µl) of NaHS (10 µM) caused rapid, short duration (<90 sec) increases in BP (mean area under the curve (AUC) = 475.4 ± 66.9 mmHg*sec, n=9) and HR (mean AUC = 19.5 ± 3.7 beats, n=9). The effects were shown to be site-specific as microinjection into non-PVN locations was without effect on BP (mean AUC = -106.2 ±71.4 mmHg*sec, n=7) or HR (mean AUC = 1.9 ± 3.8 beats, n=9). These observations identify the PVN as a CNS location at which hydrogen

sulfide may act to influence cardiovascular regulation. Funded by the CHIR. PMS is supported by a CIHR Banting and Best PhD fellowship.

2-E-80 Teneurin C-terminal associated peptide (TCAP) promotes the remodelling of dendritic spines in the hippocampus

Laura Tan[1], Susan Rotzinger[2], David A Lovejoy[3]

[1]The Salk Institute for Biological Studies, [2]University Health Network, [3]University of Toronto

The brain is a neuroplastic structure in which various stimuli can promote the remodelling of neural networks. For example, neurons in the hippocampus and amygdala can modulate their dendritic complexity in response to stimuli such as psychological stress or enrichment. Previous work has demonstrated that the novel neuropeptide, teneurin C-terminal associated peptide (TCAP), can modulate neurite outgrowth and complexity in vitro and upregulate cytoskeletal proteins such as α-actinin-4 and β-tubulin. Furthermore, TCAP has long-lasting behavioural effects which persist after the peptide has been cleared from the circulation, indicating that a lasting change must have occurred to account for these behaviours. 30 male Wistar rats were injected daily with i.c.v. TCAP (300 pmol/day) or saline (control). 24 h after the last injection, rat brains were obtained, Golgi-stained, and sectioned at a thickness of 100 µm. Individual neurons in the CA1 and CA3 regions of the dorsal hippocampus and in pyramidal-like cells in the BLA were photographed at 1000x magnification and analyzed to measure number of spines per 10 µm on the dendrites of the hippocampus and BLA. In the hippocampus, TCAP treatment increased spine density in both CA1 and CA3 neurons in all three regions measured; however, in the BLA, TCAP had no effect on spines in this region. These data suggest that TCAP possesses a role in remodelling of the hippocampus, but not the amygdala, and these morphological changes may explain long-term effects of TCAP in the modulation of anxiety-like behaviours.

2-E-81 Novel pathway for enhanced metabolic capacities underlies the neuroprotective actions of teneurin C-terminal associated peptide (TCAP)-1

Mei Xu[1], Dhan Chand[1], Tiffany Ng, Lifang Song[1], David A Lovejoy[1]

[1]University of Toronto

TCAP-1 is a member of a profoundly conserved family of peptides associated with the teneurin transmembrane proteins; it is highly neuroprotective against high alkaline, high calcium and low oxygen stressors. Upregulation of reactive oxygen species (ROS) scavenging systems such as superoxide dismutase, copper chaperone and catalase attribute to alkaline stress protection; alleviation from hypoxic stress was demonstrated through a reduction in HIF-1á expression. This study was aimed at understanding the cellular mechanisms by which TCAP-1 confers neuroprotection in vitro. Following chronic TCAP-1 treatments of 1nM, 10nM and 100nM, lactic acid concentrations were significantly reduced in a dose-dependent manner; conversely, ATP concentrations significantly increased. Interestingly, there was a significant decrease in ATP following acute treatments. Increases in ATP, in conjunction with decreased lactic acid, demonstrate that TCAP-1 mediated protection involves enhanced aerobic oxidation efficiency; this accounts for the seemingly contradictory findings of TCAP-1 rescue from hypoxic and oxidative stress. Higher energy returns per unit of oxygen would alleviate the stress of low oxygen tensions while increased mitochondrial activity would upregulate endogenous antioxidant production. The inherently large energetic cost of metabolic adaptation explains the acute ATP decline. In the absence of stress, optimized energy production could fuel the cytoskeletal observations of á-actinin-4 and â-tubulin upregulation following ERK1/2-dependant phosphorylation of stathmin and filamin a.

F – Cognition and Behaviour

2-F-61 Sensory-specific satiety in rats is influenced by repeated cycles of food restriction and binge feeding

Soyon Ahn[1], Kitty So[1], Anthony G Phillips[1]

[1]University of British Columbia

The relationship between food restriction and subsequent dysregulation of food intake is complex, variable and long-lasting. We investigated in rats whether repeated cycles of food restriction and binge feeding opportunities may alter regulation of food intake by employing a test for sensory-specific satiety. Rats that experienced repeated food restriction-binge cycles maintained heavier body weights compared to rats that remained on constant food restriction. In contrast to the control subjects, rats that alternated between food restriction and binge feeding failed to display sensory-specific satiety. During the first meal, there was a gradual decrease in the amount of food intake over a 40 min period. When presented with a second meal of the same food, these rats responded to the familiar food in a manner similar as to a novel food (i.e., comparable quantities of both types of food were consumed). Food restriction-binge feeding cycles may be considered as a form of stress, which in turn is associated with cross-sensitization to numerous behavioral responses. Therefore, we propose that stress-induced disruption of sensory-specific satiety reflects a sensitized response to food, in which the interaction between sensory and satiety factors are no longer the key regulators of food choice and meal cessation. Furthermore, a role for sensory-specific satiety in terminating food intake appeared to decline with the progression of the cycles, thereby contributing to a steady

increase in body weight of rats that experienced restriction with bouts of binge feeding opportunities.

2-F-62 Developmental decline in hippocampal neurogenesis: Transition from infantile amnesia to adult memory stability.

Katherine Akers[1], Alonso Martinez-Canabal[1], Anne L Wheeler[1], Leonardo A Restivo[1], Axel J Guskjolen[1], Sheena A Josselyn[1], Paul W Frankland[1]

[1]Hospital for Sick Children

Hippocampal neurogenesis declines steeply across development. Interestingly, this decline in neurogenesis is associated with an increased ability to form stable memories. That is, whereas infants have high rates of neurogenesis and form rapidly-decaying memories, adults have low rates of neurogenesis and form persistent memories. This inverse relationship between hippocampal neurogenesis and memory stability suggests that the addition of new neurons into hippocampal circuits may disrupt previously-formed memories. Here, we tested whether increasing neurogenesis in adults leads to forgetting, and whether decreasing neurogenesis in infants improves memory retention. In the first experiment, adult mice learned about a context and then remained sedentary or were given a running wheel for 4 weeks. We found that runner mice had more new hippocampal neurons but poorer memory for the context, indicating that increasing hippocampal neurogenesis in adults impairs previously-established memories. In the second experiment, infant mice learned about a context and then received treatment with temozolomide or vehicle for 4 weeks. We found that temozolomide-treated mice had fewer new hippocampal neurons but better memory for the context, indicating that decreasing hippocampal neurogenesis in infants enhances previously-established memories. These findings suggest that high rates of hippocampal neurogenesis may degrade pre-existing memories; therefore, the decline in hippocampal neurogenesis from infancy to adulthood may permit the formation of stable memories.

2-F-63 Site-specific disruption of conditioned fear behavior in kindled rodents

Justin Botterill[1], Axel J Guskjolen[2], Wendie N Marks[1], Lisa E Kalynchuk[1]

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

Kindling is a preparation that has been used extensively for studying epileptogenesis, behavioral comorbidities, and neural plasticity. Kindling involves brief electrical stimulation of a specific brain site. When administered daily, this preparation produces a progressive and permanent increase in seizure activity. An interesting feature of the kindling preparation is the presence of site-specific behavioral comorbidities. For instance, amygdala kindling increases anxiety and fearfulness, whereas hippocampal kindling impairs cognition. To study how aberrant neural plasticity can alter cognition and behavior, we conducted 100 kindling stimulations of the basolateral amygdala, dorsal hippocampus, and caudate nucleus. Rats then underwent trace fear conditioning, a hippocampal-dependent learning task that involves a short interval between the termination of conditioned (tone) and unconditioned (shock) stimulus. The results of our study revealed normal training for all groups; however, basolateral amygdala and dorsal hippocampal kindling significantly altered conditioned fear in a novel context tone test and when re-exposed to the training context, relative to sham control rats. Moreover, rats that received caudate kindling were not significantly different from sham controls on all measures. Our results suggest that limbic kindling significantly alters conditioned fear behavior, whereas non-limbic kindling does not. Kindling therefore provides an opportunity to investigate site-specific neural plasticity and its effects on cognition and behavior.

2-F-64 The impact of ageing on adult hippocampal neurogenesis, learning and memory

Patricia Brocardo[1], Joana Gil-Mohapel[1], Will Choquette[1], Russ Gothard[1], Jessica M Simpson[1], Brian R Christie[1]

[1]University of Victoria

The ability to generate new neurons continues in a few select regions of the adult mammalian brain, including the dentate gyrus of the hippocampus, and new hippocampal neurons are believed to contribute to hippocampal-dependent learning and memory. However, the rate of neurogenesis does not stay constant over time. We characterized how normal ageing affects not only the various stages of adult hippocampal neurogenesis, but also hippocampal-dependent spatial learning and memory in laboratory mice. Cell proliferation and neuronal differentiation significantly decreased with age, with an abrupt decline being evident in early adulthood (between 1.5 and 3 months). This is mirrored by a similar age-related decline in overall neurogenesis. A significant decrease in spatial learning and memory also occurred between 1.5 and 3 months, however performance was virtually unchanged thereafter. Concomitant with the reduction in neurogenesis was an age-related increase in hippocampal markers of oxidative stress. However, rather than starting early in adulthood, the changes in oxidative stress became more pronounced later on in life. As such, it is unlikely that oxidative stress contributes to the initial abrupt decline in neurogenesis. Overall, these results suggest that both hippocampal neurogenesis and learning and memory ability peak early in adulthood. However, the age-related decrease in the number of new neurons generated does not seem to impact the ability to learn and form new memories later on in life, indicating that the relationship between these two processes is non-linear.

2-F-65 microRNA-132 orchestrates chromatin remodeling and translational control of the SCN circadian clock

Hai-Ying Cheng[1], Matias Alvarez-Saavedra[2]

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

Mammalian circadian rhythms are synchronized to the external time by daily resetting of the suprachiasmatic nucleus (SCN) in response to light. As the master circadian pacemaker, the SCN coordinates the timing of diverse cellular oscillators in multiple tissues. The Ca(2)/cAMP response element-binding protein-regulated microRNA, miR-132, is induced by light within the SCN and attenuates its capacity to reset, or entrain, the clock. However, the specific targets that are regulated by miR-132 and underlie its effects on clock entrainment remained elusive until now. Here, we show that genes involved in chromatin remodeling (Mecp2, Ep300, Jarid1a) and translational control (Btg2, Paip2a) are direct targets of miR-132 in the mouse SCN. Coordinated regulation of these targets underlies miR-132-dependent modulation of Period gene expression and clock entrainment: the mPer1 and mPer2 promoters are bound to and transcriptionally activated by MeCP2, whereas PAIP2A and BTG2 suppress the translation of the PERIOD proteins by enhancing mRNA decay. We propose that miR-132 is selectively enriched for chromatin- and translation-associated target genes and is an orchestrator of chromatin remodeling and protein translation within the SCN clock, thereby fine-tuning clock entrainment.

2-F-66 Maternal immune activation impairs crossmodal and associative recognition memory in rat offspring

John Howland[1], Stephanie A Ballendine[1], Quentin Greba[1]

[1]University of Saskatchewan

The offspring of mothers who experienced an infection during pregnancy are more likely to develop psychiatric illnesses such as schizophrenia and autism once they reach adulthood. A rat model with high construct validity used to study this relationship involves administration of the viral mimetic polyinosinic-polycytidylic acid (PolyI:C) to pregnant dams. Given the importance of cognitive symptoms for schizophrenia, the present experiments examined the effects of prenatal PolyI:C treatment on crossmodal (tactile-to-visual) object recognition memory and object-in-place recognition memory using two spontaneous tasks. Pregnant Long-Evans rats were treated with either PolyI:C or saline on gestational day 15. PolyI:C induced weight loss in the dams for at least 48 h and a febrile response at 8 h. Behavioral testing of the offspring began in young adulthood on postnatal day 56. On the crossmodal object recognition memory test, animals treated with saline showed intact memory while animals treated with PolyI:C were impaired. Both groups of animals showed significant memory for the visual-only and tactile-only recognition tests. On the object-in-place memory task, animals treated with PolyI:C showed impaired memory while animals treated with saline were not impaired. Overall, the results demonstrate that prenatal exposure to PolyI:C impairs multisensory integration and associative memory abilities. Therefore, the PolyI:C model could be used to test novel treatment options for the cognitive deficits exhibited by human schizophrenia patients.

2-F-67 Cognition, depression and physical activity in aging

Feng Hua[1], Ruth F Santos-Galduroz[1], Marisete P Safons[2]

[1]Universidade Federal DO ABC, [2]Universidade De Brazilia

Background: Decline of cognitive capacity and depression in older people are considered a risk factor for dementia and can jeopardize their autonomy. Physical activity seems to produce positive effects on many cognitive processes, contributes reducing the suffering of the depression symptoms and allowing lower rates of depression recurrence. Objective: To evaluate the relation between physical practice and the performance in verbal fluency, verbal memory and depression scale. Methods: This was a cross-sectional study that assessed 32 elderly women (age=66,47 5,25), 16 sedentary group (SG) and 16 physical activity group (AG). The geriatric depression scale (GDS-15), animal test verbal fluency (Animal) and Rey Auditory Verbal Learning Test (RAVLT) were administered for all participants. Results: AG - age = 66,47 5,25, RAVLT = 42,12 7,18, GDS = 1,94 1,75 and Animal = 21,47 7,91. SG - age= 65,91 4,91, RAVLT = 35,94 9,84 , GDS = 3,78 3,70 e Animal = 15 3,32. Statistical analysis was performed using the Student's test t and the significance level was $p<0,05$. The data showed significant difference between SG and AG in depression symptoms $p=0,03$ and verbal fluency $p=0,04$. The difference in means were not significant in verbal memory $p=0,58$. Conclusion: These findings suggests that regular practice of physical exercise has relation with benefits in verbal fluency and depression symptoms, but in verbal memory this relation was not confirmed. The future perspective is to increase the sample and to adopt a longitudinal design.

2-F-68 Spike rate correlations vary by neuron response type during working memory in macaque prefrontal area 8A

Matthew Leavitt[1], Florian Pieper[2], Adam Sachs[3], Julio Martinez-Trujillo[1]

[1]McGill University, [2]University of Hamburg-Eppendorf, [3]Stanford University

We examined spike rate correlations between neurons in prefrontal area 8A of two macaca fascicularis during a spatial working memory task by recording the responses of multiple units using microelectrode arrays (Blackrock Inc.,

UT). The task consisted of a fixation period of 494-800ms, presentation of a circular sine wave grating at one of 16 randomly selected locations for 507ms, then extinguishing of the grating and a delay period of 494-2000ms. Finally, extinguishing of the central fixation point instructed the animals to make a saccade to the remembered stimulus location. We recorded neural activity in blocks of 32 channels and sorted spikes using Plexon software (Plexon Inc, TX). We isolated 191 single units for a total of 1170 neuronal pairs. Neurons were classified as selective (one-way ANOVA, $p<.05$) for visual stimuli (visual, n = 29, or 15%), saccades (motor, n = 22, or 12%), or both (visuomotor, n = 78, or 41%). In visual units the proportion of significant positive and negative correlations during the memory period were both significantly greater than expected by chance (positive = .22 and negative = .13, $p<0.05$). In motor and visuomotor units, only the proportion of significant positive correlations was significantly greater than chance (motor = .18, visuomotor = .17, $p<0.05$). Our results show that during working memory maintenance, visual, motor, and visuomotor units in area 8A exhibit different degrees of correlated firing. These different cell types may play different roles in the network dynamics underlying working memory maintenance by prefrontal neurons.

2-F-69 Acute stress disrupts both short- and long-term plasticity in the CA1-subiculum pathway in rats via glucocorticoid receptor activation

Matthew MacDougall[1], John G Howland

[1]University of Saskatchewan

The subiculum serves as the major output structure of the hippocampus. Exploring synaptic plasticity within this region is of great importance for understanding the dynamics of hippocampal circuitry as well as hippocampal-cortical interactions. Exposure to acute stress has been shown to dramatically alter forms of synaptic plasticity within the hippocampus proper. Using in vivo electrophysiological recordings in adult male Sprague-Dawley rats, we extend these findings and demonstrate that both short- and long-term forms of synaptic plasticity in the CA1-subiculum pathway are disrupted following exposure to 30 min of restraint stress. Robust paired-pulse facilitation is evident in control animals (38.31 ± 4.15%) but is significantly reduced after exposure to acute stress (11.76 ± 4.61%). Interestingly, two forms of long-term plasticity co-exist within the CA1-subiculum pathway following trains of low frequency stimulation (LFS; 21.30 ± 5.42%) and high frequency stimulation (HFS; 18.31 ± 5.21%) protocols. We provide evidence that both forms of long-term plasticity are similarly disrupted in rats exposed to acute stress (HFS; 2.42 ± 2.88% and LFS; -4.68 ± 8.34%) The deleterious effects on both short- and long-term form of plasticity appear to be mediated by glucocorticoid receptor (GR) activation as these disruptions can be block by pretreatment with the selective GR antagonist RU38486 (10 mg/kg; s.c.). The current results highlight the susceptibility of subicular plasticity to alterations caused by acute stressors and the role of GR activation underlying such alterations.

2-F-70 Increasing adult neurogenesis promotes forgetting

Alonso Martinez-Canabal[1], Katherine G Akers[1], Anne L Wheeler[1], Leonardo Restivo[1], Axel Guskjolen[1], Sheena A Josselyn[1], Paul W Frankland[1]

[1]Sickids Hospital

New neurons are continuously added to the subgranular zone of the hippocampus throughout the lifespan, but the functional consequences of adult neurogenesis remain unclear. While the majority of previous studies have examined the impact of increasing or decreasing neurogenesis on subsequent memory formation, few have examined the effects of similar manipulations on established, hippocampus-dependent memories. Importantly, computational models predict that addition of new neurons should lead to extensive remodeling of hippocampal circuits, and consequently degradation of established memories. Consistent with this prediction, here we show that increasing neurogenesis after training led to forgetting of contextual and spatial hippocampal memories. The degree of forgetting was strongly and negatively correlated levels of neurogenesis, consistent with the hypothesis that remodeling-induced changes in hippocampal circuits are causally related to memory loss. Furthermore, increasing neurogenesis before training had no effect on memory formation, indicating that increasing neurogenesis does not interfere with memory function in some non-specific manner. In the spatial learning paradigm, we found that subsequent reversal learning was facilitated. We therefore propose that the primary function of adult neurogenesis is to promote forgetting. Forgetting is likely adaptive as it reduces the likelihood of old memories interfering with new encoding, leading to more flexible, effective hippocampal memory function.

2-F-71 CREB in adult newborn neuron regulates memory formation

Yosuke Niibori[1], Sheena A Josselyn[1], Paul W Frankland[1]

[1]The Hospital for Sick Children

Globally reducing CREB function increases adult neurogenesis in subgranular zone (SGZ) of the hippocampus, a region that plays a central role in learning and memory. However, it is unclear whether these pro-neurogenic effects are mediated directly by neural progenitor cells in SGZ or via some indirect mechanism. To address this, we generated f/fCREB × nestin-CreERT2 (CREB-KO) mice in which tamoxyfen (TAM) treatment led to deletion of CREB in neural progenitor cells. In adult

mice, TAM treatment led to a striking, 3-fold increase in the number of immature neurons, suggesting that CREB negatively regulates early stages of adult neurogenesis. We next examined the consequences of this large increase in production of immature neurons in SGZ on hippocampal memory formation. First, we examined the effects on the generation of new memories. Eight weeks after TAM treatment, CREB-KO mice formed better contextual and spatial memories than littermate control mice, suggesting the addition of new neurons facilitated memory formation. Second, we examined the impact of increasing neurogenesis on established memories. TAM treatment impaired established memories in CREB-KO mice but not in control littermates, suggesting that the addition of new neurons after training interfered with memory persistence. These studies identify a key role of CREB in regulating neurogenesis, and suggest that large increases in neurogenesis may facilitate the generation of new memories, while at the same time degrading existing memories.

2-F-72 Alpha-7 nicotine receptor knock-out mice show sensory filtering deficits and cognitive alterations

Susanne Schmid[1], Erin Azzopardi[1], Marei Typlt[1], Bryan Jenkins[1]

[1]University of Western Ontario

Acetylcholine is an important neurotransmitter for learning and memory. Loss of cholinergic function can lead to profound memory and learning impairments. The goal of this study is to understand the role of the alpha-7 nicotinic acetylcholine receptor (nAChR7) in cognition. To do this, we attempted to characterize the cognitive and behavioural profile of nAChR7 knock out (KO) mice. We found that nAChR7 KO mice had deficits in sensory filtering using habituation and prepulse inhibition (PPI) of the acoustic startle response: KO mice had intact short-term habituation of startle (the decrease within a testing session), but impaired long-term habituation (the decrement across days of testing). KO mice also showed mildly impaired PPI compared to wild-type littermates. Systemic nicotine (1 mg/kg, subcutaneous) enhanced baseline startle amplitude and PPI in wild-type mice, but both effects were absent in KO animals. This suggests that the nAChR7 plays a role in sensory filtering, and that it also mediates nicotine-induced enhancement of PPI. Furthermore, we observed that the nAChR7 KO mice were more anxious than their wild-type counterparts, spending significantly more time along the walls in the locomotor box and in a covered arm during elevated plus maze testing. In the Barnes maze, KO mice performed as well as wild-types, and had normal short-term and long-term spatial memory. Future studies will seek to characterize if long-term habituation of emitted responses, i.e. exploratory behaviour in a locomotor box, also shows impairment.

2-F-73 Effects of prenatal stress flexible use of allocentric and egocentric memories: role of the hippocampal-prefrontal pathway

Angelica Torres Berrio[1], Yukiori Goto[1]

[1]McGill University

Stressful situations experienced during pregnancy affect the development of the fetal brain, and increase the risk of psychiatric disorders and cognitive deficits in adult offspring. Prenatal stress alters normal function of the hippocampus (HPC) and the prefrontal cortex (PFC), pivotal structures for declarative memory and executive functions, respectively. In general, the ventral HPC sends dense projections to the prelimbic and infralimbic areas of the PFC. Dysregulation in this pathway induces schizophrenia-like behavioral abnormalities in rats. However, the effects of prenatal stress on such HPC-PFC interaction and its function have not been elucidated. In experiment 1, we exposed pregnant rats to restraint stress between gestational days 14 and 21 and evaluated its effects on the flexible use of allocentric and egocentric spatial memories in adult offspring. We found that prenatal stress impaired allocentric memory and facilitated switching from allocentric to egocentric memory, but not switching from egocentric to allocentric learning. In experiment 2, we disconnected the HPC-PFC pathway by infusing tetrodotoxin (TTX) into the HPC and PFC in normal animals. We found that HPC-PFC disconnection enhanced strategy switching only from allocentric to egocentric learning. Bilateral inactivation of the HPC did not affect allocentric but impaired egocentric learning. Collectively, these results suggest that prenatal stress may affect HPC-PFC information processing that could, however, result in enhancement of flexible use of different modalities of memory.

