MANITOBA NEUROSCIENCE NETWORK
3rd ANNUAL SCIENTIFIC MEETING

JUNE 4, 2012

Special Thanks to Our Platinum Sponsor

Manitoba Health Research Council

SOCIETY FOR NEUROSCIENCE

WINNIPEG CHAPTER

MANITOBA NEUROSCIENCE NETWORK

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WELCOME

June 4th, 2012

The Manitoba Neuroscience Network represents the cornerstone approach of the Winnipeg Chapter of the Society for Neuroscience to encourage familiarity, communication and collaboration among Manitoba's broad basic and clinical neuroscience research community. The first and second annual Manitoba Neuroscience Network meetings were received with widespread enthusiasm, and we are pleased to build on this success by holding a third annual meeting.

New in 2012, the program was organized by a committee of neuroscientists, chaired by Dr. Gilbert Kirouac. The Program Committee separated the research interests of Manitoba neuroscientists into 4 broad themes and organized the 2012 and 2013 meetings around coverage of each theme, as follows:

2012 Themes:
A. Homeostasis, Neuroendocrine, Cognition, Behavior
B. Neural Excitability, Synapse, Glia

2013 Themes:
C. Developmental and Disorders of the CNS
D. Sensory and Motor Systems

For each theme, the program committee is featuring the research programs of Manitoba basic science and clinical faculty, as well as selected trainees. In addition we are honoured to host keynote speakers for each theme covered in 2012. Our speaker for Theme A will be Dr. Quentin Pittman from the Hotchkiss Brain Research Institute in Calgary, and our speaker for Theme B will be Dr. Keith Murai from McGill University in Montréal.

Consistent with previous years, there will be plenty of time for informal discussions over coffee and lunch. There will also be a poster session/competition accompanied by a reception with food and drink in the late afternoon.

We sincerely hope that you find this day useful. As always, please do not hesitate to give feedback to the organizers so that we can continue to improve the contributions the MNN makes to research excellence in Manitoba.

Chris Anderson, PhD
President, Winnipeg Chapter Society for Neuroscience
2012 MNN Meeting Chair

Gilbert Kirouac, PhD
Program Chair
2012 MNN Meeting Chair

Program Committee:
Gilbert Kirouac, PhD (Chair)
Ruth Ann Marrie, MD, PhD
James Nagy, PhD
Mark Fry, PhD

GENERAL INFORMATION

Meeting Location

Hilton Suites Winnipeg Airport
1800 Wellington Avenue
Winnipeg, MB R3H 1B2

T: (204) 783-1700
F: (204) 786-6588

Registration materials are available in the main conference foyer
Main scientific sessions will be held in Stevenson Ballroom
Poster Displays will be held as directed onsite.
Exhibitor Displays will be held as directed onsite.

Registration and Information:
The registration desk will be open starting from 8:00 am on June 4th, and will be located in the main conference foyer. Your pre-paid registration material will be available from this desk. Conference information is also available at this location. A limited number of onsite registrations may be available. Please inquire. Note that the organizers cannot guarantee the availability of conference material for onsite registrants.

Meals and Refreshments:
Coffee and snacks will be provided throughout the day. Lunch will also be provided. The afternoon poster session will be accompanied by snacks. A cash bar service will also be provided during the poster session. If you have food allergy concerns, please notify attendants at the registration/information desk. All possible will be done to accommodate special requests.

Poster Information:
Poster presentations will take place as directed onsite. Poster board assignments were sent to registrants by e-mail. They are also available in the printed program or at the registration/information desk. Posters should be mounted before 9:00 am. The main poster viewing opportunity is between 4:00 and 6:00 pm, during the reception. There will also be opportunities to see posters during coffee and lunch breaks. Poster board sizes are 54" wide by 36" high. Materials for hanging posters will be provided onsite. The Winnipeg Chapter Society for Neuroscience takes no responsibility for posters remaining after 6:00 pm.

PROGRAM

8.30 – 5.00 Registration desk open

9.00 – 9.20 Opening Remarks: Neuroscience Research Interests in Manitoba

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Theme: Homeostasis, Neuroendocrine, Cognition, Behaviour
Chair: Dr. Hugo Bergen, Human Anatomy & Cell Sciences, University of Manitoba

9.25 – 9.50 Nociceptin/orphanin FQ (N/OFQ)-induced modification of anxiety-like behaviours and time-dependent changes in N/OFQ-NOP gene expression during ethanol withdrawal

Dr. Harinder Aujla
Department of Psychology
University of Winnipeg

9.55 – 10.20 Opioid and cannabinoid homeostasis in viral epilepsy

Dr. Marylou Solbrig
Department of Medical Microbiology
University of Manitoba

10.20 – 10.40 Coffee Break, Poster Viewing

10.40 – 10.55 Not so 'bird-brained': Corvids as a model for complex social cognition

Dawson Clary
Department of Psychology
University of Manitoba

11.00 – 12.00 Keynote Lecture: Hot baby: how early inflammation programs the brain

Dr. Quentin Pittman
Department of Physiology & Pharmacology
Hotchkiss Brain Institute
University of Calgary

12.00 – 1.10 Lunch, Poster viewing

PROGRAM

Theme: Neural Excitability, Synapse, Glia: Molecular and Cellular Mechanisms
Chair: Dr. Emma Frost, Faculty of Pharmacy, University of Manitoba

1.10 – 1.35 Dysregulation of adenosine signalling in genetic mouse models: implications for stroke injury, mental illness and alcohol sensitivity.

Dr. Fiona Parkinson
Department of Pharmacology & Therapeutics
University of Manitoba

1.40 - 2:05 The role of dorsal root ganglia activation and brain derived neurotrophic factor in multiple sclerosis

Dr. Mike Namaka
Faculty of Pharmacy
University of Manitoba

2:10 - 2:25 PARP-1 and Bnip3 as mediators of mitochondrial dysfunction and neuronal death

Dr. Amit Kamboj
Division of Neurodegenerative Disorders
St. Boniface Hospital Research

2.30 - 2.50 Coffee Break, Poster Viewing

2.50 - 3.50 Keynote Lecture: Glial cells: From brain glue to synaptic plasticity

Dr. Keith Murai
Canada Research Chair & EJLB Scholar
Department of Neurology & Neurosurgery
McGill University

3.55 Closing Remarks

4.00 - 6.00 Poster Session and Reception

SPEAKER ABSTRACTS

SP-1 Harinder Aujla
Department of Psychology, University of Winnipeg

Nociceptin/orphanin FQ (N/OFQ)-induced modification of anxiety-like behaviors and time-dependent changes in N/OFQ-NOP gene expression during ethanol withdrawal

Anxiety is a key consequence of ethanol withdrawal and important risk factor for relapse. The neuropeptide nociceptin/orphanin FQ (N/OFQ), or agonists at this peptide's receptor (NOP), exert anxiolytic-like and anti-stress actions. N/OFQ dysfunction has been linked to both a high-anxiety behavioral phenotype and excessive ethanol intake. Recent studies suggest a possible link between genetic polymorphisms of the NOP transcript and alcoholism. Thus, in the present study, the effects of intracerebroventricularly (ICV) administered N/OFQ were tested for modification of anxiety-like behaviors, using the shock-probe defensive burying and elevated plus maze tests, in ethanol-dependent vs. nondependent rats, one and three weeks following termination of ethanol exposure. Additionally, Prepro-N/OFQ (ppN/OFQ) and NOP receptor gene expression was measured in the central nucleus of the amygdala, in the bed nucleus of the stria terminalis, and in the lateral hypothalamus at the same time points in separate subjects. One week post-ethanol, N/OFQ dose-dependently attenuated elevated anxiety-like behavior in ethanol-dependent rats and produced anxiolytic-like effects in nondependent controls in both behavioral tests. However, three weeks post-ethanol, N/OFQ altered behavior consistent with anxiogenic-like actions in ethanol-dependent rats, but continued to exert anxiolytic-like actions in nondependent controls. These findings were paralleled by ethanol history-dependent changes of ppN/OFQ and NOP gene expression that showed a distinctive time-course in the examined brain structures. The results demonstrate that ethanol dependence and withdrawal are associated with neuroadaptive changes in the N/OFQ-NOP system suggesting a role of this neuropeptidergic pathway as a therapeutic target for the treatment of alcohol abuse.