2-F-74 Object and spatial information processing in hippocampal area CA1

Jennie Young[1], Amy M LeMessurier[1], Thomas J McHugh[2], Toshiaki Nakashiba[1], Susumu Tonegawa[1]

[1]Massachusetts Institute of Technology, [2]RIKEN Brain Science Institute

The hippocampus plays a key role in the acquisition of new memories for places and events. In rodents, the hippocampus is believed to provide a spatial framework within which items and events can be integrated to form a coherent representation of the animal's on-going experience. PARA Sensory information arrives in the hippocampus through two parallel processing streams: place-related information from medial entorhinal cortex (MEC) and object-related information from lateral entorhinal cortex (LEC). Area CA1, the most downstream hippocampal subregion, is unique in that MEC and LEC project to separate neuronal populations. This anatomical arrangement suggests that CA1 may receive integrated

space/object information from CA3 but segregated space/object information directly from the entorhinal cortex. PARA We carried out ensemble recordings in area CA1 of awake behaving mice as they performed an object-location recognition task. We used a transgenic mouse strain in which synaptic transmission from CA3 is conditionally blocked (CA3-TeTX) to determine how the convergence of CA3, MEC, and LEC inputs contribute to the processing of object and spatial information in CA1. We find that the formation of combined object-place representations during a one-trial learning task requires CA3 input to CA1, and we present physiological data that reflects the anatomical segregation of parallel processing streams to the hippocampus. Our results support the idea that the hippocampus integrates spatial and object-related information to form a conjunctive representation of the animal's environment

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

2-H-99 Scan your brain? Diverging perspectives on neuroimaging in mental health care

Judy Illes[1], Emily Borgelt, Daniel Buchman

[1]University of British Columbia

Background: Paralleling growing media coverage of neuroimaging research is pressure from sponsors to move basic research about brain function along the translational pathway. However, contention about the potential benefits - such as early intervention, improved compliance, and tailored treatment - and potential risks - such as misinterpretation of results - surrounds translational efforts for neuroimaging in mental healthcare. Largely overlooked in practice and policy debates are the impacts anticipated by end-user stakeholders. Methods: We synthesize primary research into stakeholder perspectives on neuroimaging in mental health care, including studies on researchers, mental health practitioners, adult patients with mood disorders, psychotic disorders, or OCD, and parents of children with ADHD. Findings: Stakeholder groups may be conceptually divided in terms of their relationship to neuroimaging technology. Providers of the technology, researchers and practitioners, view neuroimaging as a potential complement to validated diagnostic techniques, not a stand-alone technology. By contrast, recipients believe neuroimaging to capture objective truths about lived experience, and expect it to profoundly improve self-understanding, stigma, and access to resources. Conclusion: Stakeholder groups diverge in their understandings and expectations of neuroimaging. The integration of these previously orthogonal perspectives offers a clear view of approaching ethics and policy challenges for translational neuroimaging in mental health.

2-H-100 Incidental Findings in Neuroimaging for Mental Health Research

Judy Illes[1], Emily Borgelt[1], James A Anderson[1]

[1]University of British Columbia

Background: Although much clarity has been achieved concerning the ethics of incidental findings (IFs) in neuroimaging research in healthy adult subjects, the ethics of IFs in vulnerable populations - such as persons living with mental illness - have yet to be addressed. Methods: This qualitative study involved interviews with neuroimagers (N=10) and a complementary document analysis of relevant consent forms and study protocols (N=38). Interviews and documents were coded for the circumstances in which they return IFs, including information provided, as well as the timeline, mode of communication, and follow-up surrounding an IF discovery. Consent language about IFs was assessed using the Flesch-Kincaid Grade Level readability test. Results: In interviews, neuroimagers expressed some concerns about IFs specific to research involving mental health populations, such as the exacerbation of symptoms like anxiety. However, these were secondary to persistent concerns about IFs generally. Neuroimagers called for explicit recommendations about IFs, accessible resources, and training in communication. Two additional themes emerged from our analysis. First, we discovered divergence between neuroimagers' reported approach to IFs and the approach described in consent documents. Second, most consent documents lacked important details about the return of IFs. Conclusion: Neuroimagers working in the mental health context would benefit from the development of clear, standardized recommendations and resources for IFs, with priority on strategies for improved consent communication.

2-H-101 Teaching neuroscience at science camp through fractal analysis

Audrey Karperien[1]

[1]CSU

Neuroscience is an engaging topic for students of any age. Computers and multimedia presentation and teaching materials make many topics and even advanced methods in the field intriguing and accessible at many levels. Even mathematically based methods such as fractal analysis can be used for fun and engaging presentations that are easily modified for adult and youth presentations at venues ranging from community interest groups to science camps. One approach that we have found successful in raising awareness as well as teaching about neuroscience is employing participants as "lab assistants" and having them work with actual data to learn about a variety of topics including cellular morphology and signal processing.

2-H-102 This is your blog on drugs: ideas for painless writing assignments in large neuroscience courses

Nicole Vittoz[1]

[1]Kwantlen Polytechnic University

Large research institutions are often criticized for ineffective teaching, especially in the sciences. Students often describe their studying for science courses as "memorizing," suggesting they are not connecting the relevance of what they learn to real life or other areas of study. Part of this stems from the obvious practical challenges of large class sizes. First- and second-year neuroscience classes often contain 100 students or more, rendering writing assignments a challenging component to incorporate into a course. In addition, the lecture experience combined with objective exams lend themselves to passive forms of learning. How, then, do we integrate a more active, engaged student experience including writing assignments with meaningful feedback? One idea is the blog assignment. Ideally, your institution installs Wordpress on their own server and integrates it with the campus-wide login system, allowing instructors to create a blog for their course and invite students as authors on it. Students can be required to blog on real-life implications of specific course topics, using self-managed sign-up through a course management system. Instructor grading and/or random-assignment peer review according to a specific rubric can be effective, efficient tools for evaluation. In small classroom settings the same systems can be applied for collaborative group projects, in which each person adds an independent, creative contribution to a growing body of engaging, interactive blog posts. Allow (screened) public commentary on the blog, sit back and watch your students debate!

IBRO – International Brain Research Organization

2-IBRO-57 Prenatal exposure to tobacco extract containing nicotinic alkaloids produces morphological and behavioral changes in newborn rats

Hanane Khalki[1]*,* Loubna Khalki[1], Abderahmane Chait[1]

[1]University cadi ayyad, faculty of Sciences Semlalia Morocco

Tobacco exposure is not only a health concern for adults but has also been shown to exert deleterious effects on the health of the fetus, newborn, child, and adolescent. Decreased cognitive function, lower Intellectual Quotient (IQ) and deficits in learning and memory in children have been associated with maternal smoking during pregnancy. In this study, we have studied the effect of a tobacco plant extract on the growth and development in the rat. The extract contained relative proportions of alkaloids, including nicotine, purified by chemical separation.Pregnant rats received oral doses of either control (NaCl) or tobacco extract during the entire gestational period.Each day, the offspring were observed for the following physical parameters: hair growth, incisor eruption and eye opening. The day of appearance of these developments was recorded. Before weaning, the offspring were examined to test their cliff avoidance response, surface righting reflex, swimming development, negative geotaxis response. Administration of tobacco extract to dams during the entire gestation period affects behavior and development in pups.The observed effects were a delay in opening eyes, incisor eruption and hair appearance, behavioral developments and an alteration in the rate of success behavior. The results suggest that tobacco extract has a significant effect on the development of behavioral patterns, orientation and motor coordination and function. They also suggest significant growth retardation and teratogenic effects.

2-IBRO-58 New mechanisms involved in the neuroprotective actions of C-Phycocyanin and Phycocyanobilin in models of brain ischemia

Javier Marín Prida[1]*,* Nancy Pavón[2], Karina Stringhetta[3], Andreia M Leopoldino[3], Alexey Llópiz Arzuaga[4], Marcela Nunes Rosa[3], José L Liberato[3], Wagner Ferreira dos Santos[3], Eduardo Pentón Arias[4], Carlos Curti[3], Gilberto L Pardo Andreu[1], Giselle Pentón Rol[4]

[1]Institute for Pharmacy and Food, Havana University, [2]International Center for Neurological Restoration, [3]Faculty of Pharmacy of Ribeirão Preto, Sao Paulo University, [4]Center for Genetic Engineering and Biotechnology

C-Phycocyanin (C-PC) has strong antioxidant and cytoprotective actions. Existing evidence suggest that Phycocyanobilin (PCB), the linear tetrapyrrole convalently attached to this biliprotein, is involved in the C-PC radical scavenging activity. Here, we evaluated the effects of both molecules in different models of brain ischemia, and also, we examined gene expression in rat brain with chronic cerebral hypoperfusion (CCH). Cell viability in PC12 neurons was assessed by MTT assay. Transient rat retinal ischemia was induced by elevating the intraocular pressure to 120 mmHg for 45 min, and 15 min reperfusion. CCH was induced by permanent ligation of both common carotid arteries in rats and gene expression was revealed by qRT-PCR performed 24h after the surgery. In PC12 cells, both the C-PC and PCB treatments were effective against H2O2 or glutamate in a dose dependent manner. In ischemic retinas, pretreatment with C-PC or PCB for 15 min showed a significant dose dependent recovery of the inner nuclear layer cell density, restoring almost completely the retina integrity. C-PC or PCB given by an intraperitoneal cumulative dose of 8 mg/Kg, or 47 and 213 µg/Kg, respectively, were able to differentially induce GFAP,

Igfbp7, NADH dehydrogenase, Baiap2, Hmox, Hsp70, Mal, MMP2, Tph2, TGF-β, Foxp3, IL-6, IL-17, IFN-γ and IL-4 genes in rat anterior brain with CCH. Together, the results of the present study suggest the therapeutic potentials and the molecular mechanisms involved in the pharmacological actions of these molecules to attenuate the dramatic consequences of ischemic stroke.

2-IBR0-59 Cholesterol modulates the rate and mechanism of acetylcholine nicotinic receptor internalization

Maria Borroni[1], Maria V Borroni, Francisco J Barrantes

[1]INIBIBB

Stability of the nicotinic acetylcholine receptor (AChR) at the cell surface is key to the correct functioning of the cholinergic synapse. Cholesterol (Chol) is necessary for homeostasis of AChR levels at the plasmalemma and for ion translocation. Here we characterize the endocytic pathway followed by muscle-type AChR in Chol-depleted cells (Chol(-)). Under such conditions, the AChR is internalized by a ligand-, clathrin-, and dynamin-independent mechanism. Expression of a dominant negative form of the small GTPase Rac1, Rac1N17, abolishes receptor endocytosis. Unlike the endocytic pathway in control CHO cells, accelerated AChR internalization proceeds even upon disruption of the actin cytoskeleton. Under Chol(-) conditions, AChR internalization is furthermore found to require the activity of Arf6 and its effectors Rac1 and phospholipase D. The Arf6-dependent mechanism may constitute the default endocytic pathway followed by the AChR in the absence of external ligands, membrane Chol levels acting as a key homeostatic regulator of cell surface receptor levels.

2-IBRO-60 Mitochondrial dysfunction involvement in excitotoxic spinal motor neuron degeneration in vivo

Luz Diana Santa Cruz[1], Sergio Guerrero-Castillo[1], Salvador Uribe-Carvajal[1], Ricardo Tapia[1]

[1]Instituto de Fisiología Celular, UNAM

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by selective and progressive motor neuron degeneration leading to paralysis and finally death. Glutamate-mediated excitotoxicity has been proposed as a probable mechanism leading to motoneuron death in ALS. Previously, our group developed an in vivo model of spinal motoneuron excitotoxic death by means of microdialysis perfusion of á-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) in the lumbar region of the rat spinal cord. This treatment produces a permanent paralysis of the ipsilateral hindlimb. To study the possible participation of mitochondrial function deficiencies in this neurodegeneration, we have tested the neuroprotective effect of various energy metabolic substrates coperfused with AMPA. These treatments prevented the paralysis and motoneuron damage and preserved motor function in the rotarod test, suggesting that mitochondrial energetic deficiencies are involved in motoneuron death. We also studied oxygen consumption and transmembrane potential in mitochondria isolated from the ventral horn of the lumbar spinal cord of rats treated with AMPA, AMPA pyruvate, pyruvate Krebs-Ringer medium or Krebs-Ringer medium. The AMPA-treated group showed decreased oxygen consumption, ADP-dependent respiratory control and transmembrane potential, and pyruvate prevented these functional deficits. Our results suggest that mitochondrial dysfunction plays a crucial role in motoneuron degeneration induced by overactivation of AMPA receptors in vivo. These mechanisms could be involved in ALS.

2-IBRO-98 Is the integrity of GPM6A´s Transmembrane Domains important in Filopodium Formation?

Karina Formoso[1], Alberto C Frasch[1], Camila Scorticati[1]

[1]Institute for research in biotechnology

Neuronal membranes and an in vitro assay demonstrated the self-association of M6a-transmembrane domains (TM). The TMs of membrane proteins are a frequent target of disease causing mutations that impair such interactions. Three nonsynonymous polymorphisms were found in the TMs coding regions of the GPM6A gene, SNP1-2 for TM2 and SNP3 for TM3. In this sense, our aim was to assess the association between M6a´s SNPs and neuroplasticity. The different mutants were overexpressed in primary hippocampal cultured neurons where all mutants displayed a reduced filopodia density. In contrast, in N2a cells only M6a-SNP3 mutant failed to induce filopodia formation. Moreover, in non-permeabilized neurons M6a-SNP3 was the only clone not recognized by the antibody directed to the extracellular loop of M6a and showed colocalization with Calnexin. In this work we provided evidence that all SNPs impaired M6a´s neuroplasticity. In the case of SNP3 the proper assembly required for surface expression might be impaired. In the case of SNP1/2 further studies are needed to analyze a possible link between TM2 mutants and reduced neuroplasticity.

Poster Session 3: Wednesday May 23 2012

A - Development

3-A-84 Contributions of dendritic growth cones and filopodia to dendritogenesis in the intact and awake embryonic brain

Sharmin Hossain[1], D. Sesath Hewapathirane[1], Kurt Haas[1]

[1]University of British Columbia

Using in vivo rapid and long-interval two-photon time-lapse imaging of brain neuronal growth within the intact and unanesthetized Xenopus laevis tadpole, we characterize dynamic dendritic growth behaviors of filopodia, branches, and dendritic growth cones (DGCs), and analyze their contribution to persistent arbor morphology. The maturational progression of dynamic dendritogenesis was captured by short-term, 5 min interval, imaging for 1h every day for 5 days, and the contribution of short-term growth to persistent structure was captured by imaging at 5 min intervals for 5h, and at 2h intervals for 10h during the height of arbor growth. We find that filopodia and branch stability increases with neuronal maturation, and while the vast majority of dendritic filopodia rapidly retract, 3-7% of interstitial filopodia transition into persistent branches with lifetimes greater than 90 min. Here, we provide the first characterization of DGC dynamics, including morphology and behavior, in the intact and awake developing vertebrate brain. We find that DGCs occur on all growing branches indicating an essential role in branch elongation, and that DGC morphology correlates with dendritic branch growth behavior and varies with maturation. These results demonstrate that dendritogenesis involves a remarkable amount of continuous remodeling, with distinct roles for filopodia and DGCs across neuronal maturation.

3-A-85 Characterization of the loukoumasome, a large intracellular organelle found in rat sympathetic neurons

Diana Hunter[1], Matt S Ramer[1]

[1]ICORD, University of British Columbia

A large, proteinaceous organelle, named the loukomasome ("doughnut" body), was identified within specific subpopulations of peripheral autonomic ganglion neurons of the rat. This organelle is striking in both its unusual size and in its distinctive conformations. Though most often found in toroidal form on the nuclear side of the trans-golgi network (TGN), the loukoumasome also takes on intermediate twisted or linear conformations when peripheral to the TGN. Furthermore, the loukoumasome associates with other organelles, notably the primary cilium, an important signaling centre, and the poorly understood nematosome. The protein composition of the loukoumasome is also currently under investigation. Immunohistochemical examination has revealed that the core is composed, at least in part, of the microtubule nucleating protein gamma-tubulin, as well as the centrosome associated proteins myosinIIb, cenexin and pericentrin, whereas the outer shell is contains β-III tubulin in addition to other undefined protein. Its presence in multiple subcellular compartments, interaction with the primary cilium and association with a motor protein implies that it may be motile, though the dynamic behaviour of this organelle has yet to be defined. Recently, other research groups have identified similarly sized and shaped structures in cancer cell lines treated with inhibitors of nucleotide synthesis. The connection between these intracellular structures is under close examination and will be discussed.

3-A-86 Semaphorin 5B regulates sensory axon innervation in the developing spinal cord

Qian Qian Liu[1], Arthur Legg[1], Wenyan Wang[1], Timothy P O'Connor[1]

[1]University of British Columbia

Semaphorins, characterized by a conserved sema domain at the N-terminus, are a large family of molecules that guide growing axons to their targets during nervous system development. Thus far, little is known about the function of the transmembrane class 5 semaphorins which are distinguished from other classes by the presence of seven type-1 and type-1-like thrombospondin repeats C-terminal to the sema domain. Here we present the expression and function of one class 5 semaphorin, Sema5B, in the developing chick spinal cord. The dynamic expression of Sema5B in the developing spinal cord appears to correlate with sensory axon targeting into the grey matter. Our research addresses the hypothesis that Sema5B regulates the timing and extent of penetration of sensory axons into the dorsal horn of the developing spinal cord. We show that sensory axon growth is inhibited by the presence of Sema5B in vitro, confirming that Sema5B can function as a repulsive guidance cue for these neurons. We also test the function of Sema5B in vivo by electroporating vectors that express short hairpin RNAs targeted against Sema5B into one side of the developing spinal cord grey matter. We demonstrate that TAG-1 cutaneous sensory axons show premature and aberrant collateral projections into the side of the spinal cord dorsal horn where Sema5B expression was reduced. Thus, Sema5B is a guidance cue that is dynamically expressed in the embryonic spinal cord and plays a critical role in the correct circuit formation of sensory axons during development.

3-A-87 Basigin maintains glial survival and morphology in Drosophila

Lindsay Petley-Ragan[1], Vanessa Auld[1]

[1]University of British Columbia

The structure and function of peripheral nerves are conserved between vertebrates and Drosophila. Axons are insulated by glial cells and a layer of extracellular matrix (ECM). Glial-ECM, glial-glial and glial-axonal adhesion are crucical for maintaining the integrity of nerves. Consistent with vertebrate studies, our lab demonstrated that although integrin plays a role in glial adhesion, other unidentified ECM receptors must also be involved. The transmembrane protein, basigin, is an ideal candidate because it contains two extracellular Ig domains capable of binding ECM components and has previously been reported to interact genetically with integrin in Drosophila. Structural analyses of basigin suggest that it dimerizes within a membrane and may bind transcellularly. We demonstrate that basigin localizes to the glial membrane contacting the ECM as well as to membranes between two glial cells, suggesting that basigin may be involved in glial-ECM and glial-glial adhesion. Basigin can regulate the actin cytoskeleton, induce matrix metalloproteinases and localize monocarboxylate transporters to the membrane. Knockdown of basigin in a subset of glia resulted in multiple glial protrusions extending into the ECM and non-autonomous glial cell death. We hypothesize that basigin maintains glial survival and morphology via regulating the actin cytoskeleton.

3-A-88 The genetic basis of cell proliferation in the rostral migratory stream of the adult mouse brain

Anna Poon[1], Daniel Goldowitz[1]

[1]University of British Columbia

Neuron production takes place continuously in the rostral migratory stream (RMS) of the adult mammalian brain. The molecular mechanisms that regulate progenitor cell division and differentiation in the RMS remain largely unknown. Here, we surveyed the mouse genome in an unbiased manner to identify genetic loci that regulate proliferation in the adult RMS. We quantified neurogenesis in adult C57BL/6J and A/J mice, and 27 recombinant inbred lines derived from those parental strains. We showed that the A/J RMS had greater numbers of bromodeoxyuridine-labeled cells than that of C57BL/6J mice with similar cell cycle parameters, indicating that the differences in the number of bromodeoxyuridine-positive cells reflected the number of proliferating cells between the strains. AXB and BXA recombinant inbred strains demonstrated even greater variation in the numbers of proliferating cells. Genome-wide mapping of this trait revealed that chromosome 11 harbors a significant quantitative trait locus at 116.75 ± 0.75 Mb that affects cell proliferation in the adult RMS. A subset of genes in the chromosome 11 quantitative trait locus region is associated with neurogenesis and cell proliferation. Our findings provide new insights into the genetic control of neural proliferation and an excellent starting point to identify genes critical to this process.

3-A-89 Characterization of Wntless in the developing cerebellum

Joanna Yeung[1], Dan Goldowitz[1], Douglas J Swanson[1], Thomas Ha[1], Yiai Tong[2], Kunho Choi[1]

[1]University of British Columbia, [2]St. Jude Children's Research Hospital

While the role of Wnt signaling in the midbrain-hindbrain organizer is well characterized, its role in subsequent development of cerebellar neurons is not well understood. To elucidate the molecular bases of cerebellar development, we studied the Pax6-deficient Small Eye mutant mouse (Sey/Sey), which is characterized by cerebellar defects and disruptions in granule cell development. A comparison of developmental transcript profiles from mutant and wildtype cerebella has identified several differentially regulated transcripts in the Pax6-mutant cerebellum. One such gene is Wntless (Wls), a highly conserved multipass transmembrane protein that is involved in Wnt signaling. In-situ hybridization and immunohistochemical analyses revealed a restrictive Wls expression in the rhombic lips during embryonic development. The localization of Wls to the cerebellar rhombic lip suggests that Wls and Wnt signaling may be implicated in early phases of granule cell progenitor development. We further examined the changes of Wls expression in the Sey/Sey cerebellum and revealed an expansion of Wls expression domain beyond the rhombic lip into the nascent EGL, consistent with the differential gene expression identified in microarray studies. We generated Wls knockout mice from genetrap ES cells. Animals heterozygous for the knockout allele appear normal and fertile. Homozygous knockouts of Wls are not viable beyond early embryonic stage (E10.5), prior to the development of cerebellum. Present studies will help elucidate the roles of Wls and Wnt signaling pathway in cerebellar development.

3-A-90 The effects of intraventricular hemorrhage on the development of the postnatal cerebellum

Ji Young Janice Yoo[1], Gloria Mak[1], Daniel Goldowitz[1]

[1]University of British Columbia

Intraventricular hemorrhage (IVH) is the most common neurological problem in premature infants and is associated with poor neurodevelopmental outcomes. With the improvement in neuroimaging technology and increased survival of premature infants, detecting IVH in the fourth ventricle of the premature brain is becoming

increasingly common. The cerebellum is a structure of the brain involved in motor control and cognitive functioning. The cerebellum is positioned immediately above the fourth ventricle, but how fourth ventricle IVH affects cerebellar development and function remains largely unknown. Therefore, our study focuses on developing a mouse model of IVH to determine the anatomical and behavioral phenotypes resulting from this perturbation. To induce a hemorrhage in the germinal matrix of the cerebellum, we inject collagenase, which breaks down surrounding blood vessel walls, into the fourth ventricle of postnatal (P) mice two days of age. Controls were injected with saline. We then analyzed cell proliferation and apoptosis in the cerebellum, as well as cerebellar size over time. Behavioral analyses were further conducted on P60 mice to assess locomotor abilities. In our model of fourth ventricle IVH, we found a delay in cerebellar development and persistent locomotor abnormalities similar to premature infants with IVH. Therefore, our study provides a novel pre-clinical model of fourth ventricle IVH that can be used to understanding the pathophysiology of this disease, as well as for the development of potential therapeutic interventions.

B – Neural Excitiability, Synapses, and Glia: Cellular Mechanisms

3-B-1 Hypocretin modulation of morphine-induced synaptic plasticity in the ventral tegmental area

Corey Baimel[1], Stephanie L Borgland[1]

[1]University of British Columbia

Dopamine neurons in the ventral tegmental area (VTA) are a key target of addictive drugs and neuroplasticity in this region may underlie some of the core features of addiction. All drugs of abuse induce an LTP-like potentiation of excitatory inputs to VTA dopamine neurons. Hypocretin (hcrt), also known as orexin, is a lateral hypothalamic neuropeptide released into the VTA that exerts modulatory effects on a variety of behaviors produced by drugs of abuse. Acute application of hcrt potentiates excitatory synaptic transmission in the VTA, and inhibition of hcrt signaling blocks both cocaine-induced plasticity and behavioral sensitization. However, the role of hcrt on the plasticity induced by other classes of abused drugs is unknown. Here we used whole-cell patch-clamp electrophysiology in rat horizontal midbrain slices to examine the role of hcrt in morphine-induced plasticity in the VTA. Systemic administration of the hcrt receptor 1 antagonist, SB 334867, blocked a morphine-induced increase in the AMPAR/NMDAR ratio. Furthermore, SB 334867 blocked morphine-induced increases in AMPAR mEPSC frequency and amplitude, as well as morphine-induced AMPAR redistribution measured by a change in rectification. These results support a role for hcrt signaling in both pre-and post-synaptic potentiation of glutamatergic transmission in the VTA, and together with previous results for cocaine, suggest that hcrt may function as a gate keeper for drug induced plasticity of dopamine neurons.

3-B-2 Gap junctions regulate the strength and plasticity of afferent input to the superficial spinal cord dorsal horn

Robert Bonin[1], Yves De Koninck[1]

[1]Université Laval

Neuronal networks within the spinal cord dorsal horn regulate the transmission of sensory information. Gap junctions (GJs) allow rapid transmission of ions and small molecules between cells, and contribute to network activity and synchrony. We explored the contribution of GJs to synaptic activity and plasticity within the superficial layers of the dorsal horn. Extracellular postsynaptic field potentials (fPSPs) were evoked in the superficial dorsal horn by stimulation of dorsal roots in an in vitro preparation. Mefloquine (4 μM) did not significantly change the input-output relationship of fPSPs when applied at concentrations that inhibit CX36-containing GJs. However, at higher concentrations that inhibit CX43-containing GJs, mefloquine (40 μM) produced a 50% inhibition of fPSPs at all stimulus intensities tested. A similar degree of inhibition was induced by CX43-selective concentrations of meclofenamate. In contrast, inhibiting glial activity with fluorocitrate inhibited fPSPs in a stimulus intensity-dependent manner. Long term potentiation (LTP) of fPSPs was induced using low-frequency (2 Hz) stimulation of dorsal roots. Following application of 40 μM but not 4 μM mefloquine, this stimulation induced long term depression of fPSPs. These results demonstrate a role of CX43-containing GJs in regulating the strength and plasticity of primary afferent input to the superficial dorsal horn. The distinct effects of CX43 inhibition on fPSP plasticity implicates a role of astrocytic gap junction activity in the metaplasticity of afferent input to the superficial dorsal horn.

3-B-3 Exogenous and endogenous insulin induces long-term depression in VTA dopamine

Stephanie Borgland[1], Shuai Liu[1], Gwenael Labouebe[1]

[1]University of British Columbia

Dopamine neurons of the ventral tegmental area (VTA) represent a critical site for food seeking and insulin may act in the VTA to suppress feeding. We demonstrated that exogenous insulin can cause a long-term depression (LTD) of excitatory synapses onto VTA dopamine neurons via endocannabinoid-mediated presynaptic inhibition of glutamate release. In the present study, we confirm exogenous insulin suppresses AMPAR- or NMDAR-mediated excitatory postsynaptic currents (EPSCs). This effect was not reversed by later application of the insulin

receptor antagonists, S961 or HNMPA[AM]3, indicating that persistent insulin receptor signalling is not required for LTD maintenance. Moreover, the CB1 receptor antagonist, AM251 or the diacylglycerol lipase inhibitor, orlistat did not reverse LTD when applied after induction. These results suggest that presynaptic mechanisms maintain insulin-induced LTD. The effect of endogenous insulin under physiological conditions was also examined. Mice were fed sweetened high fat food (SHF) or regular food (RF) for 1 hour prior to slice preparation. In contrast to RF-fed mice, exogenous insulin did not alter AMPAR EPSCs in SHF-fed mice. To test if this effect was an occlusion by endogenous insulin, we found that AM251 significantly elevated EPSCs in SHF group, but not RF group. Further, mEPSC frequency was significantly less in the SHF group compared to the RF group. Taken together, these results suggest that insulin acts in the mesolimbic reward system to suppress excitatory synaptic transmission and can potentially regulate feeding behavior.

3-B-4 Different intracellular pathways modulate GABAAR-mediated inhibition and chloride homeostasis in spinal dorsal horn.

Dominic Boudreau[1], Louis-Étienne Lorenzo[1], Jeffrey A Coull[1], Alfredo Ribeiro-da-Silva[2], Yves De Koninck[1]

[1]Centre de recherche de l'Institut universitaire en santé mentale de Québec / Université laval, [2]Department of Pharmacology & Therapeutics, McGill University

Disinhibition is an important mechanism for the increased excitability of dorsal horn neurons in neuropathic pain. While it is well established that inhibition is subject to modulation by changes in GABAA receptor (GABAAR) number or function, it is only recently that ionic modulation has been found to be an important mechanism of GABAergic plasticity through changes in chloride extrusion capacity. Brain derived neurotrophic factor (BDNF) has been shown to be implicated in neuropathic pain models and to modulate both the GABAAR and chloride homeostasis. This raises the question of whether similar intracellular pathways are implicated in these two types of modulation of the efficacy of GABAAR-mediated inhibition. Here, we show that peripheral nerve injury (PNI) changes the kinetics of GABAAR-mediated miniature inhibitory post-synaptic currents (mIPSCs) in the spinal dorsal horn and that it is reflected in the subunit composition of the receptors. Acute administration of BDNF produced similar changes. Moreover, we found that those changes in kinetics were Ca2 -dependent both in PNI and after BDNF application. PKC and CaMKII modulate both the chloride reversal potential and the kinetics of the GABAA mIPSCs. In contrast, PKA modulate chloride extrusion but not GABAAR-mediated mIPSCs. Taken together, these results indicate that modulation of inhibition through GABAAR function and chloride homeostasis involves different intracellular pathways.

3-B-5 Regulation of a Large-Conductance, Calcium-Activated Potassium (BK) Channel by cysteine string protein

Jan Braun[1], Eva Ahrendt[1], Barry Kyle[1], Andrew Braun[1]

[1]University of Calgary

n determining the amplitude and duration of action potentials, firing rates and overall excitabilty of neurons. To date, little is known about the molecular chaperone machinery that participates in the folding, maintenance and degradation of BK channels. Cysteine string protein is a synaptic vesicle-associated molecular chaperone that prevents activity-dependent neuronal degeneration by unknown mechanisms. Using CAD neuroblastoma cells that either transiently or stably express BK channels, we assessed the influence of cysteine string protein on BK channel expression using Western blot analysis, whole cell patch clamp recordings and immunocytochemistry. Our study indicates that cysteine string protein regulates the cell surface expression and activity of BK channels. We further demonstrate that mutation of the highly conserved HPD motif within the J-domain of cysteine string protein dramatically increases BK channel expression. These results provide the first evidence that cysteine string protein may influence synaptic excitability and neurotransmission by regulating BK channel expression.

3-B-6 Optogenetic analysis of neuronal excitability during global ischemia reveals selective deficits in sensory processing following reperfusion in mouse cortex

Shangbin Chen[1], Majid H Mohajerani[1], Timothy H Murphy[1]

[1]University of British Columbia

We have developed an approach to directly probe neuronal excitability during the period beginning with induction of global ischemia and extending after reperfusion using transgenic mice expressing Channelrhodopsin-2 (ChR2) to activate deep layer cortical neurons independent of synaptic or sensory stimulation. Spontaneous, ChR2, or forepaw stimulation-evoked electroencephalogram (EEG) or local field potential (LFP) records were collected from sensory cortex. Within 20 s of ischemia a >90% depression of spontaneous 0.3-3 Hz EEG and LFP power was detected. Ischemic depolarization followed EEG depression with an ~2 min delay. Surprisingly, neuronal excitability, as assessed by the ChR2-mediated EEG response was intact during the period of strong spontaneous EEG suppression. In contrast, a decrease in the sensory-evoked potential was coincident with the EEG suppression. After 5 min of ischemia the animal was reperfused and the ChR2-mediated response mostly recovered within 30 min (>80% of baseline). However, the recovery of sensory-evoked potential was significantly delayed compared to the ChR2-mediated response (<40% of baseline at 60 min). By

assessing intrinsic optical signals in combination with EEG we found that neuronal excitability approached minimal values when the spreading ischemic depolarization wave propagated to the ChR2-stimulated cortex. Our results indicate that the ChR2 EEG response recovers much faster than sensory-evoked EEG activity in vivo following ischemia and reperfusion, defining a period where excitable but synaptically silent neurons are present.

3-B-7 Metabolic communication between astrocytes and neurons via bicarbonate-responsive soluble adenylyl cyclase

Hyun Choi[1], Grant Gordon[1], Ning Zhou[1], Chao Tai[1], Jennifer Martinez[2], Teresa A Milner[3], Jae K Ryu[1], James G McLarnon[1],Martin Tresguerres[2], Lonny R Levin[2], Jochen Buck[2], Ravi L Rungta[1],Brian A MacVicar[1]

[1]University of British Columbia, [2]Department of Pharmacology, Weill Cornell Medical College, New York, [3]Department of Neurology andNeuroscience, Weill Cornell Medical College, New York

Astrocytes are proposed to participate in brain energy metabolism by supplying substrates to neurons from their glycogen stores and from glycolysis. However, the molecules involved in metabolic sensing and the molecular pathways responsible for metabolic coupling between different cell types in the brain are not fully understood. Here we show that a recently cloned bicarbonate (HCO3-) sensor, soluble adenylyl cyclase (sAC), is highly expressed in astrocytes and becomes activated in response to HCO3- entry via the electrogenic NaHCO3 cotransporter (NBC). Activated sAC increases intracellular cAMP levels causing glycogen breakdown, enhanced glycolysis and the release of lactate into the extracellular space, which is subsequently taken up by neurons for use as an energy substrate. This process is recruited over a broad physiological range of [K]ext and also during aglycemic episodes, helping to maintain synaptic function. These data reveal a novel molecular pathway in astrocytes that is responsible for brain metabolic coupling to neurons.

3-B-8 Neurexin - neuroligin signaling mediates myelination in the CNS

Michael Colicos[1], Dustin T Proctor[1], Kelvin W Poon[1], Lucas O Scott[1], Peter K Stys[1]

[1]University of Calgary

The trans-synaptic interaction between neurexin (NX) and neuroligin (NL) is a key event for synaptic maturation. NX/NL mutations have been discovered in autistic patients, leading to many studies investigating their function in the normal flow of information between neurons. However clinical and pathological observations show that gross changes to white matter exist in autistic patients, suggesting altered myelination might also play a causal role in the cognitive deficits. We hypothesized that NX-NL signaling is also involved in the process of CNS myelination. To examine this, we first transiently expressed NX1alpha in HEK cells co-cultured with cerebellar neurons and glia and found that within 24hrs the oligodendrocytes in the culture begin to myelinate the HEK cells expressing NX. Using interfering peptides in a cultured rodent cerebellar slice paradigm, we quantified by confocal microscopy myelination of Purkinje cell axons which have developed for 2 weeks in the presence of either a wildtype, secreted NL peptide (presumed to interfere with NX-NL binding by saturating the NX target) or a mutant NL peptide (R473E) which has lost its NX binding ability. In the untreated slices virtually all Purkinje axons are myelinated. In contrast, slices treated with the wt NL peptide had significantly reduced numbers of myelinated Purkinje axons. R473E peptide does not have this effect, suggesting that not only is binding to NX critical for the recognition process during myelination, but that similar, autism-associated mutations are unable to perform this function normally.

3-B-9 The fountain of synaptic youth: Extending the time window for successful induction of heterosynaptic LTP through inhibition of GluA2 endocytosis

Steven Connor[1], Yu Tian Wang[1], Peter V Nguyen[2]

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

Previously, we have established that beta-adrenergic receptor activation facilitates heterosynaptic long-term potentiation in mouse hippocampus. However, the mechanisms mediating heterosynaptic transfer of LTP have yet to be fully elucidated. Using a protocol in which prior induction of beta-adrenergic receptor-dependent LTP at one synaptic pathway (S1; homosynaptic) facilitates LTP induced at a second set of synapses converging on the same neurons (S2; heterosynaptic), we characterized the processes involved in heterosynaptic LTP. We found that, in the presence of a translation inhibitor applied during homosynaptic LTP, subsequent capture of LTP at both synaptic pathways was blocked. However, shifting application of the translation repressor emetine to coincide with S2 stimulation failed to block LTP at either pathway. To test the temporal threshold for heterosynaptic transfer of LTP, we extended the duration between stimulation at S1and S2 from 30 min to 1 hr. Heterosynaptic transfer of LTP was only observed following a 30 min delay. Interestingly, blocking GluA2 endocytosis expanded the temporal window for heterosynaptic transfer of LTP to at least 1 hr. These results suggest that beta-adrenergic receptor activation bolsters translational regulation of proteins involved in heterosynaptic plasticity and GluA2 endocytosis may serve as a rate-limiting factor in the cellular capacity for association of synaptic events over time. Taken together, our results describe a putative cellular mechanism for the remembrance of temporally spaced events during memory formation.

3-B-10 Different modes of neuronal inhibition lead to different pathways of activity dependent competition in vitro

Ainsley Coquinco[1], Max Cynader[1]

[1]University of British Columbia

Neuronal activity has long been known as an important factor regulating neuronal growth and synaptic competition. To examine the effects that differences in activity have among interacting neurons, we used a multicompartment system in which neuronal cell bodies were physically separated but their axons remained free to interact with neurons in other compartments. Primary neurons were grown in 3-compartment microfluidic devices with the lateral compartment neurons differentially labeled with either yfp- or mcherry- tagged synatophysin and allowed to grow into the the centre compartment. The neuronal activity of one lateral compartment was reduced by applying either muscimol or tetrodotoxin (TTX) for 2 days. Both treatments resulted in the treated neurons forming fewer synapses. However, TTX treatment resulted in reduced axon growth and synapse formation of the treated neurons while muscimol treatment led to increased growth and synapse formation for neurons in the untreated competing lateral compartment. The TTX induced changes required GluA2 internalization while changes due to muscimol required CamKII activity. Also, the developmental age at which the treatments induced changes differed with TTX changes beginning and ending one week later compared to muscimol. In conclusion, we created a system in which we can alter the activity of a single neuronal group while the other groups remained unaffected. Furthermore, we show that different methods of neuronal activity manipulation change the synaptic balance between competing groups of inputs by different mechanisms.

3-B-11 Dissecting mitochondrial morphology changes in cultured rat cortical astrocytes: fission vs. remodelling

Samineh Deheshi[1], Andrew Tan[1], Andrew Y Cai[1], Susu Fan[1], Annica Beckman[1], Bahram Dabiri[1], Michelle Tsang[1], Gordon Rintoul[1]

[1]Simon Fraser University

Mitochondria are dynamic semi-autonomous organelles that have their own DNA, fuse, divide and have surprising mobility within cells. We investigated the role of calcium in the regulation of mitochondrial morphology using live cell real time fluorescence microscopy. Calcium induces mitochondrial fission through Drp1 dephosphorylation by calcineurin and may predispose cells to apoptosis (Cribbs & Strack, 2007). We quantified mitochondrial morphology changes by transfecting cortical astrocytes with a mitochondrially-targeted yellow fluorescent protein and monitored mitochondrial dynamics. Our results support the Drp1 mediated mechanism of fission by demonstrating that calcium induces an increase in mitochondrial fission which can be blocked by the calcineurin inhibitors Cyclosporin A and FK506. However, by quantitatively distinguishing between mitochondrial remodelling (rounding) and fission, we observed that the changes in mitochondrial morphology induced by calcium were primarily due to remodelling in a calcineurin-insensitive manner rather than fission. We also study the impact of ROS on mitochondrial dynamics using neurotoxic ROS inducing agents. Deficits in mitochondrial dynamics have been implicated in neurodegenerative diseases. Mitochondrial remodelling as a contributor to mitochondrial morphological plasticity has been largely ignored in the literature, therefore these studies provide important insights into the regulation of mitochondrial dynamics.

3-B-12 NMDA triggers rapid microglial process outgrowth; evidence for neuron-glia communication

Lasse Dissing-Olesen[1], Brian MacVicar[2]

[1]Brain Research Center, University of British Columbia, [2]University of British Columbia

Microglia are known as the primary immune effectors in the healthy brain and even in absence of pathological stimuli microglia exhibit motile processes proposed to actively survey surrounding synapses. We hypothesized that activation of neuronal NMDA receptors leads to ATP efflux and consequently microglial process outgrowth. Using two-photon imaging in brain slices we confirmed that extracellular ATP and its hydrolyzed product, ADP, induced a characteristic microglial process outgrowth that reversed when ATP was removed or when purinergic receptors were blocked. Interestingly, we observed that a similar microglial process outgrowth occurred following NMDA receptor activation. This outgrowth was also reversible and repeatable with a second application of NMDA, demonstrating that it was not a microglial response to permanent excitotoxic damage. Furthermore the microglial response was selective to NMDA receptor stimulation as it still occurred in the presence of CNQX, TTX, Cd2 , and picrotoxin to block AMPA receptors, Na channels, Ca2 channels, and GABA activated Cl- channels, respectively. However it was blocked by AP5, the NMDA receptor antagonist. Finally, NMDA-induced microglial process outgrowth was abolished by purinergic receptor blockade, demonstrating ATP or its hydrolyzed products as key mediators. We are currently measuring ATP efflux by electrochemical recordings to determine the underlying mechanisms of NMDA- induced ATP release. We propose that NMDA-triggered process outgrowth enhances the dynamic microglial surveillance of nearby synapses.

3-B-13 Rab8 modulates metabotropic glutamate receptor subtype 1 intracellular trafficking and signalling in a PKC-dependent manner

Jessica Esseltine[1], Fabiola M Ribeiro[2], Stephen S Ferguson[3]

[1]Robarts Research Institute, [2]Universidade Federal de Minas Gerais, [3]University of Western Ontario

Metabotropic glutamate receptors (mGluR) are G protein-coupled receptors (GPCRs) that are activated by glutamate, the primary excitatory neurotransmitter in the central nervous system. Deregulation of glutamate receptors is implicated in neuropathologies such as Alzheimer's disease, ischemia and Huntington's disease among others. Group 1 mGluRs (mGluR1 and mGluR5) are primarily coupled to Gαq leading to the activation of phospholipase C and the formation of diacylglycerol and inositol 1, 4, 5-trisphosphate which results in the release of intracellular calcium stores and protein kinase C (PKC) activation. Desensitization, endocytosis and recycling are major mechanisms of GPCR regulation and the intracellular trafficking of GPCRs is linked to the Rab family of small G proteins. Rab8 is a small GTPase that is specifically involved in the regulation of secretory/recycling vesicles and has been shown to regulate the synaptic delivery of AMPA Receptors during long term potentiation and during constitutive receptor recycling. We show here that Rab8 interacts with the carboxyl-terminal tail of mGluR1a in an agonist-dependent manner and plays a role in regulating of mGluR1a signalling and intracellular trafficking. Specifically, Rab8 expression attenuates mGluR1a-mediated inositol phosphate formation and calcium release in a PKC-dependent manner, while increasing cell surface mGluR1a expression via decreased receptor endocytosis. These experiments provide us with an understanding of the role Rabs play in coordinated regulation of mGluR1a and how this impacts mGluR1a signalling.

3-B-14 Neuron-glial cell communication in the adult brain through the morphogen Sonic Hedgehog.

W. Todd Farmer[1], Emma V Jones[2], Magdalena Götz[3], Keith Murai[2]

[1]McGill University Health Centre, [2]Centre for Research in Neuroscience, McGill University Health Centre, [3]Ludwig-Maximilians-Universität München

Interactions between neurons and glia are important for proper CNS function, including synaptic development and plasticity. Although some cues involved in neuron-glial cell communication have been discovered, it is likely that many signaling molecules involved in this process still remain to be uncovered. One possible cue is Sonic Hedgehog (Shh), a morphogen known for its potent role in neural development. Using a Cre-Lox reporter system we have found that Shh is expressed in discrete populations of neurons of the cerebellum (CB) in vivo, including granule cells (GCs) and Purkinje cells (PCs). Interestingly, various gene expression databases suggest that Shh signaling components such as Smoothened (Smo), Patched1, and Patched2 are expressed in cerebellar Bergmann glia (BG), the primary glia of the cerebellum that modulate glutamatergic transmission onto PCs. We are currently testing the hypothesis that neurons secrete Shh to modify the molecular and structural properties of BGs. In vitro, Smo and Patched2 are expressed on the cell surface of CB glia. Treatment of CB glial cultures with a Shh peptide or the Smo antagonist SANT1 up-regulates and down-regulates Shh pathway components, respectively, indicating that CB glia are responsive to Shh. To investigate a role of Shh in cerebellar function in vivo, we conditionally knocked out Smo (cKO) from BG in adult mice and found that Smo cKO animals have decreased performance on a cerebellar-dependent motor learning task. Our results indicate novel role for the Shh morphogen in neuron-glia communication and adult brain plasticity.

3-B-15 QKI-5 regulates oligodendroglial cell differentiation by modulating the availability of PLP and Sirt2 mRNA for translation.

Jotham Gan[1], Shao-Ping Ji[1], J.Ronald Doucette[1], LaRhonda Sobchishin[1], Adil J Nazarali[1]

[1]University of Saskatchewan

The Quaking (Qk) gene encodes three alternatively spliced variants, QKI-5, QKI-6, and QKI-7, which are all RNA binding proteins. QKI-5 contains a nuclear localization signal, while QKI-6 and QKI-7 are cytoplasmic. Loss of QKI-6 and QKI-7 results in dysmyelination of the central nervous system and any myelin that is present is uncompacted. A mutation resulting in the loss of QKI-5 results in embryonic lethality. This suggests a role for Qk in regulating myelinogenesis as well as overall nervous system development. Our data show that QKI-5 mRNA and protein are expressed in CG4 OL cell line, prior to differentiation into myelin protein expressing cells. An increase in QKI-5 gene expression after transient transfection in CG4 OL cells with a GFP-tagged construct resulted in increases in both sirtuin-2 (Sirt2) and proteolipid protein 1 (PLP) mRNA expression. However, Sirt2 and PLP protein expression were unaffected. Also, immunocytochemistry showed that populations of CG4 cells that were transfected with QKI-5 had a significantly higher percentage of A2B5-positive cells and a lower percentage that were galactocerebroside (GalC)-positive than wild-type or blank vector transfected cells. CG4 cells transfected with a QKI-5 specific siRNA, exhibited down regulation of QKI-5 specifically without impacting QKI-6 or QKI-7 mRNA, and increased the percentage of GalC-positive cells. However, siRNA treatment had no impact on Sirt2 or PLP mRNA and protein expression. Our results

suggest QKI-5 regulates OL differentiation by modulating the availability of key mRNAs for translation.