SP-2 Dr. Marylou Solbrig
Department of Medical Microbiology, University of Manitoba

Opioid and cannabinoid homeostasis in viral epilepsy

Control of refractory status epilepticus, CNS inflammation and edema, are unmet needs in the management of viral encephalitis. Lack of effective treatments is prompting the study of neural transmitter systems with broad homeostatic and protective functions, such as opioids and cannabinoids, for lead compounds and strategies of neural protection. In two rat models of viral encephalitis based on Borna Disease Virus (BDV) and HSV-1, using neuroanatomic techniques, pharmacologic probes and EEG recording, seizures are linked to low hippocampal dynorphin and pharmacologic opioid blockade. The kappa opioid agonist U50488 is anticonvulsant. Similarly in the BDV model, cannabinoid (CB1) antagonism by SR141716 is convulsant and the anandamide transport blocker AM404 is anticonvulsant. Endocannabinoid measurements show a reciprocal relation between opioids and cannabinoids with respect to convulsant phenomena. To further evaluate potential antiepileptic drugs with an impact on disease progression, the anti-inflammatory efficacy of select, easily-administered cannabinoid agonists also are examined in the BDV rat. The general cannabinoid agonist WIN 55,212-2 reduces inflammation and increases neurogenesis, but tolerance limits the efficacy of this treatment to 1 week. CB2 receptor stimulation by HU308 affords more sustained anti-inflammatory effects, likely through a mechanism involving glial cells, in particular activated microglia in which CB2 receptors would be upregulated in response to the injury. Our pharmacologic and neurochemical studies show that opioid (mu and kappa) pathways are part of an interconnected brain network that, together with cannabinoids, participate in anticonvulsant effects during viral encephalitis. Crosstalk between these systems could have the potential to also protect against inflammation and microglial toxicity.

SP-3 Dawson Clary
Dept of Psychology, University of Manitoba

Not so 'bird-brained': corvids as a model for complex social cognition

Once thought to be only capable of producing instinctive behaviours, the avian brain is now being recognized for its capacity for complex cognition. Although the avian brain is structurally very different from the primate brain, functionally, the two are quite similar. Furthermore, corvids, the family of birds possessing the largest brains relative to body size, have been shown to possess intellectual abilities that allow them to solve cognitively demanding problems in much the same way as primates – a convergent evolution of mental abilities despite divergent neural architecture. One such example is corvids' capacity for complex social cognition – the ability to infer the thoughts, beliefs, and perceptions of other individuals. Many social corvid species have been shown to infer an observing bird's perceptions and intentions when protecting their food caches from potential pilferers. However, rarely have non-social species been tested for equivalent abilities. In this talk, I will discuss the capacity of a non-social corvid, Clark's nutcracker (Nucifraga columbiana), for complex social cognition. Through the use of both ecologically relevant food-caching experiments and non-ecologically relevant object-choice experiments, I will highlight the flexibility and limitations of nutcracker cognition and the role of sociality for the evolution of complex cognitive abilities.

SPEAKER ABSTRACTS

SP-4 Dr. Quentin Pittman
Department of Physiology & Pharmacology, Hotchkiss Brain Institute, University of Calgary

Keynote Lecture: Hot baby: how early inflammation programs the brain

Quentin Pittman obtained his BA at the University of Lethbridge and a Ph.D. (Medical Science) at the University of Calgary. This was followed by a Medical Research Council postdoctoral fellowship at McGill where he studied hypothalamic neurophysiology and a MRC Centennial Fellowship at the Salk Institute where he worked on peptide neurotransmitters.

In 1980, he returned to Calgary as an MRC Scholar, first in the Department of Pharmacology and Therapeutics and now in Physiology and Pharmacology. He was promoted through the ranks, achieving full professorship in 1988. He has remained at the University of Calgary except for sabbatical periods at the University of Geneva and at INSERM in Bordeaux. During his entire academic career, he has been continuously supported by competitive salary awards from the MRC or the Alberta Heritage Foundation for Medical Research (AHFMR). He is currently a Medical Scientist of the AHFMR, University Professor and Fellow of the Royal Society of Canada.

At the University of Calgary, Dr. Pittman has been Chairman of the Neuroscience Research Group, Assistant Dean (Medical Science) and is currently Education Director of the Hotchkiss Brain Institute. He has been active in review bodies for many scientific agencies, including the MRC, CIHR, Heart and Stroke Foundation of Canada, Human Science Frontiers Program and the NIH. He is on editorial boards of several scientific journals, was a reviewing editor for J Physiol and is currently Associate Editor for American J Physiol-Reg, Integ Comp Physiol and Frontiers in Neuroendocrine Sciences. He has been President of the Canadian Physiological Society, Councilor of the Canadian Association for Neuroscience, the International Neuroendocrine Federation and the International Union of Physiological Sciences and is currently Treasurer of the International Brain research Organization (IBRO). Dr. Pittman has trained many scientists who have gone onto successful academic and industrial careers.

Dr. Pittman has published ~300 peer-reviewed and invited articles and frequently speaks on his work that extends from cellular studies on peptide and other transmitters in brain slices to whole animal studies on the effects of early or chronic inflammation on the brain and its outputs.

SP-5 Dr. Fiona Parkinson
Department of Pharmacology & Therapeutics, University of Manitoba

Dysregulation of adenosine signalling in genetic mouse models: implications for stroke injury, mental illness and alcohol sensitivity.

Adenosine is a purine nucleoside that functions as a neuromodulator in the central nervous system. It has diverse actions, including decreasing ischemic injury, seizure activity, anxiety, and wakefulness. Adenosine has a short half life and the mechanisms that regulate its levels, and enhance or attenuate its actions, are under investigation. A transgenic mouse, expressing human equilibrative nucleoside transporter 1 in neurons and a mouse deficient in ecto-5'-nucleotidase (also known as CD73) have been used to examine the importance of these processes for regulating the levels and actions of adenosine. Experimental approaches have used cultured neurons and astrocytes, brain slice electrophysiology, behavior tests and drug sensitivity tests. From these studies, we conclude that adenosine is produced via either intracellular or extracellular pathways, depending on cell types and stimuli. Alterations in adenosine regulation may predispose individuals to neurological or psychiatric disorders.

SPEAKER ABSTRACTS

SP-6

Dr. Mike Namaka
Faculty of Pharmacy, University of Manitoba

The role of dorsal root ganglia activation and brain derived neurotrophic factor in multiple sclerosis

Multiple sclerosis (MS) is characterised by focal destruction of the white matter of the brain and spinal cord. The exact mechanisms underlying the pathophysiology of the disease are unknown. Many studies have shown that MS is predominantly an autoimmune disease with an inflammatory phase followed by a demyelinating phase. Recent studies alongside current treatment strategies, including glatiramer acetate, have revealed a potential role for brain derived neurotrophic factor (BDNF) in MS. However, the exact role of BDNF is not fully understood. We used the experimental autoimmune encephalomyelitis (EAE) model of MS in adolescent female Lewis rats to identify the role of BDNF in disease progression. DRG and spinal cords were harvested for protein and gene expression analysis every 3 days post-disease induction (pdi) up to 15 days. We show significant increases in BDNF protein and gene expression in the DRG of EAE animals at 12 dpi, which correlates with peak neurological disability. BDNF protein expression in the spinal cord was significantly increased at 12 dpi, and maintained at 15 dpi. However, there was no significant change in mRNA levels. We show evidence for the anterograde transport of BDNF protein from the DRG to the dorsal horn of the spinal cord via the dorsal roots. Increased levels of BDNF within the DRG and spinal cord in EAE may facilitate myelin repair and neuroprotection in the CNS. The anterograde transport of DRG derived BDNF to the spinal cord may have potential implications in facilitating central myelin repair and neuroprotection.