3-B-16 Modulation of PgE2 synthesis and the impact on vascular control

Rebecca Ko[1], Clare Howarth[1], Brad Sutherland[2], Hyun B Choi[1], Chris Martin[2], Janelle Pakan[1], Graham C Ellis-Davies[3], Nicola R Sibson[2], Alastair Buchan[2], Brian A MacVicar[1]

[1]University of British Columbia, [2]Oxford University, [3]Mount Sinai School of Medicine

It is critical for the maintenance of normal brain function that cerebral blood flow (CBF) is matched to neuronal metabolic demands. Neural activity can lead to elevations in astrocytic calcium (Ca) levels and arachidonic acid (AA) formation. AA is processed to produce prostaglandin E2 (PgE2), which is released from astrocyte endfeet, resulting in vasodilation. The formation of PgE2 from AA requires glutathione (GSH). It is known that following stroke, oxidative stress depletes GSH, and that there is a long-lasting failure of vasodilatory mechanisms. We investigated the hypothesis that oxidative stress reduces the efficacy of pathways underlying astrocyte-mediated increases in CBF. PARA We investigated, both in vivo and in vitro, whether lowering GSH alters PgE2 synthesis which, in turn, alters vasodilation. PgE2 efflux triggered by increased astrocytic Ca is reduced when tissue GSH levels are reduced by treatment with buthionine sulfoximine (BSO, a GSH synthesis inhibitor). BSO treatment abolishes the vasodilation evoked by astrocyte Ca transients in brain slices and alters the CBF response evoked by whisker stimulation in vivo. Our data suggest that downstream of Ca-evoked AA release in astrocytes, the component of functional hyperaemia mediated by PgE2 release from astrocytes requires GSH. PARA These data predict that GSH depletion following oxidative stress should inhibit PgE2-mediated vasodilation, possibly playing a role in the failure of vasodilatory mechanisms that contributes to increasing neuronal damage after stroke.

3-B-17 Downregulation of Nav1.3 in subfornical organ neurons following a 48 hour fast

Suman Lakhi[1], Sylvia Wong[1], Mark Fry[1]

[1]University of Manitoba

The subfornical organ (SFO) is a sensory circumventricular organ that plays a key role in detection of circulating satiety signals and regulation of energy balance. Previous work using Affymetrix microarrays revealed that a 48h fast caused at least twofold change in expression of over 600 transcripts in rat SFO. Included in the downregulated transcripts was Nav1.3, a voltage gated Na+ channel that is a major contributor to electrical excitability of SFO neurons. Quantitative PCR (qPCR) confirmed that following 48h fast, Nav1.3 levels in SFO were reduced by 54%, compared to sated controls. In order to investigate changes in electrical properties associated with the reduction of Nav1.3 transcript, we carried out patch clamp experiments on acutely dissociated SFO neurons from 48h fasted rats and sated controls and quantified whole cell voltage gated Na+ current. These experiments revealed that there was no change in voltage dependence of Na+ current activation between fasted (V1/2 = -27.2 +/- 0.3 mV n=66) and control (V1/2 = -27.7 +/- 0.2 mV, n=40). In contrast, there was a depolarizing shift in the voltage dependence of Na+ current activation between fasted (V1/2 = -39.1 ± 1.8 mV, n = 49) and control (V1/2 = -56.7 ± 0.5 mV, n = 35). These data demonstrate food restriction alters electrical properties of SFO neurons, indicating that SFO is a dynamic sensor for energy balance.

3-B-18 Presynaptic NMDA receptor-mediated regenerative glutamate release underlies the propagation of spreading depression

Aqsa Malik[1], Ning Zhou[2], Ravi L Rungta[1], Brian A MacVicar[1]

[1]University of British Columbia, [2]China Medical University

Spreading depression (SD) is characterized by a depolarizing wave that slowly propagates in the gray matter and suppresses electrical activity. SD contributes to several neurological pathologies including ischemic stroke, traumatic brain injury, and migraine with aura. The phenotype of SD propagation suggests that it arises from an unusual form of neuronal communication. Using enzyme-based glutamate electrodes, electrophysiological recordings, and intrinsic optical signals (IOS), we show that the onset of SD correlated with a transient increase in extracellular [glutamate], extracellular potential shift, and IOS change in rat cortical slices. SD-evoked glutamate release and potential shifts were blocked by inhibitors of vesicular H -ATPase, suggesting a key role for vesicular exocytosis in this mechanism. Glutamate release during SD occurs through vesicular exocytosis in the presence of TTX and Cd2 , indicating that this mechanism is independent of canonical fast neurotransmission that requires action potential propagation. Instead, by pharmacologically blocking either pre- or postsynaptic NMDARs before evoking SD, we demonstrate that glutamate release during SD requires Ca2 influx through presynaptic NMDARs. Diffusion of glutamate to adjacent synapses activates additional NMDARs resulting in a positive feedback cycle of glutamate-induced glutamate release. Our results show that Ca2 influx through presynaptic NMDARs is required for regenerative glutamate release that leads to a self-propagating wave of neuronal depolarization underlying the progression of SD.

3-B-21 An activity-dependent functional and structural framework for evoked and spontaneous activity across multiple sensory modalities and brain regions

Majid Mohajerani[1], David McVea[1], Rui Liu[1], Jeff LeDue[1], Timothy H Murphy[1]

[1]University of British Columbia

Spontaneous neuronal activity is not noise, but reflects the orchestrated action of underlying brain circuitry. Although its importance is recognized, exactly what determines the spatial and temporal dynamics of spontaneous activity and it's relation to evoked activity over spatial wide scales is unclear. Here in lightly anaesthetized adult mice using millisecond timescale bilateral brain voltage sensitive dye imaging we show that spontaneous activity is derived from a palette of common cortical activity motifs that are present during sensory processing. Correlation analysis based on both functional imaging data and the Allen Brain Institute Connectivity Atlas indicated that spontaneous and evoked activity were constrained within a common structural and functional framework. A map of intracortical structural connections was able to predict patterns of spontaneous activity. A similar constrained cortical network was found with direct cortical activation using channelrhodopsin-2. Spontaneous activity was able to encode previous experience information within wide-spread activity patterns in a regionally and temporally selective manner across sensory modalities including vision, audition, and somatosensation. These actions are consistent with spontaneous activity providing a means of encoding information while the brain is between periods of processing and indicate a common structural and functional framework for information processing during both deliberate and unprompted activity.

3-B-22 mTOR activity restores retinal ganglion cell dendritic arbors and glutamatergic inputs after axonal injury in vivo.

Barbara Morquette[1], Philippe P Roux[1], Elain Feinstein[2], Anne R McKinney[3], Adriana Di Polo[1]

[1]University of Montreal, [2]Quark Pharmaceuticals Inc, [3]McGill University

Purpose: Dendrites are major determinants of how retinal neurons integrate and process incoming information. We previously showed that retinal ganglion cells (RGCs) undergo a significant reduction of dendritic arbors soon after axonal injury. However, the molecular mechanisms that underlie injury-induced dendritic remodeling are poorly defined. Here, we investigated the role of the mTOR (mammalian target of rapamycin) pathway in RGC dendritic structure after acute optic nerve lesion. Methods: Adult transgenic mice carrying the yellow fluorescent protein (YFP) gene were subjected to optic nerve axotomy. Retinal mTOR activity was manipulated using: i) intraocular injection of siRNA against the mTOR repressor REDD2, increasing mTOR activity; and ii) intraperitoneal administration of rapamycin, an mTOR inhibitor. Results: Our data demonstrate that optic nerve axotomy leads to marked downregulation of mTOR activity in RGCs, which correlated with dendritic shrinkage at 3 days after injury. Treatment with siREDD2 stimulated mTOR activity in axotomized RGCs and promoted a significant increase in total dendritic length and field area compared to retinas treated with control siRNA. Scholl analysis revealed a marked increase in the complexity of dendritic arbors from RGCs with increased mTOR activity. mTOR activity also restores glutamatergic inputs of bipolar cells on dendritic shaft of injured RGCs. Conclusion: Our study reveals a novel role for the mTOR pathway in the rescue of dendritic structure in adult, injured RGCs.

3-B-23 Differential effects of early life maternal care on synaptic plasticity in the dorsal and ventral hippocampus

Huy-Binh Nguyen[1], Michael J Meaney[1], Tak Pan Wong[1]

[1]Douglas Mental Health University Institute

Naturally occurring variations in early life maternal care modulates hippocampal development and function to program distinct cognitive phenotypes which persist into adulthood. Adult rat offspring which received low maternal care (Low LG) are impaired in dorsal hippocampal cognition, evidenced by a reduction in long term potentiation (LTP) and impaired spatial learning. Low LG offspring simultaneously display an increase in aggression and anxiety as measured by the resident-intruder and open field paradigms, respectively. These affect-related behaviours are proposed to be more closely associated with ventral hippocampal function. Synaptic plasticity in the ventral hippocampus is mediated by distinct mechanisms, although whether maternal care alters ventral LTP is unknown. We have presently found that the early life environment differentially programs hippocampal function along the dorsal-ventral axis by enhancing LTP formation in the Schaffer Collateral CA3-CA1 synapse in the ventral hippocampus of low LG offspring relative to high LG offspring. Paired pulse facilitation was not altered at these synapses, indicating that these effects are not associated with a difference in presynaptic function. Rather, expression of the postsynaptic marker PSD95 is concomitantly increased in the ventral hippocampus of low LG offspring, potentially reflecting an increase in post-synaptic volume or spine number. Low LG offspring may thus be biased by their early environment towards a qualitatively distinct form of hippocampal cognition which preferentially supports anxiety and aggression.

3-B-24 GABAA transmission regulates dendritic spine formation in the developing organotypic hippocampal slice

Christopher Salmon[1], Emma V Jones[2], Keith K Murai[1]

[1]McGill University, [2]Research Institute of the McGill University Health Centre

The neurotransmitter γ-aminobutyric acid (GABA) plays an integral role in the mature CNS, where it is the main inhibitory transmitter. Remarkably though, GABA is excitatory in immature neurons. During this immature phase, glutamatergic synapses are actively forming and maturing on dendritic spines. To determine if early GABAergic neurotransmission influences the formation of excitatory synapses, we modulated GABAergic transmission while monitoring the formation of dendritic spines on CA1 neurons in mouse organotypic hippocampal slices. In line with previous findings, inhibiting GABAA transmission with gabazine (Gbz) or bicuculline in slices grown for 5 days in vitro (DIV) caused robust spine loss. Surprisingly, the same manipulation in younger slices (3 DIV) increased spine density by 33% while driving GABAA transmission at 3 DIV caused an opposite 25% decrease in spine density. To follow up on the effects of GABAA antagonists on spines we monitored the developmental expression of potassium-chloride cotransporter-2 (KCC2), a transporter that shifts GABA from excitatory to inhibitory by gradually moving chloride out of neurons. As expected, KCC2 levels increased between 3 and 5 DIV, suggesting the switch in the action of GABA may account for the differential effects of GABAA antagonists on spines. As KCC2 itself plays a structural role in spine stabilization, we asked if Gbz treatment increases KCC2 levels. This was not the case. Together, our findings suggest that early, excitatory GABAA transmission regulates excitatory synapse formation in the developing hippocampus.

3-B-25 Unexpected actions of BDNF on inhibitory transmission in the spinal dorsal horn

Peter Smith[1], Van B Lu[2], William F Colmers[1]

[1]University of Alberta, [2]NIAAA/NIH

Nerve injury releases brain derived neurotrophic factor (BDNF) from spinal microglia. This increases dorsal horn excitability to promote 'central sensitization' and neuropathic pain. Although impairment of GABAergic and/or glycinergic inhibition contributes to this process, certain lines of evidence suggest that GABA release in the dorsal horn may increase after nerve injury. To resolve this contradiction, we exposed rat spinal cord neurons in defined medium organotypic culture to 200ng/ml BDNF for 6d to mimic the change in spinal BDNF levels that accompany nerve injury. Morphological and electrophysiological criteria and immunohistochemistry distinguished inhibitory tonic-islet central neurons from putative excitatory delay-radial neurons. Whole-cell recording in the presence of 1ìM tetrodotoxin showed that BDNF increased the amplitude of GABAergic and glycinergic miniature (mIPSCs) in both cell types. It also increased the frequency and amplitude of spontaneous, action potential dependent IPSCs (sIPSCs) in putative excitatory neurons. By contrast, BDNF reduced sIPSC amplitude in inhibitory neurons but frequency was unchanged. This increase in inhibitory drive to excitatory neurons and decreased inhibitory drive to inhibitory neurons seems inconsistent with the observation that BDNF increases overall dorsal horn excitability. One of several explanations for this discrepancy is that the action of BDNF in the substantia gelatinosa is dominated by previously documented increases in excitatory synaptic transmission rather than by impediment of inhibitory transmission.

3-B-26 Schwann cells generated from skin-derived precursors (SKP-SCs) are practically indistinguishable from nerve-derived Schwann cells on a variety of in vitro assays

Joseph Sparling[1], Jason R Plemel[1], Wolfram Tetzlaff[1]

[1]University of British Columbia

Schwann cells (SCs) are championed by several laboratories as transplantation candidate cells to repair the injured spinal cord. Rather than generating SCs from nerve biopsies, which causes peripheral nerve damage, we favour their generation from SKin-derived Precursors (SKP-SCs). Previously, we transplanted SKP-SCs into the injured rat spinal cord and observed better integration with the astrocyte-rich host tissue than that typically reported by other groups transplanting nerve-derived SCs (N-SCs). Thus, we hypothesized that SKP-SCs may interact with astrocytes better than N-SCs. Here we describe the results of a variety of in vitro assays exploring this potential difference. SKP-SCs and N-SCs were generated from neonatal rat tissue and expanded to passage 4 under identical culture conditions. To examine the interaction of both types of SCs with astrocytes, we measured the migration of these cells on astrocytes, as well as their integration with astrocytes in a confrontation assay. These assays failed to show significant differences between SKP-SCs and N-SCs, indicating that SKP-SCs do not interact with astrocytes any better than N-SCs do in vitro. The lack of differences between these two cells types was further confirmed by immunocytochemistry, Western blot, and quantitative PCR analyses assessing a variety of proteins and genes related to SC development. In conclusion, rather than demonstrating differences between SKP-SCs and N-SCs, our in vitro comparisons served to punctuate the high degree of similarity between these two cell types.

3-B-27 Interleukin-1 beta exposure alters sensory neuron excitability at ambient levels found in the nervous system

Pat Stemkowski[1], Amany Mohamed[1], Elena Posse de Chaves[1], Peter A Smith[1]

[1]University of Alberta

IL-1â is a potent pro-inflammatory cytokine, eliciting biological effects in the attomolar (aM) range (Orencole & Dinarello, Cytokine, 1:14, 1989). The presence of the high affinity IL-1â receptor, IL-1RI, on dorsal root ganglion (DRG) neurons (Liu et al., J Neurophysiol., 95:1478, 2006) implies that IL-1â can directly affect sensory function at ambient levels (<10-16 M) found in CSF (Alexander et al., Pain 116:213, 2005). We therefore exposed defined medium cultured rat DRG neurons to 1 aM IL-1â for 5-6 days and examined changes in their excitability. DRG neurons were classified as small (<30 µm), medium (30-40 µm), or large (>40 µm). Whole-cell recordings were used to assess alterations in neuron excitability and Western blots were used to assess ERK 1/2 activation. IL-1â (1 aM) significantly reduced rheobase and afterhyperpolarization duration in medium neurons and increased repetitive discharge evoked by depolarizing current ramps. The IL-1RI antagonist, IL-1ra (100 ng/ml), attenuated IL-1â induced increases in the excitability of medium neurons. However, the effect of IL-1â exposure in large neurons was less consistent and no changes were found in small neurons. These changes were weaker but similar to those seen with 100 pM IL-1â (Stemkowski & Smith, J Neurophysiol., 107:1586, 2012). There was also a concentration dependent increase in activation of ERK1, but not ERK2. We therefore suggest that ambient levels of IL-1â exert a 'cytokinergic tone' over the uninjured sensory nervous system.

3-B-28 Cadherin/β-catenin/scribble/β-pix complexes recruit synaptic vesicles to synapses via local actin polymerization

Yu Sun[1], Shernaz Bamji[1]

[1]University of British Columbia

We have previously demonstrated that cadherin/β-catenin complexes cluster synaptic vesicles at presynaptic sites. In the present study, we demonstrate that clusters of polymerized actin can recruit and maintain synaptic vesicles to discrete sites along the axon and that cadherin/β-catenin/scribble/β-pix complexes play an important role in this event. We show that scribble, a PDZ protein and β-pix, a Rac/Cdc42 guanine nucleotide exchange factor (GEF) localize to synapses in hippocampal neurons and form a complex with cadherin/β-catenin. This protein complex enhances actin polymerization at synapses. Using an RNA interference approach, we demonstrate that scribble and β-pix are essential for clustering synaptic vesicles at nascent synapses. Indeed, in cells expressing scribble or β-pix shRNAs, the localization of synaptic vesicles is disrupted. This phenotype can be rescued by cortactin overexpression suggesting that β-pix-mediated actin polymerization regulates vesicle localization.

3-B-29 Leptin modulates excitatory synaptic transmission onto VTA dopamine neurons

Jennifer Thompson[1], Stephanie L Borgland[1]

[1]University of British Columbia

Leptin is an adipocyte-derived hormone that can act in the brain to suppress feeding. Plasticity of midbrain dopamine neurons of the ventral tegmental area (VTA) is important for encoding information about cues that predict food reward. Leptin has been demonstrated to modulate the mesolimbic dopamine system at multiple levels including inhibiting feeding behaviour, reducing motivation for food rewards, and suppressing neuronal excitability. However, it is unknown how leptin modulates excitatory synaptic transmission onto dopamine neurons. Therefore, using whole cell patch clamp in mouse midbrain slices, we tested the effects of leptin on spontaneous firing firing and evoked NMDA receptor-mediated excitatory post synaptic currents (EPSCs) onto VTA dopamine neurons. Consistent with previous reports, we found leptin (100 nM) modestly and transiently depressed firing of VTA dopamine neurons. Interestingly, leptin (100 nM) caused a long lasting inhibition of NMDAR EPSCs onto dopamine neurons. Taken together, these results suggest that leptin has inhibitory action on dopamine neuronal activity and plasticity.

3-B-30 Influences of maternal care on hippocampal long-term potentiation is related to changes in NMDA receptor function

Yiu Chung Tse[1], Rosemary C Bagot[1], Huy-Binh Nguyen[1], Alice S Wong[1], Michael J Meaney[1], Tak Pan Wong[1]

[1]Douglas Mental Health University Institute

Variations in the amount of maternal care in the form of pup licking/grooming (LG) associate with robust differences in hippocampal learning and synaptic plasticity in adult offspring. Offspring from High LG mothers displayed stronger long-term potentiation (LTP) than Low LG offspring. Since N-methyl-D-aspartate receptor (NMDAR) mediates LTP formation and its function is modulated by CORT exposure, we hypothesized that changes in NMDAR function is responsible for the influences of maternal care on LTP. Electrophysiological recording revealed higher NMDAR function in the dentate gyrus in Low LG offspring compared to High LG offspring. Synaptic NMDAR protein level was also increased in Low LG offspring. Elevated NMDAR function could result in LTP deficit in Low LG offspring, since a partial NMDAR blockade by a low dosage

of APV (0.5 μM) rescued the LTP deficit in Low LG offspring. Nonetheless, similar partial blockade of NMDAR impaired LTP in slices from High LG offspring. This study showed that the influences of low maternal care on LTP could be caused by NMDAR hyperfunction.

3-B-31 Regulation of adrenergic control of synaptic NMDA receptor in the adult hippocampus by corticosterone

Yiu Chung Tse[1], Tak Pan Wong[1]

[1]Douglas Mental Health University Institute

Stress triggers release of molecular signals that regulate learning and memory. Signals such as norepinephrine (NE) and corticosterone (CORT) synergistically facilitate memory formation. This is likely mediated by enhancing a form of synaptic plasticity, long-term potentiation (LTP), in the hippocampus. However, a history of CORT exposure at hours prior to NE treatment prevents the facilitating effects of NE on LTP and memory formation. This delay-onset effect of CORT may curtail the initial stress-induced enhancement of synaptic plasticity after termination of a stressor to limit encoding of non-salient information. Mechanisms underlying this regulatory impact of CORT on NE-induced facilitation on LTP are unknown. Since activation of N-methyl-D-aspartate receptor (NMDAR) is pivotal to the formation of hippocampal LTP, we hypothesized that NE could regulate NMDAR function in the hippocampus. In addition, NE-induced changes in NMDAR are attenuated by a history of CORT treatment. Indeed, we found that NE suppresses synaptic NMDAR function recorded from the CA1 region of adult hippocampus. At 1-2 hours after CORT exposure, this NE effect was attenuated. The impact of CORT on NE-induced suppression of NMDAR associates with a loss of GluN2B-containing NMDAR. Blockade of GluN2B-containing receptor alone reduced NE-induced suppression of NMDAR. Taken together, our findings suggest that not only is NE-induced suppression of synaptic NMDAR attenuated 1-2 hours after CORT exposure, but this CORT effect may also be mediated by a loss of GluN2B-containing NMDAR.

3-B-32 Stress resilience is related to changes in hippocampal NMDA receptor function

Tak Pan Wong[1], Ixchel Montoya[1], Yiu Chung Tse[1], Juliana Hutter[1], Alice S Wong[1], Diane C Lagace[2]

[1]Douglas Mental Health University Institute, [2]University of Ottawa

Although stress increases the vulnerability to mood disorders such as major depressive disorder, many individuals who experience stressful life events do not develop psychopathologies. These individuals display stress resilience, of which the biological underpinnings are not well understood. Since N-methyl-D-aspartate receptor (NMDAR) antagonists have acute antidepressant effects in humans and can also cause extinction of hippocampal context-dependent fear conditioning in mice, we hypothesized that resilience and susceptibility to stress may be related to changes in hippocampal NMDAR function. Using a social defeat model of depression involving chronic stress, we characterized resilient and susceptible individuals in inbred C57 male adult mice. Susceptible mice express depression-like symptoms, such as social avoidance and anhedonia in sucrose preference testing; while resilient mice express a phenotype that is similar to control mice. Hippocampal electrophysiological recordings showed that susceptible mice have larger NMDAR-mediated synaptic responses, in comparison to resilient mice. Western blot analysis of NMDAR subunits also revealed a difference in expression between susceptible and resilient mice. This work provides evidence for the importance of the NMDAR in stress resilience.

3-B-33 Dendritic spines and pre-synaptic boutons are stable despite local deep hypothermic challenge and re-warming in vivo.

Yicheng Xie[1], Shangbin Chen[1], Timothy Murphy[1]

[1]University of British Columbia

Deep hypothermia to 20 °C is used clinically for major pediatric and adult surgical procedures. Patients recovering from these procedures can exhibit neurological deficits. In this study, using in vivo 2-photon (2-P) imaging in green/yellow fluorescent protein (GFP/YFP)-expressing transgenic mice we have determined whether deep hypothermia and re-warming can have relatively covert effects on dendritic spine or presynaptic bouton stability. Each dendritic spine represents a single excitatory synapse and their number can be reflective of injury or plasticity-induced changes in network function. We report that deep hypothermia and subsequent re-warming did not change the stability of dendritic spines or presynaptic boutons in mouse somatosensory cortex measured over 8 hours. As expected, deep hypothermia attenuated ongoing electroencephalography (EEG) activity over 0.1-80 Hz frequencies. The effects of EEG activity were fully reversible following re-warming. These results are consistent with deep hypothermia being a safe treatment which could be applied clinically to those undergoing major elective surgical procedures.