SP-7

Dr. Amit Kamboj
Division of Neurodegenerative Disorders, St. Boniface Hospital Research

PARP-1 and Bnip3 as mediators of mitochondrial dysfunction and neuronal death

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 depolarizing and 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; 50 µM, 30 min), which is a direct normoxic PARP-1 activator. Hypoxic (48 hours) and MNNG-induced neuron death was significantly diminished 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 and mitochondrial integration was enhanced by normoxic PARP-1 activity. This directly implicates a role for PARP-1 in Bnip3 expression and mitochondrial activity. MitoTracker/calcein and JC-1 were used to measure mitochondrial permeability transition (MPT) and membrane potential (ΔΨm) respectively. MNNG (PARP-1)-induced neuron death was accompanied by MPT and ΔΨm collapse, which was significantly attenuated by deletion of Bnip3. These results show that PARP-1 activation leads to increased Bnip3 expression and Bnip3-mediated mitochondrial dysfunction and neuron death. Supported by the St. Boniface Hospital and Research Foundation and the Heart and Stroke Foundation of Manitoba.

SP-8

Dr. Keith Murai
Canada Research Chair & EJLB Scholar
Department of Neurology & Neurosurgery, McGill University

Keynote Lecture: Glial cells: From brain glue to synaptic plasticity

Recent work has found that communication between neurons and glial cells is important for synapse formation and plasticity in the brain. However, the molecular mechanisms that underlie these processes remain poorly understood. In this presentation, results will be shown regarding the different mechanisms used by neurons and glial cells to promote their inter-communication. These mechanisms are necessary for regulating synapse development and neural circuit function and are likely to reveal novel insight into brain disease.

POSTER JUDGING

A poster competition was held. All submitted abstracts were ranked by a panel of 4 principal investigators. The top 6 in each of the student and postdoc categories were chosen for in-person judging in poster form at the meeting yesterday. Congratulations to the following winners:

STUDENT CATEGORY WINNER:	Esteli Vasquez-Dominguez, Spinal Cord Research Centre
STUDENT CATEGORY RUNNER-UP:	Amrit Boese, Public Health Agency of Canada
POSTDOCTORAL FELLOW CATEGORY WINNER:	Dr. Amit Kamboj, St. Boniface Hospital Research
POSTDOCTORAL FELLOW CATEGORY RUNNER-UP	Dr. Ali Saleh, St. Boniface Hospital Research

POSTER #'S (* = PRESENTER)

POSTER #	AUTHOR LISTING	TITLE
P-1	Karen Bailey*, A.U. Mannan and H. Marzban	Cerebellar defects in a mouse model of Rhombencephalosynapsis.
P-2	P. Afsharinezhad*, J Kong, and H. Marzban	Patterned Purkinje Cells Degeneration in SOD1 Transgenic Mice
P-3	Shu Ying Ji, Roy Hutchings, and Eftekhar Eftekharpour*	In vitro and in vivo protective roles of Thioredoxin1in nervous system: Implication for therapeutic intervention after neural injury.
P-4	Vichithra RB. Liyanage*, Robby M. Zachariah, Carl O. Olson, and Mojgan Rastegar	Regulation of Methyl CpG Binding Protein-2 via epigenetic mechanisms in Neural Stem Cells.
P-5	Robby Zachariah* and Mojgan Rastegar	Understanding the role of MeCP2 isoforms in large-scale chromatin re-organization.
P-6	Erika Couto-Roldan*, Larry M Jordan	Serotonergic cells in the Parapyramidal Region are active during a locomotor task and receive cholinergic input.
P-7	Crystal Acosta*, Claudia Cortes, Wenjun Zhu, Emma Frost, Mike Namaka, Brian MacNeil	Antigenic induction of Nerve Growth Factor (NGF) in an animal model of Multiple Sclerosis (MS).
P-8	Wenjun Zhu, Claudia Cortés*, Crystal Acosta, Brian MacNeil, Emma Frost, Mike Namaka	Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) expression in the spinal cord associated with experimental autoimmune encephalomyelitis (EAE) induced neuropathic pain.
P-9	Wafa Kammouni*, Paul Fernyhough, Leena Hasan, Ali Saleh, Alan C. Jackson	Evidence of a neuroprotective role of nuclear factor-κB in oxidative stress induced by rabies virus in adult rat dorsal root ganglion neurons.
P-10	Shantel Gushue*, Reuben Saba, and Stephanie A. Booth	Prion-induced miRNA-146a target gene identification using proteomic, genomic and bioinformatic based approaches.
P-11	Sara I Omar*, Benedict C Albensi, Kathleen M Gough	Computational modeling of the binding of Calcium and Zinc ions to Calbindin D28k.
P-12	Kyle A. Caligiuri*, Anna Majer and Stephanie A. Booth	Investigation of the deregulated miRNome identified during acute viral infections in a murine model of HSV-1 encephalitis.
P-13	Marzena Z Kastyak-Ibrahim*, Richard Buist, Domenico L Di Curzio, Marc R Del Bigio, Benedict C Albensi, Melanie Martin	Imaging of selected brain regions in 3xTg Alzheimer's mouse model by Magnetic Resonance Microscopy.
P-14	Amrit S. Boese* and Stephanie A. Booth	Examining microRNAs in NMDA related excitotoxicity in prion induced neurodegeneration.
P-15	Katherine Cordova*, Mary-Lou Solbrig and Shadreck Mzengeza	Synthesis of 18F-4-fluorobromobutane, the radioisotope-containing portion of novel tracers for PET imaging of CB2 receptor expression.
P-16	Anna Majer*, Kathy Manguiat, Sarah Medina, and Stephanie A. Booth	The significance of pre-clinical miRNAs in prion disease.
P-17	Thatchawan Thanasupawat*, Katrin Hammje, Melanie Cieselski, Ibrahim Adham, Jean-Eric Ghia, Marc Del Bigio, Jerry Krcek, Cuong Hoang-Vu, Sabine Hombach-Klonisch, Thomas Klonisch	Human INSL5 is a novel marker of enteroendocrine cells (EEC) of the large intestine and in colon and carcinoid cancer.