3-B-34 Inflammation and hypoxia: a novel trigger for long-term synaptic depression in the hippocampus

Jingfei Zhang[1], Aqsa Malik[1], Hyun B Choi[1], Brian A MacVicar[1]

[1]University of British Columbia

Inflammation and hypoxia share similar downstream pathways leading to enhanced inflammatory reactions in macrophages and neutrophils. Similarly, in the central nervous system (CNS), concurrent neuroinflammation and hypoxia, often observed in brain trauma, stroke, and neurodegenerative diseases, are thought to synergistically enhance brain damage. However, the mechanism of this synergy and, more importantly, whether these two stressors have combined impact on synaptic function, are still poorly understood. Here we show that when hypoxia and an inflammatory stimulus (either lipopolysaccharide (LPS) or beta amyloid (Aβ)) are combined, they act synergistically within minutes to trigger long-term synaptic depression (LTD) that requires activation of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) and glutamate receptor 2 (GluR2) mediated A-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) internalization. Hypoxia-inflammation LTD is unusual in that it is independent of N-methyl-D-aspartate receptors (NMDARs), metabotropic glutamate receptors (mGluRs) or patterned synaptic activity. This type of NADPH oxidase-dependent LTD may contribute to memory impairments and synaptic disruptions in CNS disorders with neuroinflammation and tissue hypoxia.

C – Disorders of the Nervous System

3-C-19 Karyopherin-alphas and their role in TDP-43 proteinopathy

Bryson Armstrong[1], William Jia[1], Max S Cynader[1]

[1]University of British Columbia

Aberrant cellular processing and targeting of TDP-43 has been implicated in a wide variety of neurological diseases such as frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) and amyotrophic lateral sclerosis (ALS). These diseases are characterized by the sequestration of TDP-43 into the cytoplasm of afflicted neurons, leading to the formation of ubiquitinated, cytoplasmic inclusions and an increased susceptibility to cellular insults. While the underlying causes of TDP-43 proteinopathy are unknown, we are investigating the role of a protein family known as the karyopherins in the nuclear targeting of TDP-43. Using co-immunoprecipitation in SH-SY5Y we determined that a major binding partner of TDP-43 is karyopherin-alpha 2 (KPNA2). Next, utilizing a high density peptide array comprised of overlapping peptide sequences from TDP-43 we found six regions where KPNA2 may directly interact with TDP-43. From these regions we developed six small, cell penetrating peptides designed to specifically inhibit the interaction between KPNA2 and TDP-43. Through the use of these synthetic peptides, we aim to determine if the disruption of TDP-43 nuclear import is sufficient to induce TDP-43 proteinopathy in vitro. By investigating the mechanism of TDP-43 proteinopathy we hope to elucidate the underlying causes of a number of debilitating neurological diseases.

3-C-20 The microtubule-associated protein p600 mediates neuronal survival through Ca2+ homeostasis and signaling

Camille Belzil[1], Gernot Neumayer[1], Alex P Vassilev[2], Kyoko L Yap[3], Hiroaki Konishi[4], Serge Rivest[5], Michael A Colicos[1], Mitsuhiko Ikura[3], Yoshihiro Nakatani[6], Minh Dang Nguyen[1]

[1]Hotchkiss Brain Institute, University of Calgary, [2]NICHD, National Institute of Health, [3]Ontario Cancer Institute, University of Toronto, [4]Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, [5]Centre de Recherches du Centre Hospitalier de l'Université Laval, Université Laval, [6]Dana Farber Cancer Institute, Harvard Medical School

In acute and chronic neurodegeneration Ca2+ mishandling and disruption of the cytoskeleton compromise neuronal integrity, yet how these pathological cellular events interface remains largely unexplored. We now report that the microtubule-associated protein p600 promotes neuronal survival through Ca2+ signaling. In primary cultured hippocampal neurons depleted of p600, glutamate-induced Ca2+ influx through NMDA receptors initiates a degenerative process characterized by ER stress, fragmentation and ER Ca2+ release via IP3 receptors. Stabilization of microtubules by taxol does not rescue neuronal survival, indicating that p600 does not promote survival through its MT-associated function. In search of an alternate mechanism, it was found that p600 forms a complex with CaM(calmodulin)/CaMKIIα after glutamate treatment. An atypical p600/CaM interaction was mapped to a C-terminal domain on p600. A peptide disrupting the p600/CaM interaction causes a 50% decrease in cell viability following endogenous NMDA receptor activation, indicating that a direct p600/CaM interaction promotes neuronal survival in physiological conditions. p600 is similarly required for neuronal survival in vivo as indicated by the analysis of hypomorph p600 null mice: Spontaneously active neurons on the cortical plate of these animals degenerate as evidenced by an increase in cleaved caspase 3. Additionally, fewer neurons are found in the cortical plate of these animals. In sum, we identify p600 as a cytoskeletal molecule with a role in Ca2+ signalling that may be disrupted in neurodegeneration.

3-C-35 A switch in synaptic association of Neuroligin proteins in successful aging

Cyril Bories[1], Zoé Husson[1], Yves De Koninck[1]

[1]Université Laval

Growing number of evidences suggest that imbalance in excitation/inhibition (E/I) ratio toward inhibition underlie age-related behavioural deficits. Here we unveil stereotyped exploratory behaviour in aged rats. We then assessed synaptic proteins in prefrontal cortex and confirmed an association between E/I ratio (PSD95/Gephyrin) and stereotypy occurrence. Because stereotyped behaviours are also hallmark of psychiatric-related disorders and that change E/I ratio is also suspected to contribute to these disorders we hypothesized that molecular pathways such as autism-related Neuroligin also implicated in the control of E/I ratio, may be affected in aging process. Consistently we reveal an imbalance of Neuroligin2/Neuroligin1 ratio toward Neuroligin2. Finally when segregating aged animals into impaired and unimpaired groups we unveil an unexpected shift of association of Neuroligin2 with PSD95 in aged unimpaired animals. This shift may underlie distinct trajectories in the evolution in E/I balance and may be a mechanism to preserve synaptic balance in successful cognitive aging.

3-C-36 Understanding the role of FMRP in synaptic plasticity in both the male and female brain

Crystal Bostrom[1], Brennan Eadie[2], Brian R Christie[1]

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

Fragile-X syndrome (FXS) is the most common form of inherited intellectual impairment. FXS is caused by transcriptional repression of the Fmr1 gene on the X chromosome and results in the loss of Fragile-X Mental Retardation Protein (FMRP). FMRP is thought to be involved in the trafficking and translational regulation of mRNAs, and has been associated with mRNAs coding for proteins involved in neuronal development and synaptic function. We have recently shown that Fmr1 knockout (KO) causes significant impairments in structural and functional plasticity in the dentate gyrus (DG) subfield of the hippocampus in male mice. FXS has been studied far less intensively in females, who are most often heterozygous (Het) for the mutation. The current study tested the hypothesis that functional plasticity in the DG is impaired in the mouse model of FXS in females. Field electrophysiology revealed a significant impairment in long-term potentiation in male Fmr1 KO and female Fmr1 Het mice that was associated with decreased N-methyl-D-aspartate receptor function. Conversely, female Fmr1 KO mice did not show reduced synaptic plasticity. These findings suggest that a reduction in FMRP can also impair synaptic plasticity in female mice, but that the complete absence of the protein engages compensatory mechanisms. Future studies will seek to identify these mechanisms.

3-C-37 Hyperexcitable and hypoexcitable reciprocally connected neurons in the thalamus of a genetic model of absence epilepsy

Stuart Cain[1], John R Tyson[1], Terrance P Snutch[1]

[1]University of British Columbia

We previously identified the first functional genetic mutation in the Cacna1h (Cav3.2 T-type calcium channel) gene of the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) absence epilepsy model, which correlates with seizure expression. In Cav3.2 channels expressed in HEK cells the GAERS mutation induces a robust gain-of-function, selectively in Cav3.2 splice variants containing exon 25. Reticular thalamic nucleus (nRT) neurons, which express both Cav3.2 and Cav3.3 T-type channels, exhibit more sustained burst firing in GAERS than in control animals in a frequency and age-dependent manner. Furthermore, the expression of the mutation-sensitive Cav3.2 splice variant increases with development, peaking when the animals begin to experience seizures. We hypothesize that increased expression of the hyperexcitable Cav3.2 splice variant with development leads to more sustained burst firing in the nRT, promoting the propagation of seizures in this model. Contrary to the enhanced burst firing profile observed in the nRT, we found that ventrobasal thalamic (VB) neurons, which primarily express Cav3.1, display decreased burst firing properties. Significantly more current injection is required to generate rebound bursts in the VB neurons of both neonatal and adult GAERS compared to control animals in thalamic slices, correlating with an increase in the current density of the HCN channel-mediated Ih current. We predict that this occurs via a mechanism whereby the VB neurons have developed a compensatory decrease in burst firing ability as a result of the hyperexcitable upstream nRT.

3-C-38 Effects of mPFC NMDA receptor dysfunction beginning in adolescence on sociability and preference for social novelty in adult mice.

Ryan Craft[1], Thuyanh Nguyen[1], Janet Finlay[1]

[1]Western Washington University

Deficits in social functioning are a hallmark symptom of schizophrenia and autism. Severity of social interaction deficits can predict an individual's overall quality of life. Current theories of schizophrenia and autism suggest that N-methyl-D-aspartate (NMDA) receptor dysfunction in development may be a biological substrate. Our study examined whether NMDA receptor dysfunction in the medial prefrontal cortex (mPFC) beginning in adolescence

(PN28-30) impairs social interaction in the adult. mPFC NMDA receptor dysfunction was induced by microinjection of AAV-Cre recombinase into the mPFC of transgenic mice floxed at the NR1 subunit of the NMDA receptor - producing NMDA receptor dysfunction in all areas exposed to AAV-Cre. Social interaction and social novelty preferences were examined by assessing latency and amount of time adult mice spent in chambers containing (1) an unfamiliar conspecific versus an empty cage and (2) a newly introduced conspecific versus a familiar conspecific. Relative to control, NR1 knockout mice (n=12-14/group) exhibited reduced latency to enter a chamber containing an unfamiliar conspecific (4.8 ± 1.8 vs. 24.4 ± 7.6 s). Additionally, control mice preferred a chamber containing a mouse versus a chamber containing an empty cage (159.5 ± 17.9 vs. 88.7 ± 14.8 s). This preference is not apparent in NR1 knockout mice (137.4 ± 17 vs. 114.8 ± 18.9 s). The present results support our hypothesis that mPFC NMDA receptor dysfunction beginning in adolescence may contribute to impaired social interaction in conditions such as schizophrenia and autism.

3-C-39 Characterizing cellular changes in the DRG following SCI: focus on vasculature

Mark Crawford[1], Leanne M Ramer[1], Wang Dan Yang[1], Adrian P Van Stolk[1], Matthew S Ramer[1]

[1]University of British Columbia

While a great deal is known about cellular reactions in the spinal cord following injury, less is known about comparable changes occurring in the periphery. This is important since peripheral plasticity of neurons and glia may underpin debilitating sequelae of spinal cord injury (SCI) such as pain and autonomic dysreflexia. Here we present a preliminary investigation of neuronal and non-neuronal alterations in dorsal root ganglion (DRG) cytoarchitecture. Three lines of evidence lead to our hypothesis that angiogenesis occurs in the DRG following SCI: 1. Neuronal hypertrophy: Sensory neurons expressing the vanilloid receptor TRPV1 undergo selective hypertrophy in L6/S1 DRG neurons one month following SCI. Subpopulations of DRG neurons known to co-localize with TRPV1 (P2X3/IB4) do not increase in size after SCI. However, a subset of TRPV1-positive DRG neurons express GFRα3, the receptor for the GDNF family member artemin, but not P2X3/IB4. Hypertrophy occurs within this TRPV1 subset. Interestingly, artemin is expressed by vascular smooth muscle cells. 2. Sympathetic sprouting: Sympathetic axons are not normally present within the DRG (except for where they innervate DRG vasculature). SCI prompts sympathetic ingrowth and formation of pericellular "baskets." 3. Non-neuronal cellular changes after SCI: Macrophages accumulate and glial reactivity increases in the DRG following SCI. Based on the findings of others, we reason that macrophages may provide endothelial tip cell fusion sites in post-SCI DRG angiogenesis.

3-C-40 Exome sequencing in familial bipolar disorder

Cristiana Cruceanu[1], Amirthagowri Ambalavanan[1], Dan Spiegelman[2], Julie Gauthier[2], Ronald G Lafrenière[2], Martin Alda[3], Guy A Rouleau[2], Gustavo Turecki[1]

[1]McGill University, [2]University of Montreal, [3]Dalhousie University

Bipolar disorder (BD) is a complex psychiatric condition characterized by manic and depressive episodes. In spite of the strong support for the role of genetics, previous approaches have had minimal success in identifying disease-causing genes. This is likely because of high levels of heterogeneity in the sample sets used, but this problem may be minimized by focusing on a well-defined sub-phenotype of BD. Our group has led efforts to characterize BD patients that respond positively to Lithium (Li) therapy, and shown that Li-response clusters in families. Research in BD genetics to date has presumed that common variants in a small subset of genes are the cause for BD. Our hypothesis is that BD is caused by highly penetrant rare variants in a large number of different genes across the population. We focused on a well-defined clinical subtype of BD (Li-responsive) to minimize clinical heterogeneity, and we used next-generation exome sequencing in all affected individuals from multi-generational family units. To identify relevant BD susceptibility genes we prioritized rare variants that segregate with affected status within each family. We found that each family shared a limited number of potentially highly penetrant (e.g. protein-truncating or missense) or functionally relevant (e.g. 3'UTR, 5'UTR, splicing) variants. By focusing on rare variants, rather than common variants, we hope to have narrowed in on the key genes and biochemical pathways that play an important role in bipolar disorder and can lead directly to clinically relevant diagnostic and therapeutic applications.

3-C-41 Characterization of juvenile ferrets following induction of hydrocephalus with kaolin

Domenico Di Curzio[1], Terry L Enno[1], Richard J Buist[1], Marc R Del Bigio[1]

[1]University of Manitoba

Hydrocephalus is a common neurological condition in humans characterized by altered cerebrospinal fluid (CSF) flow with enlargement of ventricular cavities in the brain. A reliable induced model of hydrocephalus in ferrets would be beneficial to test preclinical hypotheses. Our objective is to characterize behavioural, structural, and histological changes in juvenile ferrets. Ferrets were given a kaolin (aluminum silicate) injection at 14 days into the cisterna magna, which causes an inflammatory scar that obstructs CSF flow. Two days later, magnetic resonance imaging was used to assess ventricle size, which was repeated weekly until 8 weeks age. Behaviour was also examined

thrice weekly. Compared to age-matched saline-injected controls, hydrocephalic ferrets weighed significantly less, their postures were impaired, and they became hyperactive until severely debilitated. They developed moderate to severe ventriculomegaly (ventricle to brain area ratio 11.6% vs 1.9%, $F(1,64) = 37.935$, $p < .001$) and displayed gross white matter destruction. Reactive astroglia and microglia detected by GFAP and Iba1 immunostaining were apparent in white matter, cortex, and hippocampus. There was an age-related increase in Active Caspase 3 staining for apoptosis (4.4 to 8.8% vs 6.7 to 1.2%) and Ki67 staining for cellular proliferation (5.8 to 8.3% vs 3.4 to 15.1%) in the subventricular zone and dentate gyrus. The hydrocephalus induced periventricular disturbances match those shown by other species; the ferret should prove useful for testing hypotheses about white matter damage and protection.

3-C-42 Blocking aggregation does not prevent the cytotoxicity induced by mutant tdp-43

Rui Liu[1], William Jia[1], Max S Cynader[1]

[1]Brain Research Center, University of British Columbia

TAR DNA-binding protein 43 (TDP-43) is a protein that is thought to be involved in the pathology of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD). Several TDP-43 mutations have been found to be associated with familial ALS and FTLD. In patients with these neurodegenerative diseases, TDP-43 does not remain in its normal nuclear location, but instead forms insoluble aggregates in both the nucleus and cytoplasm of affected neurons. Accumulating evidence shows that TDP-43 is abnormally cleaved by caspase-3 and that the truncated inclusions are toxic to cells. These mislocalized, truncated, and aggregated proteins appear to be an important part of the pathogenesis of these neurodegenerative diseases. To obtain new insights into the role of aggregation in the cell death process, we used high density peptide array analysis to identify regions in TDP-43 that are bound by TDP-43 itself. Based on the identification of zones of possible self binding, we designed candidate peptides that might be able to block TDP-43 self binding and aggregation. We found that two of the synthetic peptides identified with this approach could effectively inhibit the formation of TDP-43 protein aggregates in a concentration-dependent manner in Hela cells in which a mutated human TDP-43 gene was overexpressed. However, despite blocking aggregation, these peptides did not reduce or prevent cell death. Our results suggest that TDP-43 aggregation is associated with the cell death process rather than being a direct cause.

3-C-43 Neuroprotective mechanisms of the antidepressant drug phenelzine and its metabolite B-phenylethylidenehydrazine

Erin MacKenzie[1], Dmitry Matveychuk[1], Emerson Nunes[1], Andrew Holt[1], Kathryn G Todd[1], Mee-Sook Song[1], Glen B Baker[1]

[1]University of Alberta

The antidepressant drug phenelzine (PLZ) and its recently characterized metabolite β-phenylethylidenehydrazine (PEH) have been shown by us to be neuroprotective in a gerbil model of global ischemia. We have conducted studies on possible neurochemical mechanisms contributing to their protective effects, and the results are presented here. PLZ inhibited the activities of monoamine oxidase (MAO) and GABA-transaminase (GABA-T), resulting in a decrease in the formation of toxic amine oxidation products and an increase in rat brain levels of GABA (300-400% of control levels at peak), respectively. In vitro, PLZ inhibited human polyamine oxidase (PAO; which is overexpressed in Alzheimer's disease) and sequestered the reactive aldehyde formaldehyde (formed by the action of PAO). It also altered glutamate transporter expression, decreased astrocytic glutamate levels, upregulated neuronal and astrocytic BDNF mRNA, and enhanced astrocytic BDNF release. Like PLZ, PEH inhibited the activity of human PAO, elevated rat brain levels of GABA and sequestered formaldehyde (shown by mass spectrometric analysis). Unlike PLZ, PEH did not appreciably inhibit MAO activity ex vivo, and therefore would likely not be a risk factor for causing blood pressure increases when administered with tyramine-rich foods. The protective mechanisms of PLZ and PEH suggest that these drugs may be effective for the treatment of or for the delay of disease progression in conditions where toxic aldehydes and/or glutamate contribute to the degenerative processes. Funds provided by CIHR and the University of Alberta.

3-C-44 Early detection of Alzheimer's disease via accurate measurements of hippocampal volume

Ammar Mahmood[1]

[1]Simon Fraser University

Alzheimer's dementia is a serious neurodegenerative disease associated with the progressive degeneration of critical areas in the nervous system, notably the cerebral cortex and hippocampus. The incidence of such neurodegenerative diseases has steadily increased in western societies over the past several decades. Despite the fact these illnesses vary in severity, these dementias typically involve a substantially decreased quality of life for those affected sufferers. This is compounded by the fact numerous conditions do not currently have significant treatment strategies for disease modification. Therefore, there is an urgent necessity to develop biomarkers that

may assist in the detection of Alzheimer's disease in its preliminary stages. One such promising biomarker that may assist in the detection of Alzheimer's disease is the volume of the hippocampus. A collection of segmentations generated through automatic algorithms were subsequently manually corrected to show that a decrease in hippocampal volume is correlated with the progression of Alzheimer's dementia. The significance of manual corrections lies in obtaining accurate hippocampal volumes with relation to disease status, as automatic algorithms tend to overestimate volumes and potentially mask the effects of disease. Using magnetic resonance images, the volumes of the left hippocampus from a set of 486 subjects with mild and severe Alzheimer's were used to assess their ability to inform about presence of dementia as compared to healthy-age matched controls upon manual correction of segmentations.

3-C-45 Immunomodulatory and neuroprotective properties of the antioxidant TEMPOL, a potential therapeutic in multiple sclerosis

Sarah Neil[1], Elena Cavazzi[1], Katerina Dorovini-Zis[1], Jacqueline Quandt[1]

[1]University of British Columbia

Reactive oxygen and nitrogen species are implicated in inflammatory-mediated damage to the central nervous system (CNS) in multiple sclerosis (MS), as contributors to vascular changes, demyelination, axonal loss, and neurodegeneration. We have shown that oral administration of the antioxidant TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), a stable nitroxide radical, reduces incidence and severity of disease in an animal model of MS, experimental autoimmune encephalomyelitis (EAE). We hypothesize that TEMPOL limits disease early by regulating pathogenic autoimmune responses, limiting alterations to the blood-brain barrier (BBB), and providing neuroprotection from oxidative damage. TEMPOL alters the magnitude of T cell responses in vitro and also reduces the proinflammatory nature of immune cell responses in EAE. Flow cytometry revealed fewer immune cells in the CNS and enrichment of CD8 T cells in lymphoid tissues of TEMPOL-fed EAE mice compared to controls. Using brain microvessel endothelial cells as an in vitro model of the BBB, we have shown that TEMPOL enhances BBB integrity at rest and limits barrier changes secondary to inflammatory mediators such as tumor necrosis factor (TNF)-alpha. In preliminary studies, TEMPOL limits cytokine-mediated upregulation of cell adhesion molecules that mediate immune cell recruitment across the BBB. Taken together, these immunomodulatory properties identify TEMPOL as a promising novel therapeutic in the treatment of MS.

3-C-46 Myelin inhibits oligodendrocyte maturation by increasing transcriptional inhibitors

Jason Plemel[1], Sohrab Manesh[1], Benjamin Richards[1], Lisa Anderson[1], Joseph S Sparling[1], Wolfram Tetzlaff[1]

[1]University of British Columbia

Myelin loss is a hallmark of multiple sclerosis and promoting central nervous system myelin repair has become a major therapeutic target. The mechanism underlying the failure of oligodendrocyte maturation in the CNS remains unknown, but it is hypothesized that environmental cues act to inhibit the maturation of oligodendrocyte precursor cells (OPCs). One factor that is known to inhibit OPC maturation is myelin, and given the presence of myelin debris following demyelination and trauma, this mechanism seems likely to play a role in impairing CNS remyelination. We demonstrate that OPCs cultured on myelin have a robust inhibition of their maturation, characterized by decreased expression of immature and mature oligodendrocytes marker, impaired production of myelin gene products as well as stalled morphological complexity. OPCs in contact with myelin exit the cell cycle and down regulate OPC markers normally and contact with myelin did not increase astrogenesis or cell death. Measuring the expression of transcription factors that prevent OPC differentiation into mature oligodendrocytes, we found that contact with myelin increased the expression of Inhibitor of Differentiation family (ID) members 2 and 4. Forced expression of ID2 and ID4 in OPCs has been shown to decrease the percentage of cells expressing mature oligodendrocyte markers; hence, enhanced expression of ID2 and ID4 due to myelin exposure is sufficient to block OPC maturation. In conclusion, upregulation of ID2 and ID4 provide a mechanistic explanation how myelin blocks oligodendrocyte lineage cell maturation.