POSTER #'S (* = PRESENTER)

POSTER #	AUTHOR LISTING	TITLE
P-18	Stephanie A. Booth*, Anna Majer, Kathy Manguiat, Sarah Medina, Bernard Abrenica and Yulian Niu	Decoding pre-clinical genomic programs in prion disease reveals in vivo evidence for the early induction of NMDA receptor-mediated signaling.
P-19	Edna Esteli Vasquez-Dominguez*, Brent Fedirchuk	Acetylcholine induces a hyperpolarization of voltage threshold in neonatal rat spinal motoneurons.
P-20	Brendan Olynik*, Robby Zachariah, Vichithra Liyange, Carl Olson and Mojgan Rastegar	A TALE of Homeobox Transcription Factors; Their Role in Neural Regulation of Stem Cells.
P-21	Teng Guan*, Chengren Li, Jiming Kong	BNIP3 and OGD-induced oligodendrocyte death: Role in white matter injury in cerebral ischemia.
P-22	Dali Zhang*, Wei Xiong, Stephanie Chu, Chao Sun, Benedict C. Albensi, Fiona E. Parkinson	CD73 is not required for hypoxic inhibition of synaptic activity in hippocampus.
P-23	Darrell R. Smith*, Subir K. Roy Chowdhury, Katie Frizzi, Lakshmi Kotra, Nigel A. Calcutt and Paul Fernyhough	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.
P-24	Kanami Orihara*, Sonia Charran, Redwan Moqbel	NMDA receptor activation induces amphiregulin, a potential survival factor production from CD4+ T cells.
P-25	Ngoc H. On*, and Donald W. Miller.	Lysophosphatidic acid (LPA)-induced enhancement of blood-brain barrier (BBB) permeability as a potential method for enhancing drug delivery to the brain.
P-26	Stephanie Chu*, Fiona E. Parkinson	Effect of human equlibrative nucleoside transporter 1 and ecto-5'nucleotidase (eN) in adenosine formation by astrocytes under ischemic conditions.
P-27	Reuben Saba, Sarah Medina* and Stephanie A. Booth	Prevalence of SNPs in the miRNA-binding regions of genes implicated in neurodegeneration.
P-28	Hanifi Soylu*, Dali Zhang, Richard Buist, Melanie Martin, Benedict C Albensi, Fiona E Parkinson.	Effect of neuronal expression of human equilibrative nucleoside transporter (hENT1) and caffeine on cerebral blood flow and cortical stroke in mice.
P-29	Marie-Krystel Gauthier*, Kamilla Kosciuczyk, Laura Tapley and Soheila Karimi	Modulatory effects of Neuregulin-1 on oligodendrocytes differenciation and preservation after spinal cord injury.
P-30	James Nagy, Wendy Bautista*	Connexin36 at nerve terminals in the vestibular, cochlear and hippocampal systems: mixed chemical/electrical transmission in mammalian CNS.
P-31	Sheryl L. Herrera*, Krista Hewlett, Jonathan D. Thiessen1, Richard Buist, James Peeling, Dale Corbett, Christopher Bidinosti, Melanie Martin	Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy Identify Abnormalities in the Development of a Rodent Model of Covert Stroke.
P-32	Jillian LeMaistre*, Lingling Lu, Hope Anderson, Christopher Anderson	Endogenous D-serine and NMDA receptor activation mediate astrocyte-induced cerebral vasodilation.
P-33	Ping Lu*, Amit Kamboj, Christopher M. Anderson	The role of FoxO3a in PARP-1-induced Bnip3 signaling pathway during hypoxia in cortical neurons.
P-34	Amit Kamboj*, Ping Lu, Spencer B. Gibson, Christopher M. Anderson	Bnip3 up-regulation and mitochondrial dysfunction in PARP-1 induced neurotoxicity.
P-35	Subir K. Roy Chowdhury*, Darrell R. Smith, Jason Schapansky, Ali Saleh, Suzanne Gomes, Eli Akude, Dwane Morrow, Nigel A. Calcutt and Paul Fernyhough.	Impaired AMP-activated protein kinase signaling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes.

POSTER #'S (* = PRESENTER)

POSTER #	AUTHOR LISTING	TITLE
P-36	Ali Saleh*, Darrell R. Smith, Lori Dunn, Corina Mertens, Abigail R Mateo, Randy Van der Ploeg, Cory Toth, Douglas W. Zochodne and Paul Fernyhough PFernyhough@sbrc.ca	The role of receptor for advanced glycation end products (RAGE) in sensory neurons isolated from normal rats.
P-37	Xiaoyu Chen*, Yonghui. LI, Sa Li, Hugo Bergen, Gilbert J. Kirouac	Orexins are involved in the expression of fear and anxiety in a rat model of post-traumatic stress disorder (PTSD).
P-38	Domenico L. Di Curzio*, Terry L. Enno, Richard J. Buist, & Marc R. Del Bigio	Characterization of Juvenile Ferrets Following Induction of Hydrocephalus with Kaolin.
P-39	Zhizhi Sun*, Vinith Yathindranath, Stephanie Chu, Fiona E. Parkinson, Torsten Hegmann, Donald W. Miller	Characterization of Aminosilane Coated Iron Oxide Nanoparticles for Brain Targeted Delivery.
P-40	Eric Platt*, Ken Oikawa, Gary L. Odero, Avril Hatherell, Melanie Neuendorff, Michael Bernstein, Benedict C. Albensi	NF-kappa b p50 subunit knockout impairs late LTP and alters long term memory.
P-41	M. Rak*, M. Kastyak-Ibrahim, C. Liao, M. Unger, C.J. Hirschmugl, B. Albensi, KM Gough	Label-free Imaging of Brain Tissue with High Resolution FTIR-FPA.

POSTER ABSTRACTS

P-1 Cerebellar defects in a mouse model of Rhombencephalosynapsis.

K. Bailey*, A.U. Mannan and H. Marzban.
Department of Human Anatomy and Cell Science, University of Manitoba Email: marzban@cc.umanitoba.ca

The cerebellum, which is involved in motor and non-motor functions, is a remarkable model system for studying brain defects. Rhombencephalosynapsis (RES) is a cerebellar malformation defined as complete or partial absence of the vermis, and fusion of hemispheres across the midline. Cerebello-trigeminal-dermal dysplasia, also known as Gomez Lopez Hernandez Syndrome (GLHS), is a neurocutaneous syndrome characterized by RES, scalp alopecia, and in some cases trigeminal anesthesia, ataxia and small stature. A mutant mouse called nax, which has a spontaneous mutation in lysosomal acid phosphatase (Acp2), presents similar phenotypic characteristics to GLHS including a lack of vermis, ataxia, whole body alopecia and small stature. In nax mice the cerebellum is underdeveloped with a prominent dysgenesis of the anterior vermis lobules but a relatively normal posterior cerebellum. The altered morphogenetic program of the anterior cerebellum results in fissures that normally run in the mediolateral direction to be replaced by anteroposterior directed fissures. At the cellular level, immunohistochemical analyses substantiated the gross anatomical defects and revealed a disorganized layering of cerebellar neurons. In addition, immunohistochemical analysis has confirmed that part of the cerebellum in nax mice is missing as fewer stripes of typical gene expression were found. Since the vermis is anatomically absent it seems likely that this is the portion missing, however medial gene expression patterns in the nax cerebellum were found to be similar to those normally expressed in the vermis. This suggests that in nax mice the vermis fails to form during development and later somatosensory afferents induce gene expression characteristic of the vermis in an attempt to compensate for its absence. Although it needs to be confirmed, it is promising that the vermis is absent and midline fusion of the hemispheres occurs in nax mice, therefore, providing an excellent model to study RES and GLHS. Supported by the Manitoba Health Research Council.

P-2 Patterned Purkinje Cells Degeneration in SOD1 Transgenic Mice

P. Afsharinezhad*, J Kong, and H. Marzban.
Department of Human Anatomy and Cell Science, University of Manitoba, Email: marzban@cc.umanitoba.ca

In despite of the uniform histological appearance of cerebellum, complex zones and parasagittal banding pattern can be revealed by the use of immunocytochemical markers, functional mapping, and the terminal field distribution of the afferents. Although the pattern of Purkinje cell death is complex, but it has been shown that it is aligned with patterned zones and stripes in cerebellum. Human superoxide dismutase 1 gene (SOD1) is responsible for producing superoxide dismutase enzyme, which functions to neutralize supercharged oxygen radicals within the cells. A mutation in this gene causes a neurodegenerative disease called amyotrophic lateral sclerosis (ALS). SOD1 transgenic mice with a mutated SOD1 gene are used as animal model to study ALS. P-003However, the wildtype SOD1 transgenic mice have shown adult P-004onset ataxia. It was expected that all Purkinje cells of wildtype SOD1 transgenic mice express calcium binding protein D28k (CaBP) uniformly in the cortex of cerebellum. However, Purkinje cells of these transgenic mice showed a patterned down-regulation of CaBP and subsequently cerebellar Purkinje cell loss. Immunohistochemistry of anti-CaBP and anti-Hsp25 (Heat-shock protein) revealed that the subset of Purkinje cells express CaBP is mirrored in the expression pattern of Hsp25 in the central and nodular zones. In addition, study with the two well-known zone and stripe markers (anti-Zebrin II and anti-PLCβ4) shown that down-regulation of these genes in the cerebellum. It is revealed down-regulation of anti-zebrin II in the Hsp25-regions of central and nodular zones. In more advanced cases, it is revealed down-regulation of PlcB4 in the regions of anterior zones. Nissl's staining indicated distinct differences between normal and abnormal Purkinje cells proceeding to the Purkinje cell degeneration. Further experiments are needed to be performed to explore different aspects of patterned Purkinje cells degeneration in SOD1trangenic mice as a model for ALS, but it is clearly shown that the Purkinje cells degeneration occurs and that is aligned with the zones and stripes in cerebellum.

Supported by the MHRC.

P-3 In vitro and in vivo protective roles of Thioredoxin1 in nervous system: Implication for therapeutic intervention after neural injury.

Shu Ying Ji, Roy Hutchings, and Eftekhar Eftekharpour*.
Neuroprotection Laboratory, Spinal Cord Research Centre, Department of Physiology, University of Manitoba

Thioredoxin1 is (Trx1) a small and ubiquitously expressed protein in all living organisms. It was originally identified as a cytosolic source of reducing equivalents and as an oxidant scavenger; however during the last decade Trx central role in functional regulation of many enzymes and transcription factors has been recognized. The redox regulatory role of Trx1 is implicated in all aspects of cell survival: including anti-apoptosis, anti-oxidation and cell proliferation. The complex protective roles of Trx1 have been shown for many diseases including diabetics, lung disease, cardiac and brain ischemia but has not been addressed for spinal cord injury (SCI). We have used a clinically relevant model of SCI to test the potential protective effects of recombinant human Trx1 (rhTrx1) during the acute stage of injury. The rhTrx1 was administered intrathecally during the first week of injury. Spinal cords were extracted at the termination of 7 days and were examined for cell and tissue preservation. Our results showed a decrease in apoptosis which was associated with enhanced tissue preservation. Parallel in vitro studies in neural cell cultures confirmed the anti-apoptotic effects of rhTrx1-therapy in a model of oxidative stress which was associated with a significant decrease in protein oxidation. Trx1 has also been linked to enhancement of cell proliferation in a wide variety of cells. We show that rhTrx can significantly enhance cell proliferation in adult neural stem cells cultures, suggesting that rhTrx1 therapy might promote the proliferation of endogenous neural stem cells in vivo, which will maximize the regenerative capacity of the injured tissue. These observations are the first indication of potential therapeutic properties of rhTrx for the treatment of SCI, and can be expanded to other diseases that are associated with enhanced cell death.

P-4 Regulation of Methyl CpG Binding Protein-2 via epigenetic mechanisms in Neural Stem Cells

Vichithra RB. Liyanage*, Robby M. Zachariah, Carl O. Olson, and Mojgan Rastegar
Regenerative Medicine Program, Faculty of Medicine, University of Manitoba, Department of Biochemistry and Medical Genetics, University of Manitoba, Canada. Email: rastegar @cc.umanitoba.ca

Methyl CpG Binding Protein 2 (MECP2) is a transcriptional regulator capable of recognizing and binding to methylated DNA. Mutations in MECP2 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, 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.

P-5 Understanding the role of MeCP2 isoforms in large-scale chromatin re-organization

Robby Zachariah* and Mojgan Rastegar
Regenerative Medicine Program, Department of Biochemistry and Medical Genetics, University of Manitoba, Canada Email: rastegar@cc.umanitoba.ca

Methyl CpG Binding Protein 2 (MECP2) is a transcriptional regulator capable of recognizing and binding to methylated DNA. Mutations in MECP2 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, 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.

P-6 Serotonergic cells in the Parapyramidal Region are active during a locomotor task and receive cholinergic input.

Couto-Roldan Erika*, Jordan Larry M.
Physiology Department, University of Manitoba. Email: larry@scrc.umanitoba.ca

Serotonin can activate the Central Pattern Generator (CPG). It has been shown that a discrete group of serotonergic neurons located in the Parapyramidal Region (PPR) produce locomotor-like activity when stimulated in the isolated neonatal rat brainstem-spinal cord preparation. 5-HT2A and 5-HT7 receptor antagonists are able to block locomotor-like activity evoked in this preparation. Furthermore, spinal cords of 5-HT7 knockout animals (5-HT7-/- mice) rarely exhibited 5-HT induced-well coordinated fictive locomotion, and the activity evoked can be blocked by ketanserine. Subdural application of SB-269970 alters locomotor activity in wild-type mice. Cholinergic input from the Mesencephalic Locomotor Region (MLR) to reticulospinal neurons that produce locomotion has been demonstrated. Here we tested the hypothesis that serotonergic cells in the PPR are active during a locomotor task and receive cholinergic input. Serotonergic neurons are detected using ePet/EYFP mice; in this model 5-HT neurons in the brain express EYFP. We subjected the animals to a locomotor task on a treadmill for 60 minutes. The animals were anaesthetized, perfused and the brainstem was removed. C-fos, a marker of neuronal activity, and choline acetyltransferase (ChAT), enzyme that catalyzes acetylcholine synthesis, inmunohistochemistry was performed on brainstem slices.

Expression of c-fos remained the same in raphe serotonergic cells in animals subjected to the locomotor task in comparison to control ones. A slight (not statistically significant) increase in the number of c-fos expressing serotonergic cells in the PPR region at the level of the 7th cranial nerve (7n PPR) of animals subjected to a locomotor task in comparison with control animals was observed. We found in the ventral medulla a small region (approximately 100 µm rostrocaudal extent) located at the level of the 7th cranial nucleus where expression of c-fos was dramatically increased after the locomotor task. An interesting finding was the close localization of serotonergic and cholinergic terminals on cells along the Lateral Paragigantocellularis Nucleus (LPGi). We can conclude that a small region in the ventral medulla,at the level of the 7th cranial nucleus, increases its activity during a locomotor task, as revealed by c-fos inmunohistochemistry. Serotonergic neurons in the PPR are slightly more active during a locomotor task. This effect is not observed in raphe neurons. Neurons located in the LPGi receive cholinergic and serotonergic input.

P-7 Antigenic induction of Nerve Growth Factor (NGF) in an animal model of Multiple Sclerosis (MS).

Crystal Acosta*, Claudia Cortes, Wenjun Zhu, Emma Frost, Mike Namaka, Brian MacNeil
Faculty of Pharmacy, University of Manitoba.