3-C-47 Aberrant localization of FUS and TDP43 is associated with misfolding of SOD1 in Amyotrophic Lateral Sclerosis

Eddie Pokrishevsky[1], Leslie I Grad[1], Ian Mackenzie[1], Neil R Cashman[1]

[1]University of British Columbia

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by the progressive death of motor neurons; the precise pathological mechanism of which remains unknown. Several studies have hypothesized that the cytosolic trapping and aggregation of the predominantly nuclear Fused in Sarcoma (FUS) and TAR-DNA Binding Protein 43 (TDP43) are the potential cause of ALS. Similarly, misfolding of Cu/Zn Superoxide Dismutase (SOD1) has been associated with both sporadic and familial forms of ALS, even in the absence of SOD1 mutations. Aberrant misfolding,

localization and aggregation of all three proteins results in clinically indistinguishable motor neuron disease, and therefore could be involved in a common pathological pathway. Here, we show by both immunofluorescence microscopy and immunoprecipitation that expression of cytosolic FUS variants, but not wild-type, is associated with the misfolding of SOD1, as determined by conformational-specific antibodies. Additionally, we find that over-expression of human wild-type, or expression of a cytosolic mutant TDP43, also induce SOD1 misfolding. Patient spinal cord necropsy immunohistochemistry revealed misfolded SOD1 in perikarya and motor axons of SOD1-familial ALS, and in motor axons of R521C-FUS familial ALS and sporadic ALS with cytoplasmic TDP43 inclusions. Our data demonstrate for the first time a direct relationship between expression of ALS-linked FUS and TDP43 mutant, and SOD1 misfolding, suggesting that all three key proteins implicated in ALS associate in a common pathological process.

3-C-48 Mechanisms and efficacy of transient aortic occlusion for treatment of acute ischemic stroke

Gomathi Ramakrishnan[1], Bin Dong[1], Glenn Armitage[1], Kathryn Todd[1], Ashfaq Shuaib[1], Ian R Winship[1]

[1]University of Alberta

The occlusion of a blood vessel results in ischemic stroke. Irreversible brain damage results in the ischemic core immediately downstream of the occlusion, where perfusion falls below 20% of baseline blood flow. The area surrounding the core with partially preserved blood flow is called the ischemic penumbra. This partial maintenance of blood flow in the penumbra is due to the presence of auxiliary channels of blood flow termed the cerebral collaterals. Augmenting blood flow through these collaterals may reduce damage due to stroke. In our study we used transient occlusion of descending aorta (TOA) for 45 minutes to increase the global cerebral perfusion after occlusion of middle cerebral artery (MCAo). The blood flow maps were measured using Laser Speckle Contrast Imaging (LSCI) prior to and during TAO. LSCI maps during TAO shows increase in collateral blood flow with no change in MCA-ACA anastomoses but with increase in blood vessel diameter. Infarct volume was measured at one week after thromboembolic MCAo involving injection of either a small or large blood clot. After injection of a small clot, TAO reduces the infarct volume primarily by shifting the clots downstream and preserving the striatum. After "large clot" MCAo, the LSCI maps show a much more dramatic change in collateral blood flow. Ongoing studies are confirming the neuroprotective efficacy and shift vs collaterals mechanism in these larger MCAo groups. Given the high failure rate of thrombolysis, augmenting collateral blood flow offers an important alternative neuroprotective strategy for ischemic stroke.

3-C-49 Chronic Nicotine Upregulates α4* Nicotinic Receptors in Glutamatergic and GABAergic Neurons of the Medial Prefrontal Cortex

Anthony Renda[1], Raad Nashmi[1]

[1]University of Victoria

Nicotine addiction is the leading cause of premature and preventable mortality worldwide and is responsible for 37,000 deaths per year in Canada alone. Nicotine activates neuronal nicotinic acetylcholine receptors (nAChRs), which are a major class of ligand-gated cation channels that enhance neuronal excitability and neurotransmitter release. Chronic nicotine exposure in smokers' brains results in increased nAChR expression in various brain regions. In order to accurately quantify nicotine-induced changes subcellularly in specific neuronal subtypes of various brain regions we have genetically engineered mice to express α4 nAChR subunits tagged with yellow fluorescent protein. These mice have been exposed to chronic nicotine via mini-osmotic pump implants (2 mg/kg/hr for 10 days) in order to investigate nicotine-induced receptor changes in the medial prefrontal cortex, a brain region implicated in executive control over regions of reward and habit formation. We observed an upregulation of α4* nAChRs on the somata of both glutamatergic and GABAergic neurons in all layers (1-6) of the medial prefrontal cortex. Chronic nicotine exposure also upregulated α4* receptors in the medial perforant pathway of the hippocampus. We propose that nicotine-induced receptor changes in the prefrontal cortex not only modifies local circuit activity but also impacts neuronal function in the brain's reward centre through their long range projections. Future, experiments will examine the effect of chronic nicotine exposure to an oral self-administration choice of nicotine vs water.

3-C-50 Microtubule stability, Golgi organization, and transport flux require dystonin-a2/MAP1B interaction

Scott Ryan[1], Kunal Bhanot[1], Andrew Ferrier[1,3], Yves De Repentigny[1], Alphonse Chu[2], Alexandre Blais[2], Rashmi Kothary[1,3,4]

[1]Ottawa Hospital Research Institute, [2]Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, [3]Department of Cellular and Molecular Medicine, University of Ottawa, [4]Department of Medicine, University of Ottawa

Loss of function of dystonin cytoskeletal linker proteins causes neurodegeneration in dystonia musculorum (dt) mutant mice. While much investigation has focused on understanding dt pathology, divergent functions of dystonin isoforms remain unclear. Here, we highlight novel functions of the dystonin-a2 isoform in mediating microtubule (MT) stability, Golgi organization, and flux through the secretory

pathway. Using dystonin mutant mice combined with isoform-specific loss of function analysis, we find dystonin-a2 is bound to MT associated protein 1B (MAP1B) in the centrosomal region, where it maintains MT acetylation. In dt neurons, absence of the MAP1B/dystonin-a2 interaction results in altered MAP1B perikaryal localization, leading to MT deacetylation and instability. Deacetylated MTs result in Golgi fragmentation and prevent anterograde trafficking via motor proteins. Maintenance of MT acetylation through trichostatin A (TSA) administration or MAP1B overexpression in vitro, mitigates the observed defect. These aberrations are apparent in pre-phenotype dorsal root ganglia (DRG) and primary sensory neurons, suggesting they are causal in the dt disorder.

3-C-51 Loss of neuronal potassium/chloride cotransporter 3 (KCC3) is responsible for the degenerative phenotype in a conditional mouse model of hereditary motor and sensory neuropathy associated with agenesis of the corpus callosum

Masoud Shekarabi[1], Randal Moldrich[2], Rasheed Sarah[1], Adele Salin-Cantegrel[1], Janet Laganière[1], Daniel Rochefort[1], Pascale Hince[1], Karine Huot[1], Rebecca Gaudet[1], Nyomann Kurniawan[2], Susana Sotocinal[3], Jennifer Ritchie[3], Patrick A Dion[1], Jeffrey S Mogil[3], Linda Richards[2], Guy A Rouleau[1]

[1]CHUM Research Centre, University of Montreal, [2]The University of Queensland, [3]McGill University

Disruption of the potassium/chloride cotransporter 3 (KCC3), encoded by the SLC12A6 gene, causes hereditary motor and sensory neuropathy associated with agenesis of the corpus callosum (HMSN/ACC), a neurodevelopmental and neurodegenerative disorder affecting both the peripheral nervous system and CNS. However, the precise role of KCC3 in the maintenance of ion homeostasis in the nervous system and the pathogenic mechanisms leading to HMSN/ACC remain unclear. We established two Slc12a6 Cre/LoxP transgenic mouse lines expressing C-terminal truncated KCC3 in either a neuron-specific or ubiquitous fashion. Our results suggest that neuronal KCC3 expression is crucial for axon volume control. We also demonstrate that the neuropathic features of HMSN/ACC are predominantly due to a neuronal KCC3 deficit, while the auditory impairment is due to loss of non-neuronal KCC3 expression. Furthermore, we demonstrate that KCC3 plays an essential role in inflammatory pain pathways. Finally, we observed hypoplasia of the corpus callosum in both mouse mutants and a marked decrease in axonal tracts serving the auditory cortex in only the general deletion mutant. Together, these results establish KCC3 as an important player in both central and peripheral nervous system maintenance.

3-C-52 Skin changes in amyotrophic lateral sclerosis

Sneha Sheth[1], Pierre Zwiegers[1] , Christopher A Shaw[1]

[1]Neural Dynamics Research Group

There is a growing body of literature on the unique skin changes that occur in ALS patients, which has largely been overlooked by most researchers in the West. This dates back to the nineteenth century when Charcot first observed that ALS patients do not develop bedsores as other bedridden patients, and was subsequently verified in literature (Toyokura, 1975, 1977; Furukawa and Toyokura,1976). The most characteristic skin change is the "delayed return phenomenon" of pinched skin. Morphological skin changes include collagen fibril abnormalities and presence of amorphous material between collagen bundles, while at a biochemical level there is an increase of MMP-9 which leads to an overall decrease in collagen IV perhaps due to its high turnover rate during degradation. While the skin changes may seem merely coincidental in the motor neuron disease, their common ectodermal origin may provide some insight into the pathogenesis of the disease or reveal mechanisms common to both. Since there has not been much research published on these skin changes in mice with the ALS phenoytype, the current study will demonstrate if there are any differences in collagen IV and MMP-9 levels in the skin and spinal cord of transgenic mice with mSOD mutation, through western blot and immunohistochemistry analysis. The results from this study can be used to track the temporal progression of these skin changes with respect to motor neuron death which could potentially lead to the development of a novel diagnostic tool using skin to track the onset and/or progression of ALS.

3-C-53 Connexin43 in reactive astrocytes promotes glioma invasion

Wun Sin[1], Annie Aftab[1], John Bechberger[1], Justin Leung[1], Helen C Chen[1], Christian C Naus[1]

[1]University of British Columbia

The lethality of glioblastomas is mainly due to difficulty in complete surgical removal of invasive glioma cells at the tumor-host interface. Therefore, the interaction of tumor cells with its microenvironment has a pivotal role in determining the extent of glioma invasion into brain parenchyma. In this study, we examined whether intercellular interaction mediated by the connexin family of proteins is critical in this process. In particular, we focused on the role of connexin43 (Cx43) that is widely expressed in adult glial cells encountered by the invading glioma cells. In order to alter Cx43 levels in glioma and host cells in an immune-competent animal, we employed syngenic intracranial implantation of fluorescently-labeled GL261 glioma cells into C57BL/6 mice that were either wild type or conditional knockout. We observed the colocalization of the

astrocyte maker glial fibrillary acidic protein (GFAP) and Cx43 protein directly surrounding the tumor mass. Single or small clusters of GL261 cells that broke away from the tumor core contributed to the uneven tumor-host interface, evidence of an invasive front. Such invasion was reduced when Cx43 was eliminated in Nestin-Cre:Cx43fl/fl mice where Cx43 was deleted from neural progenitors and astrocytes, and when glioma cells with knockdown Cx43 expression were introduced into wild type brains. Glioma invasion was not attenuated by expression of a channel-defective Cx43 mutant (T154A) in glioma cells. Our results demonstrate that glioma invasion into brain is supported by Cx43-mediated interactions between glioma and astrocyte

3-C-54 A regional and subcellular examination of dystrophin localization in the mouse cerebellum

Wanda Snow[1], Mark Fry[1], Judy E Anderson[1]

[1]University of Manitoba

Duchenne muscular dystrophy (DMD) results from an absence of dystrophin, normally present in both muscle and brain tissue, including Purkinje neurons of the cerebellum. A lack of dystrophin in these neurons has been postulated to account for cognitive deficits associated with DMD. Studies examining cerebellar dystrophin localization thus far, however, have emphasized the vermal region, associated with motor function. There are no detailed reports examining dystrophin distribution in the lateral region, implicated in cognition. We examined dystrophin localization regionally (vermal and lateral) and subcellulary (soma, proximal, and distal dendritic regions) in the mouse cerebellum using immunocytochemistry and found homogeneous localization patterns in both regions, namely punctate labeling of dystrophin along the somatic and dendritic membranes. Dystrophin resided along the entire length of Purkinje dendrites. Quantitatively, we noted a 30% increase in the density of dystrophin puncta in Purkinje neurons of the lateral region relative to the vermal. Subcellulary, dystrophin density was highest in Purkinje neuron somata. These data provide additional support for the theory that an absence of dystrophin in cerebellar Purkinje neurons negatively impacts cerebellar functioning and extend this to the lateral functional zone, the cerebellar region associated with cognition. Based on the present findings, future research examining the cerebellum's role in cognitive dysfunction in DMD and related animal models of the disorder should focus on the lateral cerebellum

3-C-55 Overexpression of APP increases RCAN1 expression by inhibiting its degradation

Weihong Song[1], Yili Wu[1]

[1]University of British Columbia

Alzheimer's disease (AD) is the most common cause of dementia in aged population. Extracellular neuritic plaques, intracellular neurofibrillary tangles and neuron loss are three pathological hallmarks of AD. Down syndrome (DS) patients inevitably develop characteristic AD neuropathology after middle age. Amyloid ?À-protein (A?À), the proteolysis product of amyloid precursor protein (APP), is the major component of neuritic plaques. Overexpression of APP increases the risk of AD. In addition to plaque formation, previous study showed that A?À increased regulator of calcineurin 1 (RCAN1) expression at mRNA level. RCAN1 is highly expressed in human brain and is significantly increased in the brain of AD and DS patients. Our previous work has shown that RCAN1 overexpression could induce neuron apoptosis by caspase 3 activation. In this study, we found that APP overexpression significantly increases RCAN1 protein expression in both human cells and APP transgenic mice. We also found that the induction of RCAN1 by APP overexpression depends on holo-APP?Cbut not A?À, secreted APP and other APP cleavage products. Furthermore, we have determined that RCAN1 upregulation by APP is occurred at post-transcription level and it is caused by inhibiting RCAN1 degradation.

3-C-56 Ketogenic diet improves forelimb motor function after spinal cord injury

Femke Streijger[1], Ward T Plunet[1], Jae H Lee[1], Jie Liu[1], Clarrie K Lam[1], Brett J Hilton[1], Bas L Fransen[1], Keely A Matheson[1], Peggy Assinck[1], Brian K Kwon[1], Wolfram Tetzlaff[1]

[1]ICORD, University of British Columbia

High fat, low carbohydrate ketogenic diets (KD) were developed in the 1920s and are today an established and effective non-pharmacological treatment for some forms of drug-resistant epilepsy. Here, we investigated the efficacy of KD as a treatment for acute cervical spinal cord injury (SCI) in rats. Starting 4 hours following C5 hemi-contusion injury animals were fed either a standard carbohydrate based diet or a KD formulation with lipid to carbohydrate plus protein ratio of 3:1. The forelimb functional recovery was evaluated for 14 weeks, followed by quantitative histopathology of the spinal cord. Post-injury 3:1 KD treatment resulted in increased usage and range of motion of the affected forepaw. Furthermore, KD improved pellet retrieval with recovery of wrist and digit movements. Importantly, after returning to a standard diet after 12 weeks of KD treatment, the improved forelimb function remained stable. Histologically, the spinal cords of KD

treated animals displayed smaller lesion areas and more grey matter sparing. In addition, KD treatment increased the number of glucose transporter-1 positive blood vessels in the lesion penumbra and monocarboxylate transporter-1 expression. Post-injury KD effectively promotes functional recovery and is neuroprotective after cervical SCI. These beneficial effects may at least in part be mediated via increased vascular density and expression of transporters important for energy metabolism. Our data suggest that current nutritional treatment standards which include higher carbohydrate contents should be revisited.

3-C-57 Retromer (VPS35) dysfunction in Parkinson's disease.

Lucia Tapia[1], Austen A Milnerwood[1], Carles Vilarino-Guell[1], Matthew J Farrer[1]

[1]University of British Columbia

Parkinson's disease (PD) is a debilitating neurodegenerative disease that affects 1-2% of the population by 65 years, increasing to 4-5% by 85. We recently discovered a novel mutation within vacuolar protein sorting 35 (VPS35) in autosomal dominant late-onset parkinsonism. VPS35 is a central component of the retromer cargo-recognition complex, critical for membrane-protein recycling and endosome-trans-Golgi trafficking. A greater understanding of VPS35 biology and pathophysiology is essential to the development of therapeutic interventions aiming to prevent or delay progression of this devastating disease. We have explored the sub-cellular localization of wild-type (WT) and mutant VPS35 proteins together with the previously described VPS35-specific cargo binding proteins to determine pathophysiological alterations in protein trafficking. Additionally, we are looking for new binding partners whose intracellular transport may be altered by VPS35 mutation. Interestingly, VPS35 has been shown to participate in the traffic of a number of proteins involved in other neurodegenerative diseases including sortilin -mediated progranulin (PGRN) linked to fronto-temporal dementia and processing of the amyloid precursor protein (APP) linked to Alzheimer's disease. Thus, our goal is to determine which cellular processes are perturbed by VPS35 mutations, and how these disturbances relate to those of other known PD gene mutations. Upon an improved understanding, novel therapeutics can be envisaged that will alleviate motor and non-motor symptoms.

3-C-99 A parkinsonian-like motor phenotype responsive to dopaminergic agonists in LRRK2-G2019S transgenic mice

Mattia Volta[1], Mariangela Calcagno[2], Carmela Paradiso[2], Francesco Longo[2], Heather Melrose[3], Michele Morari[2], Matthew J Farrer[1]

[1]University of British Columbia, [2]University of Ferrara, [3]Mayo Clinic

The G2019S mutation in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene is the most common cause of familial and sporadic Parkinson's Disease. However, mice bearing G2019S mutation did not show a parkinsonian-like motor impairment. We therefore undertook an age-related behavioral analysis of mice expressing the human mutant LRRK2 gene (BAC hLRRK2-G2019S) compared with LRRK2 / and LRRK2-/- mice. Motor behavior was assessed at 3, 6 and 12 months by using the previously validated bar, drag and rotarod tests. Dopaminergic agonists (L-DOPA and pramipexole) were administered systemically (i.p.) to rescue motor deficits. At 3 months, no differences in motor behavior were detected. At 6 months, G2019S mice showed an increase in immobility time compared to LRRK2 / mice whereas LRRK2-/- mice were less akinetic. In the drag test, G2019S mice showed impaired stepping activity whereas LRRK2-/- mice increased motor abilities. Mice aged 12 months showed a similar motor profile, but increased rotarod performance was observed in LRRK2-/- compared to LRRK2 / mice. Pharmacological testing was performed on 6-month old mice. Systemic administration of L-DOPA (10 mg/Kg plus benserazide 12 mg/Kg) selectively reversed stepping deficits in G2019S mice. Likewise, pramipexole (0.001 mg/Kg) caused a reversal of stepping deficits in G2019S mice, while increasing akinesia and reducing stepping activity in LRRK2-/- mice. We propose LRRK2 to age-dependently inhibit motor function, while the pathogenic G2019S mutation confers a parkinsonian phenotype which is partly responsive to dopaminergic agonists.

3-C-100 The evolution of multiple sclerosis lesions using magnetic resonance phase images

Vanessa Wiggermann[1], Enedino Hernandez Torres[1], Irene Vavasour[1], Corree Laule[2], Anthony Traboulsee[1], Wayne Moore[1], Alex MacKay[1], David Li[1], Alexander Rauscher[1]

[1]University of British Columbia, [2]Vancouver General Hospital

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system. Characteristic for MS is the development of focal lesions due to episodic inflammation resulting in demyelination of nerve fibres, axonal loss and progressive neurodegeneration. We used a novel magnetic resonance (MR) imaging method that is based on the MR signal's phase for the investigation of newly forming MS

lesions. It was previously shown that MR phase can be used to probe brain tissue structure at high spatial resolution and with high sensitivity. Twenty patients with relapsing-remitting MS were serially scanned for 7 months at one-months intervals. New lesions were identified by enhancement with gadolinium contrast agent, which is indicative of blood brain barrier damage. We observed a rapid reduction in phase between one month prior and after the month of enhancement (first appearance in T1 scans). Phase values more than two months before enhancement correspond to the values observed in normal appearing white matter. After enhancement phase was similar to gray matter. These results are in good agreement with MRI studies that used myelin or diffusion tensor imaging. They are also in good agreement with theoretical and experimental work which showed that phase is reduced when tissue structure changes from anisotropic to isotropic structure. However, the reasons for changes in phase are not yet completely understood. The observed phase shift in this study is assumed to be due to structural changes. Other contributing factors might be magnetic susceptibility changes or chemical exchange.

3-C-101 Implication of the role of ubiquitin carboxy-terminal hydrolase L1 in Alzheimer's disease pathogenesis

MingMing Zhang[1], Yu Deng[1], Yawen Luo[1], Shuting Zhang[1], Haiyan Zou[1], Fang Cai[1], Weihong Song[1]

[1]University of British Columbia

Amyloid-â precursor protein (APP) is cleaved by BACE1, the â-secretase in vivo to yield CTFâ C99, which is then cleaved by ã-secretase complex to produce amyloid â protein (Aâ), the central component of neuritic plaques in AD pathology. BACE1 is essential for Aâ production and plays a crucial role AD pathogenesis. Previously we reported that BACE1 is ubiquitinated and its degradation is mediated by the ubiquitin-proteasome pathway (UPP). However the exact mechanism underlying regulation of BACE1by the UPP remains elusive. Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), a neuronal-specific de-ubiquitinating enzyme. is reported to be down-regulated in AD brains. In addition, gad mice, a UCH-L1 knockout mouse model, show an accumulation of APP and Aâ in gracile tract. We hypothesized that UCH-L1 plays its role in AD pathogenesis by down-regulating BACE1 level and subsequent CTFâ and Aâ levels. UCH-L1 was stably expressed in HEK293 cells to generate HUCH cells. We found that BACE1 is accumulated in a time-dependent manner in HUCH cells when treated with UCH-L1 inhibitor. The half life of BACE1 is decreased in HUCH cells compared to controls. Level of C99 and Aâ are decreased when UCH-L1 is over-expressed. More importantly, we showed in vivo that in the neocortex of gad mice, BACE1 and C99 levels are higher than that of their wildtype littermates. Our data indicated that UCH-L1 accelerates BACE1 degradation, and administration of UCH-L1 might reduce the level of BACE1 and Aâ in brain, thus slowing down the development of Alzheimer's Disease.

3-C-102 Distinct differences in protease activation in two models of traumatic brain injury

Si Zhang[1], Luba Kojic[1], Michelle Tsang[1], Jie Liu[1], Dhananjay Namjoshi[1], Cheryl L Wellington[1], Wolfram Tetzlaff[1], Max S Cynader[1], William Jia[1]

[1]University of British Columbia

We investigated distribution and activity of MMP2, MMP9, ADAM10, ADAM17 in two brain trauma models, open head injury (OHI) and close head injury (CHI) in mice. Protease activity was assessed by in situ zymography and distribution examined with immunohistochemistry at 10 minutes, 1 hour and 24 hours post-injury. At 1h post-injury of OHI, upregulation of protease activity was found in dorsal cortex, in area CA3 of hippocampus and in thalamus on side of the brain ipsilateral to the injury. Increased expression of MMP2 was found in peri-injury areas of cortex, co-localized with NeuN (neuron) or GFAP (glia) positive cells. For CHI, increased expression of MMP2 and ADAM17 was found in dorsal cortex, hippocampus, and thalamus at both sides of brain at 1h post-injury although it was more obvious on ipsilateral side. Quantitative studies revealed intensities of immunostaining after CHI were increased in NeuN or GFAP positive cells as well as extracellular matrix (ECM). Percentages of protease-positive neurons in these brain areas were not changed. In contrast, increased expression of MMP2 was found in NeuN and GFAP positive cells in deeper layers of dorsal cortex in OHI animals but not in sham controls. We also found increased nuclear localization of ADAM-17 from 1h to 24 hours after open head TBI. These results suggest that OHI and CHI have distinct responses in protease activation in the brain after TBI. Further investigations are ongoing to further compare the two models and to delineate the pathways of the protease activation.

D – Sensory and Motor Systems

3-D-61 Adaptation to prism-induced altered visual input during visually demanding walking tasks generalizes in a task-specific manner

Scott Alexander[1], Brent W Flodin[1], Daniel S Marigold[1]

[1]Simon Fraser University

Prism glasses disrupt the normal relationship between visuomotor and proprioceptive-motor reference frames resulting in motor error. We previously showed that prism-induced adaptation could transfer, or generalize, between a precision walking task that required subjects to step to 2 sequential targets (with the right then left foot) and a

walking task that required subjects to position their right foot beside an obstacle and then step laterally over it with their left foot (Alexander et al. 2011). Thus, both tasks required a visually guided right-left stepping pattern. However, only measures of the left leg showed generalization. We suggested that since the tasks required a coordinated step with the left foot, proprioceptive feedback caused by the stepping pattern might have enhanced the storage of the adaptation and contributed to the specific generalization pattern. In this study, we used rightward shifting prisms with a modified protocol. Groups 1 and 2 performed tasks similar to our previous study, but stepped in a left to right pattern in both tasks. Group 3 included a third target in the precision walking task, creating a right-left-right stepping pattern. All groups adapted, and we found strong aftereffects when prisms were removed. Groups 1 and 2 showed right leg measures generalized ($p<0.05$); group 2 also showed generalization of the left leg. The addition of the third stepping target for group 3 appeared to eliminate the presence of generalization. These findings support our previous work, and argue for task- and limb-specific generalization. Funded by: NSERC.

3-D-62 Imaging membrane depolarization during spontaneous infraslow activity in mouse cortex

Allen Chan[1], Majid H Mohajerani[1], Yu Tian Wang[1], Tim H Murphy[1]

[1]University of British Columbia

Neuronal activity occupies a wide temporal landscape ranging from 600 Hz to 0.01 Hz. While much is known about the determinants and function of higher frequency activity, much less is known about very low frequency forms of activity. We investigated an infraslow (<0.1 Hz), spontaneous form of brain activity in the anesthetized mouse cortex. Infraslow activity manifested as slow, DC fluctuations in EEG signal as well as in alternating fluctuations in power spectrum, centered at 1 Hz, from both EEG and cortical local field potential recordings. In addition, we used voltage-sensitive dye imaging to investigate the spatiotemporal structure of this activity. We found that infraslow activity exhibited discrete regional activation, was pervasive and active over primary, secondary and association cortices, and typically exhibited bilateral synchrony across hemispheres. We tested the hypothesis that cortical excitability may also fluctuate with ongoing infraslow activity. We measured sensory evoked responses (0.1 Hz), either electrical stimulation of forelimb or light stimulation of the eye, while monitoring ongoing, baseline spontaneous infraslow activity. We found that the trial-to-trial variability of sensory evoked responses, as measured from EEG and voltage-sensitive dye recordings, positively co-varied with the phase of the infraslow fluctuation. Also, we found light-evoked responses from layer-5, ChR2-expressing mice demonstrated a similar relationship indicating that these changes in excitability may be intrinsic at the level of the cortex. These data are suggestive

3-D-63 Neural correlates of perception during actions requiring memory

Leanna Cruikshank[1], Jeremy B Caplan[1], Anthony Singhal[1]

[1]University of Alberta

The N170 event-related potential (ERP) component reflects visual perceptual processes and is known to have a source in the lateral occipital cortex (LOC). Convergent evidence from neuropsychological and neuroimaging studies suggests that the LOC is recruited for action tasks in which visibility of a target is unavailable and perceptual memory must be used instead. We tested whether the N170 reflects the contribution of additional ventral stream processes required for actions in which vision of a target is occluded, predicting that the amplitude of the ERP in the latency range of the N170 would be larger when perceptual mechanisms are engaged to a greater extent. Participants were auditorily cued to touch target dots on a touchscreen. Two viewing conditions varied with respect to the contribution of the ventral stream during response initiation. In condition 1, the target disappeared with movement initiation whereas in condition 2, it disappeared with the cue to respond. The N170 during the initiation phase of trials was larger in amplitude for condition 2. This effect was observed over temporal electrode sites, likely reflecting an overlap between auditory cue-related processes and additional perceptual processes within regions in the temporal cortex. A second experiment ruled out the possible confound of the offset of the visual stimulus. Results indicate that the N170 may be a marker of neural activity within the ventral stream, supporting the notion that actions initiated in the absence of a visual target rely more on perceptual representations.

3-D-64 Comparing subthalamic nucleus and substantia nigra pars reticulata electrical stimulation

Jay Jantz[1], Masayuki Watanabe[1], Douglas P Munoz[1]

[1]Queen's University

In the basal ganglia (BG), the 'indirect' and 'hyperdirect' inhibitory pathways converge in the subthalamic nucleus (STN). We investigated whether the STN is involved in saccade initiation, and if so, what pathway through the BG is involved. We paired sub-threshold electrical stimulation of the STN and SNr in rhesus monkeys with a behavioral examination of saccadic eye movements. If STN and SNr stimulation effects are similar, then the STN likely mediates saccade initiation through an STN-SNr-SC pathway. Otherwise, the STN may mediate saccade initiation through an STN-GPe-SNr-SC path. Two monkeys performed the following paradigms: (1) view a blank screen freely (free viewing), (2) generate a saccade (2) toward (pro-saccade)

or (3) away from (anti-saccade) a peripheral visual stimulus. Sub-threshold stimulation in STN in the free-viewing task produced a bias of saccades toward the contralateral hemifield with respect to stimulation site, whereas stimulation of the SNr produced an ipsilateral bias of saccades. Stimulation in either the STN or SNr during pro- and anti-saccade tasks either prolonged reaction times for saccades bilaterally, or contralateral to the stimulation site. Opposite behavioral effects of STN and SNr stimulation during the free-viewing task challenge the STN-SNr-SC pathway for saccade initiation in this task. However, similarities between STN and SNr stimulation effects in the pro- and anti-saccade tasks suggest that the STN is involved in a task-dependent modulation of saccade initiation, through multiple pathways depending on cognitive demand.

3-D-65 Electrophysiological investigation of spatiotemporal tuning in mouse primary visual cortex

Emily LeDue[1], Michelle Y Zou[1], Nathan A Crowder[1]

[1]Dalhousie University

Humans can discriminate the speed of a moving visual stimulus; however, how speed is processed by the visual system is not well understood. In primates motion processing occurs most extensively in the middle temporal area, but a portion of complex cells in macaque primary visual cortex (V1) also show speed tuning. Conversely, simple cells respond preferentially to a certain temporal frequency rather than speed. The speed of a sine-wave grating is defined as the ratio of the temporal and spatial frequencies. The mouse has become a popular model of cortical processing due to its ease of use for in vivo imaging and because genetic manipulation of the mouse is well established. Unlike in the macaque, mouse V1 neurons do not appear to show speed tuning and preferred speed changes in proportion to the spatial frequency of a stimulus. To confirm these findings and further evaluate the mouse as an animal model to study motion processing, we used single unit in vivo electrophysiology to measure spatiotemporal tuning in mouse V1. We presented neurons with drifting sine-wave gratings of various spatial and temporal frequency combinations to generate spatiotemporal response functions. We quantitatively assessed the speed tuning of neurons using two methods: 1) we fit our spiking responses with a two-dimensional Guassian function and 2) we performed a partial correlation analysis to determine whether model responses selective for speed or temporal frequency better described our data. Our results substantiate earlier findings that very few neurons in mouse V1 code for stimulus speed.

3-D-66 Local pairwise correlations and network states in cat primary visual cortex

Martin Spacek[1], Nicholas V Swindale[1]

[1]University of British Columbia

We used high density 54-channel silicon polytrodes to record single unit spiking activity in primary visual cortex of anesthetized cats. We simultaneously recorded from up to 150 neurons spanning up to 2 mm of cortical depth. Multi-hour recordings were made under both spontaneous and visually stimulated conditions, including naturalistic movies. Spike trains were binned and digitized. Pairwise correlations were calculated by taking the correlation coefficient between each binary signal pair. As reported in other brain areas (Schneidman et al, 2006; Schlens et al, 2006), mean pairwise correlations were very weak (< 0.05). Pairwise correlations decreased gradually with increasing pair separation. Subgroups of neurons were randomly selected, and the state of each subgroup at a given time bin was represented as a binary word of its member neurons. Observed probabilities of some states deviated by up to 10,000x from those expected given the near independence indicated by weak pairwise correlations. A maximum entropy model that considered weak pairwise correlations had better predictive power than the independence assumption, but still deviated by up to 100x from observed probabilities for some states. Although inclusion of closely spaced pairs improved model performance more than did distant pairs, all pairs were needed for maximum performance. These results suggest that higher order correlations may be present (Ohiorhenuan et al, 2010), and that even the weakest pairwise correlations between distant neuronal pairs can significantly affect the behaviour of cortical networks.

3-D-67 Pursuit eye movements and motion prediction in patients with schizophrenia and untrained normals

Miriam Spering[1], Elisa C Dias[2], Jamie Sanchez[2], Alexander C Schütz[3], Daniel C Javitt[2]

[1]University of British Columbia, [2]Nathan Kline Institute for Psychiatric Research, [3]University of Giessen

Tracking moving objects with smooth pursuit eye movements is essential for many everyday tasks. These continuous, slow eye rotations critically support vision by centering and stabilizing moving images on the fovea; they prevent motion blur and enhance visual acuity. We recently discovered a strong perceptual benefit during pursuit in a trajectory prediction task [Spering et al. J. Neurophysiol., 2011]. Observers in this study were healthy adults experienced in laboratory pursuit tasks. Here we ask how the ability to predict motion trajectories is affected by impairments in pursuit eye movements in patients with schizophrenia. Observers (11 patients, 12 age-matched, untrained normals) judged whether a linearly moving target

("ball") would hit/miss a stationary vertical line segment ("goal"). Ball and goal were shown briefly (200-500ms) on a computer monitor and disappeared before the perceptual judgment was prompted. We manipulated eye movements: observers had to track the ball with their eyes (50% trials) or fixate on the goal while the ball moved towards fixation. Trajectory prediction was overall better in normals (75% correct) than in patients (68% correct). Interestingly, this finding was uncorrelated with pursuit quality: velocity gain and direction error were equally low in both groups. Pursuit was better in trials with longer presentation duration, but only normals showed better performance when stimuli were presented for the longer period vs. shorter. These findings indicate that pursuit that reaches a minimum quality criterion may improve motion prediction.

3-D-68 Spontaneous spikes are synchronised to multiple frequency components of the local field potential

Nicholas Swindale[1], Martin A Spacek[1]

[1]University of British Columbia

Spontaneous activity in cortical neurons is often assumed to be a random process, possibly occurring as the result of random firing of other neurons accentuated by fluctuations in transmitter release. Here we show that this is unlikely to be the case for many spontaneously occurring spikes in the visual cortex. We made simultaneous recordings of spike activity and local field potentials (LFP) with 54-channel polytrodes in area 17 of anesthetised cats. Spectral analysis of the raw LFP recordings revealed peaks at frequencies that varied in intensity with position along the electrode and over time. Spike-triggered averages of the LFP (ST-LFP waveforms) were calculated and analysed in the time and frequency domains. ST-LFP waveforms for individual neurons were multiply periodic with energy preceding and following spikes typically for 200 - 500 msecs. Spectral analysis of these waveforms revealed multiple narrow frequency peaks that were sub-sets of those present in the raw LFP signal, but which varied distinctively from cell to cell. This kind of phase synchronisation could occur if recorded neurons are members of networks of cortical neurons which fire in oscillatory patterns, contributing to LFP frequencies. If so, the particular ST-LFP frequencies should also be detectable in the spontaneous spike trains of individual neurons. However, this was often not the case. We suggest that at least some oscillatory inputs may originate outside of the cortex and that individual cortical neurons are responsive to specific frequency and phase combinations of these inputs.

3-D-69 The representation of visual salience in the superior colliculus

Brian White[1], Takuro Ikeda[1], David Berg[2], Laurent Itti[2], Douglas P Munoz[1]

[1]Queen's University, [2]University of Southern California

In natural scenes, salient stimuli are often brought into and out of a neuron's response field (RF) by virtue of the ongoing eye movements that characterize typical natural viewing. Neurons are often characterized by artificially presenting stimuli abruptly in their RF, but less is known about how the brain encodes higher order properties that render a stimulus salient. We explored the representation of salience in the superior colliculus (SC), a primitive brain structure in the control of visual attention and eye movements. Rhesus monkeys viewed dense arrays of oriented color stimuli (>200 items) on a large, high resolution monitor (1920x1080 pixels; 82x52 deg). Salient items were brought into and out of the RF of a neuron by either a saccade or smooth pursuit movement guided by a goal-related stimulus that always ran orthogonal to the RF. We compared visual activation between popout-, conjunction-, and single item conditions. We found a robust salience response in single units and local field potentials in the superficial and intermediate SC layers. Although the 1st order transient associated with an abrupt onset dominated the initial response, salience responses emerged on subsequent volleys of activity. For some neurons, we observed a gradual increase then decrease in activation as the RF was dragged over a salient item via a pursuit movement. Because this response was observed in the superficial SC layers, it may arise from visual cortical inputs, not parietal/frontal cortex. The SC might propagate this signal to other brain areas via the thalamus.

3-D-70 Optical imaging of the visual cortex of both hemispheres through the intact skull

Kaiyun Yang[1], Jeffrey LeDue[1], Max Cynader[1]

[1]Brain Research Centre

Optical imaging of intrinsic signals (IOS) has greatly facilitated the study of the visual system, and been established as an effective measurement approach to ocular dominance plasticity. Here we report a method for monitoring bilateral changes induced by monocular deprivation in the developing visual cortex through the intact skull. Juvenile mice were anesthetized with isoflurane for surgery and imaging. After the skull was exposed, the surface was coated with a thin layer of clear dental acrylic/nail polish to keep the skull transparent and prevent drying. Contrast-modulated noise covering large areas of the visual field was used for binocular stimulation and for acquisition of visual responses in primary visual cortex (V1) of both hemispheres. Visual stimuli restricted to the central visual field (45¢ª) were used to identify and obtain the responses of the binocular zone (V1b). We were able to

map V1 and V1b in both normal and monocularly deprived mice, and used two-way Gaussian fitting of evoked maps to compare the size and response amplitude of V1/V1b in two hemispheres, we found a significant decrease in both the size and amplitude of the evoked response in the V1/ V1b contralateral to the deprived eye. Spontaneous metabolic activity were recorded with the screen turned off. We performed single pixel correlation analysis, and found that after monocular deprivation, a single pixel in the contralateral V1/V1b showed higher correlation with a more diffuse area, indicating a change in functional coupling to neighboring cortical areas resulting from deprivation.

E – Homeostatic and Neuroendoctrine Systems

3-E-89 Distal and local origins of limbic-related projections to the paraventricular nucleus of the hypothalamus

Adam Anonuevo[1], Innala Leyla[1], Victor Viau[1]

[1]University of British Columbia

Limbic structures have few direct projections to the paraventricular nucleus (PVN) of the hypothalamus, but nonetheless exert pronounced effects on basal and stress-induced activity of the hypothalamic-pituitary-adrenal (HPA) axis. Intervening cell groups within the hypothalamus and bed nucleus of the stria terminalis likely provide the bulk of most limbic connections to the parvicellular HPA-regulating division of the PVN. However, diffuse clusters of afferents surrounding the PVN are in position to integrate descending information from a variety of limbic structures to control HPA axis activation. Such candidates occupy portions of the anterior hypothalamic area, perifornical area, medial zona incerta, and subparventricular zone. Based on our previous mapping of afferents to the PVN, and in agreement with previous connectivity studies, we found little to no uptake of retrograde tracer within limbic structures in animals bearing discrete injections of fluorogold (FG) into the PVN proper. However, animals bearing FG injections that were biased towards the zona incerta showed doubly labeled (AR FG) cells in the prefrontal cortex, whereas double labeling was found in the medial amygdala in animals with injections localized to the ventral margin of the PVN. We are now targeting surround-projecting limbic cell groups using injections of FG outside the PVN periphery. We also propose to coinject FG and anterograde tracer to confirm the afferent connectivity of the PVN surround. This strategy provides a framework for extending our current understanding of limbic PVN regulation.

3-E-90 Ghrelin modulates somatodendritic dopamine concentration

Stephanie Borgland[1], Frances Xia[1]

[1]University of British Columbia

Ghrelin is a peptide hormone that is released from the stomach and can act in the brain as a potent modulator of food intake. Although many studies have focused on its actions in the hypothalamic nuclei, recent evidence suggests that ghrelin may also regulate the motivation to feed by interacting with the mesolimbic dopaminergic pathway. In response to salient environmental stimuli, dopamine (DA) neurons of the ventral tegmental area (VTA) burst fire resulting in increased extracellular DA in projection areas, including the nucleus accumbens. However, DA can also be released within the VTA either somatodendritically or from synaptic DA input from its own axon collaterals and those from the substantia nigra pars compacta. Growth hormone secretagogue receptors are located on VTA DA neurons, where ghrelin potentiates excitatory synaptic transmission and enhances downstream DA release in nucleus accumbens. However, it is unknown if ghrelin modulates somatodendritic DA in the VTA. DA release in mouse midbrain slices was evoked using electrical stimulation mimicking burst firing (40 Hz 5 pulses), and DA concentration was monitored in real-time using fast-scan cyclic voltammetry. The active, acylated form of ghrelin significantly increased DA concentration in the VTA, likely by enhancing its release rather than inhibiting reuptake. In contrast, the inactive form of ghrelin had no effect on somatodendritic dopamine concentration in the VTA. The effect of ghrelin on somatodendritic DA may be one of the mechanisms by which ghrelin promotes the motivation to obtain food.

3-E-91 Stat3 Signaling in midbrain dopamine neurons regulates locomotor activity, operant learning and dopamine tone

Maria Fernandes[1], Sandeep Sharma[1], Shabana Mogra[1], Shizuo Akira[2], Stephanie Fulton[1]

[1]CRCHUM & University of Montreal, [2]Osaka University

The hormone leptin plays a critical role in energy balance and modulates neural circuits regulating appetite, locomotion and reward via its receptor (LepRb). Leptin acgivation of the transcription factor Stat3 is the primary means by which leptin modulates gene expression. Dopamine (DA) neurons originating in the midbrain and their limbic targets are an essential component of the neural circuitry underlying locomotor behaviour and motivation. Leptin targets the midbrain to decrease feeding, locomotion and DA tone, but it remains to be resolved which cells and signaling pathways in the midbrain mediate these actions of leptin. To determine the contribution of Stat3 in midbrain DA neurons we generated DA-specific Stat3 knockout (KO) mice (DATcreStatflox/flox). Our results

showed that male KO mice consume less food and weigh less on a chow diet. Male KO mice are also more active as observed by increased ambulatory activity and wheel running. Both male and female KO mice show significant impairments in food-motivated learning in an operant conditioning task. In addition, we find that protein expression for tyrosine hydroxylase (TH) and D1, but not D2, receptor is significantly reduced in the nucleus accumbens (NAc) of KO mice. Reductions in NAc TH and D1R expression in male KO mice are consistent with hyperactivity and impaired operant learning and suggest that loss of Stat3 decreases DA tone. Collectively, these data suggest a novel role for LepRb-Stat3 signaling in DA neurons in the regulation of locomotor activity, feeding, food-motivated operant learning and DA tone.

3-E-92 Oxysterols and cholesterol measures in human post-mortem prefrontal cortical brain tissue.

Erika Freemantle[1], Naguib Mechawar[2], Gustavo Turecki[2]

[1]McGill University-Douglas Hospital, [2]McGill University

Brain oxysterol levels, enzymatic oxidation products of cholesterol, have been proposed to reflect the dynamic process of physiological synapse maintenance and repair of nerve terminals within the central nervous system (CNS), due to the turnover of membrane cholesterol (CHL). Modifications of these have important implications in neurological conditions, especially in neurodegenerative pathology and disorders wherein alterations of synaptic plasticity or cell signalling are implicated, such as depression. Oxysterols are CHL derivatives which may diffuse across the blood brain barrier and thus has been hypothesised as a mechanism by which the brain can eliminate excess cholesterol to maintain a steady state. Relations of 24-hydroxycholesterol and 27-hydroxycholesterol specifically may provide a depiction of CNS CHL homeostasis. Delineating the complexity of lipid metabolism dynamics in the human post-mortem central nervous system, however, necessitates a combined approach of gene expression and metabolite measures. Thus, the objective of this study is to integrate oxysterol measures and gene expression measures in an effort to delineate how they may relate to neurological conditions, such as depression. Using post-mortem human prefrontal cortex from the Quebec Suicide Brain Bank, quantification of metabolite and gene expression measures will be combined. This research aims to provide a characterisation of enzymatic oxidative CHL homeostasis in post-mortem human prefrontal cortex with relevance to neurological disorders, such as major depression.

3-E-95 Ghrelin stimulates neurite outgrowth in tyrosine hydroxylase neurons of the arcuate nucleus

Mark Fry[1], Karen Oswald[1]

[1]University of Manitoba

Ghrelin and leptin are thought to play roles in regulation of energy balance in part via trophic activity altering synaptic connectivity of hypothalamic arcuate nucleus (ARC) neurons. For example, administration of ghrelin altered the synaptic profile of POMC neurons in the ARC, with more inhibitory and fewer excitatory synapses. However, although the tyrosine hydroxylase (TH) neurons of the ventrolateral ARC also strongly express ghrelin receptors, trophic effects of ghrelin on these neurons are unknown. In order to investigate this, we prepared acutely dissociated cultures of ventrolateral ARC neurons from six week old male transgenic mice expressing GFP under the control of the TH promoter. We treated the cultures for 5 days with either 100 nM ghrelin or 10 uM GHRP-6[D-Lys3] (ghrelin antagonist) and compared cell morphology to untreated controls. Specifically, ghrelin treatment caused a significant increase in the number of neurites compared to control and antagonist (2.42±0.18 ghrelin; 1.62±0.14 control; 1.53±0.19 antagonist; $p<0.05$ K-W ANOVA). Ghrelin also caused a significant increase in the total neurite length compared to control and antagonist (69.8±7.0µm ghrelin; 43.70±5.9µmm control; 42.8±7.8µm; $p<0.05$ K-W ANOVA). Lastly, ghrelin significantly increased the number of branches and varicosities compared to control and antagonist. These data suggest that ghrelin may play a role in the long term regulation of synaptic connectivity of TH neurons in the ARC. Additionally, like the NPY and POMC neurons, TH neurons may play a role in the regulation of energy balance.