MS is a neurological, autoimmune disease of the central nervous system (CNS) characterized by inflammation, demyelination, and loss of axons. Peripheral T-cells are activated by CNS-like antigens. Subsequently, T-cells and cytokines cross the blood brain barrier into the dorsal root ganglia (DRG) and spinal cord where they continue the inflammatory process and induce disease. The resultant death of oligodendrocytes and loss of myelin leads to the degeneration of axons consequently compromises neuronal signaling. During an immune response inflammatory cytokines in the DRG subsequently induce neurotrophins such as NGF. The current study is designed to determine the role of nerve growth factor (NGF) in MS. We hypothesize that the pathogenic DRG acts as a reservoir for cytokines and neurotrophins. Lewis rats were divided into naïve control (NC), active control (AC) and active experimental autoimmune encephalomyelitis (EAE) groups. EAE was induced with guinea pig myelin basic protein. Comparative, time dependant analysis of NGF in DRG and spinal cord was conducted using immunohistochemistry, western blot, and quantitative real time PCR. Levels of NGF protein are increased in the DRG and spinal cord in both the AC and EAE rats. In the DRG a significant increase in NGF levels were observed between AC and EAE 12 days post induction (dpi) ($p<0.05$) and NC and EAE 9 ($p<0.01$), 12 ($p<0.01$), 15 ($p<0.001$), and 18 ($p<0.001$) dpi. A significant increase was observed between AC and EAE 12 dpi ($p<0.05$) and NC and EAE 12 ($p<0.001$) and 18 ($p<0.001$) dpi in the spinal cord. These data suggest CNS inflammation results in an increase in NGF expression levels in the DRG but not in spinal cord. EAE induction results in increased NGF in the spinal cord but not in the DRG. This implies that the NGF in the spinal cord is locally derived rather than transported in from the DRG. The study of neurotrophins in MS offers potential insights into new treatment strategies for this incurable disease.

P-8 Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) expression in the spinal cord associated with experimental autoimmune encephalomyelitis (EAE) induced neuropathic pain.

Wenjun Zhu, Claudia Cortés*, Crystal Acosta, Brian MacNeil, Emma Frost, Mike Namaka.
Faculty of Pharmacy, University of Manitoba.

Neuropathic pain has been reported to be the second worst disease associated symptom in multiple sclerosis patients. Further, the onset of neuropathic pain is often described by patients as occurring prior to the time of diagnosis. Therefore, the study of the molecules involved in this process is crucial for early detection of this disabling disease. Chemokines, such as CX3CL1 (fractalkine) are key mediators controlling the response of leukocytes to areas of inflammation. Recent evidence has shown that CX3CL1 and its receptor (CX3CR1) are known to be involved in the pathogenesis of neuropathic pain. Studies show that neuropathic pain results in the synthesis and release of CX3CL1 and CX3CR1 in the spinal cord microglia. Therefore, antagonism of CX3CR1 may be a promising novel strategy to reduce neuropathic pain. We proposed that CX3CL1 and CX3CR1 expression is up regulated in spinal cord of rats with experimental autoimmune encephalomyelitis (EAE). In this study, we used the EAE rat model of MS to determine the role of CX3CL1 during the antigenic induction of neuropathic pain. Protein and gene expression was analyzed using ELISA and real time RT-PCR respectively. We show a significant increase in CX3CL1 mRNA and protein expression in spinal cord ($p<0.005$) compared to active control rats at 12 days post induction. The receptor CX3CR1 is also significantly increased at the protein and mRNA level, at day 12 ($p<0.005$). These results correlate with peak neurological disability. Further, we detected markedly increased immunoreactivity for CXCL1 in astrocytes and neurons, and for receptor CX3CR1 in neurons and microglia. Our data showed a significant overexpression of the chemokine CX3CL1 in spinal cord astrocytes and neurons in EAE rat compare to a healthy controls. Therefore we propose that over expression of this chemokine is induced in response to inflammatory stimulus, and through its receptor CX3CR1 results in neuropathic pain.

P-9 Evidence of a neuroprotective role of nuclear factor-kB in oxidative stress induced by rabies virus in adult rat dorsal root ganglion neurons.

Wafa Kammouni*[1], Paul Fernyhough[2], Leena Hasan[3], Ali Saleh[2], Alan C. Jackson[1].
[1]Departments of Internal Medicine (Neurology), [2]Pharmacology and Therapeutics, 3Medical Microbiology, University of Manitoba and Division of Neurodegenerative Disorders, St. Boniface Hospital Research Centre. Email: kammouni@cc.umanitoba.ca

Recent studies in an experimental model of rabies showed major structural changes in the brain involving neuronal processes that are associated with severe clinical disease. Cultured adult rat dorsal root ganglion (DRG) neurons infected with the CVS strain of rabies virus showed axonal swellings and immunostaining for 4-hydroxy-2-nonenal (4-HNE), indicating evidence of lipid peroxidation associated with oxidative stress, and reduced axonal growth versus mock-infected DRG neurons. We have evaluated whether nuclear factor (NF)-κB might act as a critical bridge linking CVS infection and oxidative stress. On Western immunoblotting, CVS infection induced expression of NF-κB p50 subunit versus mock infection. Ciliary neurotrophic factor, a potent activator of NF-κB, had no effect on mock-infected rat DRG neurons and reduced the number of 4-HNE-labeled puncta. SN50, a peptide inhibitor of NF-κB, and CVS infection had an additive effect in producing axonal swellings, indicating that NF-κB is neuroprotective. The fluorescent signal for subunit p50 was quantitatively evaluated in the nucleus and cytoplasm of mock- and CVS-infected rat DRG neurons. At 24 hrs post-infection (p.i.) there was a significant increase in the nucleus:cytoplasm ratio, indicating increased transcriptional activity of NF-κB, perhaps as a response to stress. At both 48 and 72 hrs p.i. there was significantly reduced nuclear localization of NF-κB. CVS infection may induce oxidative stress by inhibiting nuclear activation of NF-κB. A rabies virus protein may directly inhibit NF-κB activity. Further investigations are needed to gain a better understanding of the basic mechanisms involved in the oxidative damage associated with rabies virus infection.

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P-10 Prion-induced miRNA-146a target gene identification using proteomic, genomic and bioinformatic based approaches

Shantel Gushue*,[1,2] Reuben Saba,[1,2] and Stephanie A. Booth[1,2].
[1]University of Manitoba, [2]National Microbiology Laboratory, Winnipeg, MB, Canada. Email: shantelgushue@phac-aspc.gc.ca.

Increasing evidence supports the involvement of microRNAs (miRNAs) in inflammatory and immune processes in prion neuropathogenesis. MiRNAs are small non-coding RNA's that regulate gene expression at the post-transcriptional level and are involved in numerous cellular processes. One miRNA in particular, miR-146a, has gained considerable attention given its role in modulating the innate immune response, inflammatory signaling and neurodegenerative disorders. We determined that this miRNA is over-expressed in a mouse prion disease model, concurrent with the onset of prion deposition and appearance of activated microglia. Furthermore, we found that as well as modulating the innate immune response in microglial cells, miR-146a appeared to also be capable of regulating morphological changes that accompany microglial activation as well as phagocytic mediators of the oxidative burst. We hypothesized that alterations in the proteome induced by miR-146a overexpression or knock-out will lead to the identification of key host proteins involved in disease. We developed an in vitro system that mimics miR-146a overexpression/knockdown within the context of an inflammatory response in a mouse microglial cell-line. Employing quantitative proteomics, we systematically identified those protein targets whose expression is modulated by miR-146a. Comparing our proteomic data with genomic data and bioinformatically predicted targets, we determined a short-list of potential targets. Validation of binding of miR-146a to its predicted target sites was performed for a number of these genes. We have determined a number of novel miR-146a regulated proteins that play important roles in microglial immune function including phagocytosis, cellular movement and shape, and antimicrobial/antitumoral responses. Identification of these targets not only gives insight into the biology and function of this specific miRNA, but manipulation of its control over the immune pathways involved in neurodegeneration may be an avenue to design new therapeutics.