3-E-97 Sex differences in serotonin and 5HT-1A receptor regulation of the hypothalamic-pituitary-adrenal (HPA) axis.

Nirupa Goel[1], Leyla Innala[1], Victor Viau[1]

[1]University of British Columbia

The higher incidence of anxiety and depressive disorders in females may be linked to central interactions between serotonin (5-hydroxytryptamine, 5-HT) and the hypothalamic-pituitary-adrenal (HPA) stress axis. We investigated the serotonergic mediation of HPA output responses to stress in adult male and female rats by administering a selective 5-HT 1A receptor antagonist (WAY-100635, 0.5 mg/kg, sc) prior to 30 min of acute restraint exposure. This dose of WAY attenuated ACTH and corticosterone (CORT) responses in males, but not in females. To investigate a possible sex difference in drug metabolism and to determine the most effective dose for studying 5-HT 1A regulation of the HPA axis, we employed a range of WAY doses (0, 0.5, 1, 3 and 10 mg/kg) prior to

restraint stress in male and female rats. Relative to their vehicle counterparts, WAY-treated males showed attenuated ACTH responses with the .5, 1, and 3 mg/kg doses. In contrast, WAY-treated females showed stress-induced ACTH levels comparable to those receiving vehicle. Females also secreted greater levels of CORT than males across all doses of WAY, providing little evidence to suggest a sex difference in drug delivery or metabolism. Immunocytochemical analyses of tryptophan hydroxylase within forebrain regions indicate higher levels in females compared to males. In situ hybridization analysis suggests lower 5-HT 1A mRNA in females than males in the dorsal raphe. Taken together, sex differences in HPA responses may be explained by differences in 5-HT 1A receptors and 5-HT input to the HPA axis.

3-E-98 Vasopressin V1a receptor regulation of cellular activation and HPA axis responses to repeated restraint

Megan Gray[1], Leyla Innala[1], Clara Myung[1], Jacques Flores[1], Victor Viau[1]

[1]University of British Columbia

Previously we found that continuous central antagonism of the vasopressin V1a receptor impedes endocrine stress responses from declining during 5 consecutive exposures of daily 3h repeated restraint. Ventricular antagonism had no effect on basal AM and PM endocrine levels in stress naïve rats. Antagonism also had no effect on acute glucocorticoid responses, but attenuated habituation of ACTH and corticosterone responses on the last day of restraint. Having established that stress habituation relies on V1a receptors, we next examined where our antagonist might be acting centrally. Relative to vehicle controls, antagonism blunted acute stress-induced increases in Fos-positive cells in the dorsomedial hypothalamus (DMH) and ventrolateral septum (vlSEPT). Antagonism also impeded habituation of Fos responses in the hypothalamic paraventricular nucleus, medial amygdala, anterior bed nucleus, DMH and vlSEPT. Stress-induced Fos increases in the CA1 hippocampal region did not habituate in controls, and this increase was blocked by antagonism on the last day of restraint. A follow up study has found that relative to stress naïve controls, repeated restraint increases V1a receptor binding in the bed nucleus and septum. These findings suggest that this form of adaptation requires a shift towards V1a receptor utilization and is associated with site specific changes in receptor binding levels. Having showed that select forebrain regions are sensitive to vasopressin antagonism we now have a framework for examining the anatomical specificity of these effects in greater detail.

F – Cognition and Behaviour

3-F-71 Interstimulus interval-dependent phenotype in avr-14 mutants

Ricardo Bortolon[1], Catharine Rankin[1]

[1]University of British Columbia

avr-14 encodes a chloride-permeable glutamate receptor subunit in C. elegans, homologous to Cys-loop receptors including vertebrate glycine and GABA receptors. avr-14 is expressed in the mechanosensory neurons of the tap withdrawal response. Mutations of avr-14 produce two contrasting habituation phenotypes to tap reversal: faster habituation of magnitude at a 10-second ISI and slower habituation of magnitude at a 60-second ISI. By systematically varying ISI we determined that the breakpoint from more rapid habituation to slower habituation is between 30 and 45 sec ISIs. The phenotype at a 60-second ISI though is attenuated in the presence of food, suggesting the possibility of dopaminergic modulation. Interestingly, spontaneous recovery from habituation is more rapid at all ISIs in avr-14 compared to wild-type. There are two isoforms of avr-14; a mutation affecting only the avr-14a isoform leads only to decreased habituation of frequency at a 10-second ISI and no other disruptions. This suggests that the avr-14b isoform plays an important role in short-term habituation.

3-F-72 mPFC neural dynamics during exploration and exploitation

Barak Caracheo[1], Jeremy Seamans[1]

[1]University of British Columbia

Foraging generally involves two distinct phases. First, during exploration, an organism samples available options in order to identify action-outcome contingencies. Then, during exploitation, it settles on the actions that produce the most desirable rewards. To investigate the neural basis of these processes, arrays of 16 tetrodes were implanted into the medial prefrontal cortex of rats trained to perform a three-lever exploration-exploitation task. In each session a different lever was rewarded and the rat was required to deduce which it was through a trial and error process. At the beginning of the session rats pressed each lever approximately 33% of the time, consistent with an explorative state but as they progressed through the task they transitioned towards the rewarded lever pressing it above 80% of the time, consistent with an exploitative state. The instantaneous firing rates of recorded neurons were plotted against each other in an N-dimensional space and the dynamics of state transitions were characterized using unsupervised clustering and markov processes. It was observed that behavioural transitions and neural state transitions were correlated. Exploration was associated with increased variance in the network activity patterns while during exploitation the variance in the network activity

decreased. It is hypothesized that increased variance in mPFC network activity facilitates transitions between alternative options during exploration while reductions in variance during exploitation help to focus attention on contingencies that maximize reward.

3-F-73 Sex differences in spatial learning affect adult hippocampal neurogenesis but not activation of new neurons in response to memory retrieval

Carmen Chow[1], Stephanie E Lieblich[1], Jonathan R Epp[2], Liisa A Galea[1]

[1]University of British Columbia, [2]Hospital for Sick Children

Adult hippocampal neurogenesis is associated with hippocampus-dependent learning and memory. Previous research shows that the time of training relative to the probe trial in the Morris water maze task may be important for neurogenesis (Epp et al., 2007). In male rats, cell survival was increased if spatial training occurred 6 to 10 days after an injection of the DNA synthesis marker, bromodeoxyuridine (BrdU), but training on days 1 to 5 or 11 to 15 did not significantly affect cell survival when rats were perfused 16 days after BrdU injection. Because sex differences have been reported in cognition, with males outperforming females in spatial tasks it is unclear whether spatial learning would influence hippocampal neurogenesis in the same way in males and females. Therefore, this study aimed to compare sex differences in hippocampal neurogenesis relative to training in a learning and memory task. Female and male rats were exposed to training in the spatial or cued version of the Morris water maze 6 to 10 days after one injection of BrdU (200mg/kg). Twenty days following BrdU injection, all animals were given a 30-second probe trial and perfused. Males showed better learning in the spatial task than females. Spatial learning increased the density of BrdU-labeled cells relative to cue training only in males, but both males and females showed an increase in cell activation (BrdU co-labeled with immediate early gene zif268) after spatial but not cue training . This study highlights the importance of sex on neural plasticity and cognition. Supported by CIHR.

3-F-74 Cortical dynamics in offline sleep-like brain activity

Anastasia Greenberg[1], Clayton T Dickson[1]

[1]University of Alberta

The medial prefrontal cortex (mPFC) is critical for decision making involving cost/benefit evaluations. we used a task that consisted of 4 discrete blocks of trials in which a response on safe lever always delivered one food pellet, whereas a response on risky lever delivered four food pellets with decreasing probability (100, 50, 25, 12.5%) as blocks of trials progressed. Rats trained on the task were then implanted with 16 drivable tetrodes bilaterally into the mPFC. From the unit recordings we generated population instantaneous firing rate (iFR) vectors and using various statistical methods we evaluated the distinction between clusters of points in the N-dimensional space (where N=number of recorded units). Individual mPFC neurons displayed behaviorally correlated discharge patterns in response to a number of distinct aspects of the task, including large/risky vs. small/safe lever presses and reward epochs across trial blocks. At the population level we reliably observed distinct network activity states when animals were making risky decisions under the different probabilities of risk. These network states displayed adaptive encoding in that they became more distinct from one another as the relative difference in risk increased. This analysis reveals that mPFC networks are not encoding pure representations of risky lever reward probability levels but rather are forming relative representations of probability that indicate the size of the changes in probability over multiple blocks.

3-F-75 Diverse network representations of risky decision-making in mPFC

Shirin Hadizadeh[1], Nina Di Pietro[1], James Hyman[1], Stan Floresco[1], Eldon Emberly[2], Jeremy Seamans[1]

[1]University of British Columbia, [2]Simon Fraser University

The medial prefrontal cortex (mPFC) is critical for decision making involving cost/benefit evaluations. we used a task that consisted of 4 discrete blocks of trials in which a response on safe lever always delivered one food pellet, whereas a response on risky lever delivered four food pellets with decreasing probability (100, 50, 25, 12.5%) as blocks of trials progressed. Rats trained on the task were then implanted with 16 drivable tetrodes bilaterally into the mPFC. From the unit recordings we generated population instantaneous firing rate (iFR) vectors and using various statistical methods we evaluated the distinction between clusters of points in the N-dimensional space (where N=number of recorded units). Individual mPFC neurons displayed behaviorally correlated discharge patterns in response to a number of distinct aspects of the task, including large/risky vs. small/safe lever presses and reward epochs across trial blocks. At the population level we reliably observed distinct network activity states when animals were making risky decisions under the different probabilities of risk. These network states displayed adaptive encoding in that they became more distinct from one another as the relative difference in risk increased. This analysis reveals that mPFC networks are not encoding pure representations of risky lever reward probability levels but rather are forming relative representations of probability that indicate the size of the changes in probability over multiple blocks.

3-F-76 The role of group 1 metabotropic glutamate receptors in neurodegenerative diseases

Alison Hamilton[1], Rebecca DeVries[1], Amanda Martyn[1], Vania F Prado[1], Fabiola M Ribeiro[2], Stephen S Ferguson[1]

[1]Robarts Research Institute, University of Western Ontario, [2]Universidade Federal de Minas Gerais

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with extensive neuronal loss. Much of the neuronal death which occurs in AD is attributed to the excessive production of neurotoxic beta amyloid 1-42 (Aâ42) oligomers, which bind to neuronal synapses causing disruption of singalling. Many sites have been proposed as the Aâ binding site and evidence is now indicating that group 1 metabotropic glutamate receptors (mGluR1 and mGluR5) act as a scaffold for Aâ1-42. Pathophysiological alterations of group 1 mGluRs are already implicated in a number of neurodegenerative diseases including Huntington's disease and Parkinson's disease, so a role in AD is not surprising. These receptors are members of the GPCR superfamily and are coupled to the heterotrimeric protein Gáq. mGluR1/5 signaling plays an important role in modulation of synaptic activity and in neuron survival. In addition Aâ has been suggested to complex with cellular prion protein PrPc. We suggest that Aâ complexes with PrPc and that this complex signals via group 1 mGluRs, causing disruption to receptor signaling and trafficking. Here we show that Aâ1-42 oligomer alters group 1 mGluR signaling particularly in the presence of PrPc. Additionally we show that the knock out of mGluR5 in the APPswe/PS1Δe9 mouse model of AD reduces anxiety and increases exploratory behaviour while altering group 1 mGluR signaling. We believe that exploration of the role of mGluR1/5 in AD present us with an exciting opportunity to identify therapeutic strategies for this neurodegenerative disease.

3-F-77 Reduced anxiety and defensive behaviours in bax knockout mice

Melissa Holmes[1], Angela C Luedke[1], Pierre Boucher[1], Lee Niel[2]

[1]University of Toronto Mississauga, [2]University of Guelph

Developmental neuronal cell death is critically regulated by the pro-death protein Bax. Bax-/- mice exhibit increased neuron number, the elimination of several neural sex differences, and altered socio-sexual behaviours. Here we examined the effects of Bax gene deletion on anxiety and defensive behaviors. We first compared male and female wildtype and Bax-/- mice on the elevated plus maze. Bax-/- mice of both sexes made more entries into and spent more time in the open arms, indicating decreased anxiety compared to wildtype animals. Next, we exposed mice to two odours: trimethylthiazoline (TMT), an olfactory component of fox feces which rodents find aversive, and butyric acid (BA), an aversive odour without ecological significance. Each odour was presented individually and all animals were tested with both odours in a counterbalanced design. TMT was consistently more aversive than BA across a variety of behaviours (e.g., mice spent more time away from the odour source). Overall, Bax -/- mice showed less avoidance of both TMT and BA, displaying fewer stretch approaches and more contacts with the vial than wildtypes. Finally, no effect of genotype was seen in baseline olfactory behaviour; all mice were able to locate a buried food item, demonstrating that Bax-/- mice do not have impaired olfaction per se. Collectively, these data indicate a reduction in anxiety and defensive behaviours in Bax-/- mice, suggesting that alterations in cell number affect more general mechanisms of fear and anxiety in addition to behaviours directly related to reproduction.

3-F-78 Representing context in anterior cingulate cortex ensembles

James Hyman[1], Liya Ma[1], Emili Balaguer-Ballester[1], Daniel Durstewitz[1], Jeremy K Seamans[1]

[1]University of British Columbia

Representations of context serve to guide many aspects of behavior and influence the way stimuli or actions are encoded and interpreted. The medial prefrontal cortex (mPFC), including the anterior cingulate subregion, has been implicated in contextual encoding, yet the nature of contextual representations formed by the mPFC is unclear. Using multiple single-unit tetrode recordings in rats we found that different activity patterns emerged in mPFC ensembles when animals moved between different environmental contexts. These differences in activity patterns were significantly larger than those observed for hippocampal ensembles. Population activity patterns were not identical upon repeated exposures to the very same environment. This was partly because the state of mPFC ensembles appeared to systematically shift with time, such that we could sometimes predict the change in ensemble state upon later re-entry into one environment based on linear extrapolation from the time-dependent shifts observed during the first exposure. We also observed that many strongly action-selective mPFC neurons exhibited a significant degree of context-dependent modulation. These results highlight potential differences in contextual encoding schemes by the mPFC and hippocampus and suggest that the mPFC forms richcontextual representations that take into account not only sensory cues but also actions and time.

3-F-79 Effect of neonatal immune-activation in mice carrying a mutation in schizophrenia-susceptibility gene, Dysbindin-1

Sara Jamali[1], Sanjeev K Bhardwaj[1], Lalit K Srivastava[1]

[1]Douglas Mental Health University Institute, McGill University

Genetic factors and early adverse environmental events interact to contribute to the pathogenesis of schizophrenia. Here, we investigated if immune-activation during development interacts with a susceptibility gene to produce schizophrenia-related phenotypes. We injected mice with a loss of function mutation in dysbindin-1, a schizophrenia-risk gene and controls with either Poly I:C, a viral mimic or saline, at postnatal days (PD)5,6 and 7. At PD60, possible gene-environment (GxE) interaction was studied using tests of behaviours relevant to schizophrenia as well as examination of postnatal neurogenesis. Our data showed a significant effect of genotype on spontaneous locomotion as dysbindin-1 homozygous mice displayed increased locomotor activity. Further, we observed a significant effect of genotype as a decrease in the number of newborn cells in the glomerular layer of OB. Analyses of the data did not reveal an interactive effect between dysbindin-1 and Poly I:C exposure as far as spontaneous locomotion, pre-pulse inhibition of the acoustic startle, novel object recognition memory, elevated plus maze, fear memory, the number of newborn cells in the dentate gyrus and granular cell layer of the olfactory bulb are concerned. These preliminary results demonstrate lack of an interactive effect between this schizophrenia candidate gene and this viral mimic at neonatal periods on selected behavioral and neurobiological measures. Further investigation using different doses of the immune activator and/or different timing of treatment are needed to fully test GxE hypothesis.

3-F-80 Functional interaction between a7 nicotinic receptors and T cell receptors

Pragya Komal[1], Geoff Gudavicius[1], Chris Nelson[1], Raad Nashmi[1]

[1]University of Victoria

T cell receptors (TCRs) are expressed on T lymphocytes and play a major role in adaptive immunity. TCR proteins and other immune molecules are found in the CNS and can influence synaptic transmission. a7 nicotinic acetylcholine receptors (nAChRs) are a major class of ligand-gated ion channels in the brain that can directly mediate cholinergic postsynaptic transmission. We examined whether activation of TCRs can alter a7 nicotinic receptor function. Whole-cell patch-clamp recordings were performed in Jurkat cells transfected with a7 nicotinic receptors. Concanavalin A (conA) activation of TCRs decreased ACh mediated a7 nicotinic currents as compared to control. To examine the downstream signaling mechanism of TCR mediated attenuation of a7 nicotinic activity, we explored the role of tyrosine kinase activation. Genistein treatment prior to conA stimulation, rescued the ACh mediated response. To examine whether TCR activation can modulate a7 nicotinic receptor activity in the CNS, whole-cell recordings were performed in prefrontal cortical brain slices using a a7 specific agonist PHA543613. ConA treated slices showed a significant reduction in a7 nicotinic currents in layer 1 interneurons. We explored the mechanism of TCR mediated down regulation of a7 nicotinic currents. Using bungarotoxin labelling of surface a7 receptors, we found that TCR activation resulted in a decrease in surface a7 receptors due to internalization. Furthermore, noise fluctuation analysis of a7 mediated currents showed a significant decrease in single channel conductance with conA stimulation.

3-F-81 The strength of visual representations in dorsolateral prefrontal cortex neurons depends on working memory requirements

Diego Mendoza-Halliday[1], Julio Martinez-Trujillo[1]

[1]McGill University

Simple visual features such as visual motion direction are encoded in the firing patterns of neurons in macaque dorsolateral prefrontal cortex (dlPFC). Such representations persist in the absence of visual input during tasks requiring holding a stimulus direction in working memory. How working memory requirements influence the strength of dlPFC representations remains unclear. We clarified this issue by recording the activity of 155 dlPFC neurons from two macaques while they performed two variants of a match-to-sample task in which they compared the motion direction of a sample stimulus to that of a test stimulus. In the memory task, sample and test were separated by a delay period during which the sample was removed from the display, thus requiring the monkey to remember its direction. In the no-memory task, the sample remained present during the entire trial, and therefore working memory was not required. ROC analysis was used to quantify each neuron's motion direction representation strength. In approximately half of the neurons, representations were stronger during the delay period of the memory task than during the equivalent period of the no-memory task. These results show that dlPFC neurons maintain visual representations in the absence of sensory input, and that in many neurons the strength of these representations is in fact reduced in the presence of sensory input, when working memory is not required.

3-F-82 Identifying pathological neurocognitive function in migraine populations

Marla Mickleborough[1], Christine M Chapman[1], Andreea S Toma[1], Jeremy H Chan[2], Todd C Handy[1]

[1]University of British Columbia, [2]Dartmouth College

People with migraine headache consistently report heightened cognitive sensitivities to visual stimuli in-between headache events, yet clinical efforts to confirm what neurocognitive systems are contributing to these effects have been inconclusive. Here we examined interictal neurocognitive function in migraine using a novel paradigm designed to assess not the functioning of a specific neurocognitive system per se, but rather, how a stimulus is processed and evaluated as a system-integrating event. Two groups of participants--migraineurs (N = 29) and non-migraine controls (N = 29)--were asked to view a set of 232 unfamiliar commercial logos in the context of a target identification task as their brain electrical responses were recorded via event-related potentials (ERPs). The set of logos was viewed serially in 10 trial blocks, with data analysis focusing on how brain responses changed across time within each group. For the controls, the depth of their implicit cognitive analysis of the logos rapidly stabilized with repeated exposure, as measured via the amplitude of the late positive potential (LPP). In contrast, migraineurs showed an increasing depth of implicit cognitive analysis with repeated exposure, an effect that arose despite no explicit instructions to consciously think about or evaluate the stimuli. Our findings suggest that cognitive diagnostics in migraine may benefit by targeting pathologies not in individual neurocognitive systems per se, but rather, pathologies in how stimuli are processed and evaluated as integrated perceptual/cognitive events.

3-F-83 High field structural MRI reveals specific episodic memory correlates in the subfields of the hippocampus, independent of salivary cortisol.

Scott Travis[1], Yushan Huang[1], Nicholas Coupland[1], Kathy Hegadoren[1], Esther Fujiwara[1], Ashley Radomski[1], Rawle Carter[1], Peter Seres[1], Nikolai Malykhin[1]

[1]University of Alberta

There is debate about the localization of various aspects of episodic memory in the hippocampus. Recent magnetic resonance imaging studies suggest a specialization of hippocampal subfields in different memory tasks. Cortisol dysfunction also changes subfield volumes. Past structural MRI studies have only examined global HC. The purpose of the present study was to determine the association between volumes of HC subfields performance on standard memory tests in a population of young healthy subjects. We also examined salivary cortisol levels and their effects on subfields and memory. We recruited 22 healthy participants, and acquired MRI data to obtain volumes of the cornu ammonis, dentate gyrus, and subiculum using a manual protocol. Participants were administered the Wechsler Memory Scale to assess auditory memory (AMI), visual memory (VMI), visual working memory (VWMI), immediate memory (IMI), and delayed memory (DMI). Pearson correlation coefficients revealed DG and CA volumes in the tail were positively correlated with WMS performance on VMI, IMI, and DMI. CA within the HC body positively correlated with VMI and DMI. Volumes of SUB, and volumes in the HC head failed to correlate with performance in any index of the WMS. No cortisol measures correlated with memory scores or subfields. Our data show that even in healthy young adults, performance on memory tests could be pinpointed to volumes of the DG and CA subfields in the hippocampus. These findings have direct applicability to a range of neurological and psychiatric disorders.

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3-IBRO-58 Antidepressant activity of the macerate of G.dalenii in depression-like features associated with epileptic seizures and that associated with chronic exposure to stress: a comparative study

Gwladys Temkou Ngoupaye[1,2], Willie M.U. Daniels[3], Elisabeth Ngo Bum[1]

[1]Department of Biological Science, University of Ngaoundér, Cameroon, [2]Department of Animal Biology, University of Dschang, Cameroon, [3]Discipline of Human Physiology, School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa.

The aim of our study was to study the effect of the macerate of G.dalenii on the pathophysiology of depression associated with temporal lobe epilepsy (TLE) and the more common depression that develops following chronic exposure to stress and secondly to compare its effect on these two precipitation states. We used the atropine-pilocarpine paradigm as an animal model of TLE, and animals exposed to chronic restraint stress served as our stress-related comparison group. The levels of ACTH, corticosterone and adrenal gland weight were assessed, as well as the concentration of hippocampal BDNF. Our results showed that the macerate of G.dalenii significantly reduced the immobility times in the forced swimming test, in both the TLE and chronic stress group when compared to control. This reduction was less important in rats chronically stressed than those with TLE. A similar pattern was observed when the HPA axis parameters were analysed. The macerate of G.dalenii significantly reduced the levels of ACTH, corticosterone but not the adrenal gland weight in the TLE and stressed group than the saline. In both the TLE group and the chronic stress group a significant increase in BDNF levels was observed when compared to the control group with the increasing greatest in chronically stressed animals. Our data confirmed the antidepressant activity of the macerate of G.dalenii in depression associated with temporal lobe epilepsy (TLE) and the more common depression that develops following chronic exposure to stress.