This research project was funded by the Natural Sciences and Engineering Research Council of Canada through the Post Graduate Scholarship, PrioNet Canada, the Public Health Agency of Canada and the University of Manitoba.

P-11 Computational modeling of the binding of Calcium and Zinc ions to Calbindin D28k

Sara I Omar*[1], Benedict C Albensi[2], Kathleen M Gough[3]
[1]Department of Chemistry, University of Manitoba, [2]Department of Pharmacology and Therapeutics, St. Boniface Hospital Research Centre, [3]Department of Chemistry, University of Manitoba. Email: sara.ibrahim.omar@gmail.com

Neurodegeneration in Alzheimer's disease (AD) is characterized by multiple abnormalities including the disruption of calcium homeostasis. We suspect a possible role for Calbindin D28k (CB), one of the proteins responsible for calcium homeostasis; it can act as a calcium buffer and as a sensor. CB has 6 calcium binding domains called EF-hands, each composed of a helix-loop-helix sequence. Although very little is known about the apo (unbound) form of CB, its holo form (fully bound) has been resolved by NMR. The protein is capable of binding only four Ca2+, in EF-hands 1, 3, 4 and 5. Studies have shown that CB can also bind Zn2+, which is known to be elevated in AD and which is linked with inflammatory processes. The objective of this research is to achieve a clearer understanding of the factors that control the binding of these cations, through computational calculations. Software packages employed include a molecular mechanics program for ligand docking: Autodock (autodock.scripps.edu) and molecular dynamics simulations program to permit flexibility in the protein backbone: NAMD, (http://www.ks.uiuc.edu/Research/namd/). Semi-empirical calculations are being run to help explain why EF2 and 6 lose their calcium binding ability. Docking calculations show that, upon equilibration of the holo protein, the angle between the helices of EF2 become obtuse, while the angle between the helices of EF6 becomes acute. We propose that these deviations from the optimal perpendicular configuration of the loop residues in functional EF hands may be the reason why these hands lose their calcium binding capability. In ongoing research, docking calculations with Zn2+ as ligand are being conducted to investigate its potential interference with calcium binding. Therefore, these results have important implications for the neuroprotective capability of CB for calcium binding and calcium homeostasis in AD.

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P-12 Investigation of the deregulated miRNome identified during acute viral infections in a murine model of HSV-1 encephalitis

Kyle A. Caligiuri*, Anna Majer and Stephanie A. Booth.
Department of Medical Microbiology and Infectious Diseases, University of Manitoba and Molecular PathoBiology Unit, Public Health Agency of Canada. Kyle. Email: caligiuri@phac-aspc.gc.ca

We seek to gain a better understanding of the transcriptomic changes that take place during an acute viral infection of the brain. We chose to use a murine host and Herpes Simplex Virus Type-1 (HSV-1) as the infectious trigger, causing HSV-1 encephalitis (HSVE). HSV-1 is generally a persistent, harmless virus that may resurface frequently throughout the life of the host. In rare cases, HSV-1 may cause HSVE which has a ≥70% mortality rate if untreated. It is also the major cause of all sporadic viral encephalitis cases. MicroRNAs (miRNAs) are a major class of post-transcriptional gene regulators of which many are deregulated in the presence of HSV-1 infection. Specifically, several families of miRNAs have been shown to be highly up-regulated in vivo as determined by next generation sequencing (NGS) and TaqMan Low Density Arrays (TLDAs), and a subset of these miRNAs were subsequently validated individually by real-time PCR. TLDA data yielded 58 up-regulated miRNAs found in vivo along with 5 miRNAs that were down-regulated. These results correlated well with the NGS and previous in vitro data. Target prediction software analysis yielded a wealth of potential targets to investigate via luciferase assays. In particular, the potential for these miRNA families to influence apoptosis mechanisms was investigated via ATP/ADP Ratio Assays. It was also found that the family of miR-141/200c has the potential to inhibit apoptosis in vitro. Considering the widespread infectivity of HSV-1 throughout the population and the potential seriousness of HSVE if untreated, this information may yield insight into how miRNAs can be manipulated for therapeutic benefits against acute viral infections of the brain. Supported by the Public Health Agency of Canada through the Biotechnology Initiative of Government Laboratories – Genomics and Proteomics, and the University of Manitoba.

P-13 Imaging of selected brain regions in 3xTg Alzheimer's mouse model by Magnetic Resonance Microscopy.

Marzena Z Kastyak-Ibrahim[1*], Richard Buist[2], Domenico L Di Curzio[3], Marc R Del Bigio [3,4], Benedict C Albensi[5], Melanie Martin[1,2,5]
[1]Physics, University of Winnipeg, [2]Radiology, [3]Human Anatomy and Cell Science, [4]Pathology and [5]Pharmacology and Therapeutics, University of Manitoba

Alzheimer's disease (AD) is the most common cause of dementia in the ageing populations worldwide. In Canada, the number of people living with AD or a related dementia is expected to reach 1.1 million in 2035, if nothing improves in early diagnostics and treatment. Amyloid plaques, one common feature of AD, are not always present and their appearance may occur in the late stage of the disease when treatment is not the most effective. Therefore, targeting early and easier to detect changes, preceding plaque formation is crucial. The aim of this study was to develop methods to obtain information about temporal and spatial changes occurring in selected regions of the mouse brain and validate these methods using the 3xTg mouse model of AD.
In our study, we used a combination of MRI methods including: a T2 weighted method (98 x 98 µm2 in plane resolution and 250 µm slice thickness, with adjacent axial slices covering the 6 mm region including corpus callosum, fornix and entire hippocampus), an EPI-DTI method (195 x 195 µm2 in plane resolution 500 µm slice thickness; imaging time 48 min) and ex vivo DTI method (98 x 98 µm2 in plane resolution and 500 µm slice thickness; imaging time 21 min) to monitor changes in 3xTg AD mice and control mice. Animals were imaged at 11, 13, 15, and 17 months. Mice were then perfused, brains were imaged ex vivo and frozen.
The combination of these MRI methods allows the monitoring of in vivo temporal and anatomical changes occurring in selected regions of mouse brains, with high spatial resolution, and short scan times.. MRI data obtained with these methods can be used to determine the volume of the brain or selected brain region (for example hippocampus or ventricles) and areas where water diffusion is altered, as well as diffusion parameters (FA). Additional MRI measurements performed ex vivo allow for higher in-plane resolution giving more detailed anatomical information. Correlation with histological and biochemical data obtained post mortem will help us to determine the nature of changes observed in vivo in selected brain regions of 3xTg AD mouse model.

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P-14 Examining microRNAs in NMDA related excitotoxicity in prion induced neurodegeneration.

Amrit S. Boese* and Stephanie A. Booth.
Department of Medical Microbiology, University of Manitoba. Email: amrit.boese@phac-aspc.gc.ca

Prion Diseases are a set of neurodegenerative diseases that lead to death; examples include Creutzfeld-Jakob Disease in humans, mad cow disease and scrapie in sheep. Prion induced neurodegeneration commences long before clinical symptoms appear. The purpose of our study is to elucidate the relationship between microRNAs (miRNA) and neurodegenerative processes that occur throughout the course of disease using a murine model. Previous miRNA profiling at end stages of scrapie infection in mice revealed a number of disrupted miRNAs including miRNA-128 and miRNA-342-3p. In particular, we are interested in the relationship between miRNA-128 and miRNA-342-3p and NMDA related excitotoxicity that is apparent at preclinical stages of scrapie infection in mice. Both miR-128 and miR-342-3p computationally target subunits of the NMDA receptor, namely GRIN2B and GRIN2D. To test the relationship between miRNA-128 and miRNA-342-3p with NMDA receptor subunits, a luciferase reporter assay was utilized to test miRNA binding with the 3'UTR of GRIN2B and GRIN2D, respectively. Furthermore, GRIN2B has an altered expression during preclinical stages of prion infection in mice thus GRIN2B was knocked down in primary murine hippocampal cells and miRNA alterations were detected. Finally, miR-128 and miR-342-3p were overexpressed and knocked down in primary murine hippocampal cells to determine global gene alterations. To determine if GRIN2B is downregulated due to extrasynaptic excitotoxicity, assays inducing extrasynaptic or intrasynaptic NMDA signalling were performed and miRNA expression changes were examined. Our results indicate there is a relationship between NMDA excitotoxicity and miRNAs that are found during prion induced neurodegeneration. Supported by PrioNet Canada and Public Health Agency of Canada.

P-15 Synthesis of 18F-4-fluorobromobutane, the radioisotope-containing portion of novel tracers for PET imaging of CB2 receptor expression.

Katherine Cordova[1*], Mary-Lou Solbrig[2] and Shadreck Mzengeza[1]
[1]Department of Radiology, University of Manitoba, [2]Department of Internal Medicine, University of Manitoba

Objective: The objective of this project was the synthesis of the radioisotope-containing portion of PET radiotracers for monitoring CB2 receptor expression in Multiple Sclerosis. 18F-4-fluorobromobutane is a radioactive [18F]fluoroalkylating agent that will be used to radiolabel biomolecules that have high affinity for CB2 receptors.

Methods: The synthesis of 18F-4-fluorobromobutane was achieved by [18F]fluorination of 1,4-dibromobutane using the Siemens Explora programmable automated synthesis module. 18F-Fluoride was produced via 18O(p,n)18F nuclear reaction using the Siemens Eclipse-RD 11 MeV cyclotron by bombarding 18O-water with a proton beam. 18F-Fluoride was delivered from the cyclotron to the automated synthesis module where 1,4-dibromobutane was labeled with 18F via substitution to yield 18F-4-fluorobromobutane. The product was purified by passing through alumina solid extraction cartridge. The product was then spotted on a TLC plate, developed in acetonitrile and scanned on the Bioscan radioTLC scanner.

Results: The Rf of 18F-4-fluorobromobutane produced was identical to an authentic commercially available non-radioactive standard. The product was easily purified using alumina cartridge. The non-decay corrected yield of the product was 6.5%.
Conclusion: The overall results indicate that 18F-1-bromo-4-fluorobutane can be synthesized using the Siemens Explora synthesis module. The radioisotope-containing portion of the CB2 receptor PET radiotracer was successfully synthesized. Future work will involve coupling this radioactive portion to molecules with high affinity for CB2 receptors.

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P-16 The significance of pre-clinical miRNAs in prion disease.

Anna Majer[1,2*], Kathy Manguiat[1], Sarah Medina[1], and Stephanie A. Booth[1,2]
[1]Molecular PathoBiology, Public Health Agency of Canada and [2]Medical Microbiology, University of Manitoba.
Email: Anna.Majer@phac-aspc.gc.ca

The development of prion disease occurs as a result of the conversion and subsequent accumulation of the normal cellular form of the prion protein (PrPC) to the abnormal, infectious form (PrPSc). Nevertheless, it remains unclear how these changes cause neuronal connections to be severed, leading to prion-induced neuronal degeneration. Investigating pre-clinical stages of prion disease may help identify key regulators of the neuronal survival/death pathways that become deregulated by PrPSc accumulation. miRNAs are a major class of gene regulators that fine tune gene expression at the post transcriptional level and many have been implicated in neurodegenerative diseases. We aim to identify miRNAs that are deregulated during early prion disease and investigate the function of these miRNAs on cellular mechanisms of prion-induced neurodegeneration. Hippocampal CA1 regions were removed from both scrapie and control mice using laser capture microdissection at 40, 70, 90, 110, 130 and terminal days post inoculation. Total RNA was extracted and samples screened for miRNA levels using TaqMan low density arrays. A subset of miRNAs that were up-regulated in infected samples at pre-clinical disease was further validated using real-time PCR. We confirmed the expression level of one miRNA via in situ hybridization. Bioinformatic miRNA target prediction yielded a list of genes involved in neuronal-specific function of which many are implicated in neuroprotective mechanisms. Characterizations of these miRNAs in primary mouse hippocampal neurons are currently ongoing. MicroRNAs we found to be deregulated during pre-clinical stages of prion disease may help our understanding of early mechanisms of prion-induced neurodegeneration.

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P-17 Human INSL5 is a novel marker of enteroendocrine cells (EEC) of the large intestine and in colon and carcinoid cancer.

Thatchawan Thanasupawat*[1], Katrin Hammje[2], Melanie Cieselski[2], Ibrahim Adham[3], Jean-Eric Ghia[4,5], Marc Del Bigio[1,6], Jerry Krcek[1,7], Cuong Hoang-Vu[2], Sabine Hombach-Klonisch[1,8], Thomas Klonisch[1,7,9]
Depts. of [1]Human Anatomy & Cell Science, [4]Internal Medicine, [5]Immunology, [6]Pathology, [7]Surgery, [8]Obstetrics, Gynecology & Reproductive Medicine, [9]Medical Microbiology & Infectious Diseases, Faculty of Medicine, University of Manitoba, 130 Basic Medical Sciences, 130-745 Bannatyne Ave., Winnipeg, Manitoba, R3E 0J9, Canada; [3]Institute of Human Genetics, University of Göttingen, Germany, [2]Clinics of General, Visceral & Vascular Surgery, Martin Luther University (MLU), Germany.

Introduction: The insulin superfamily comprises insulin, insulin-like growth factors (IGF-1 and -2), relaxin, and the insulin-like (INSL) peptides 3-7. INSL5 shares high sequence homology with INSL7/ relaxin 3. INSL5 is the ligand for the orphan receptor RXFR4. Insl5-KO mice show mild hyperglycemia suggesting a role for Insl5 in glucose metabolism. Enteroendocrine cells (EEC) constitute the largest endocrine systems in the body. EEC are endoderm-derived and develop from the same pluripotent stem cells as the other intestinal epithelial cell lineages. EEC alone and in interaction with the enteric nervous system have important roles in the gut-brain, gut-pancreas, and immuno-endocrine axis.

Hypothesis: Human INSL5 and RXFP4 are a new signalling system in the normal and neoplastic colon.
Methods: Immunohistochemistry, immunofluorescence (IF), PCR, colitis and Insl5-KO mouse models
Results: Immunoreactive human INSL5 was detected in synaptophysin+ EEC within the mucosa of the normal colon, in colon carcinoma and carcinoid tumours. RXFP4 was expressed in colonocytes. Acute inflammation of the large intestine did not affect Insl5+ EEC. Insl5 was not essential for the formation of synaptophysin+ EEC in the mouse gut.

Conclusion: We show for the first time the tissue localization of human INSL5 and RXFP4 as a novel autocrine/ paracrine ligand receptor system in the normal large intestine, colon cancer cell lines, colon cancer tissues, and carcinoid tissues.

P-18 Decoding pre-clinical genomic programs in prion disease reveals in vivo evidence for the early induction of NMDA receptor-mediated signaling

Stephanie A. Booth[1,2*], Anna Majer[1,2,] Kathy Manguiat[1], Sarah Medina[1], Bernard Abrenica[1] and Yulian Niu[1]
[1]Molecular PathoBiology Unit, Public Health Agency of Canada, Winnipeg, MB, Canada; [2]Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada.

In prion diseases neurons ultimately undergo necrosis and apoptosis, yet the up-stream pathways which trigger the damage and dysfunction of nerve cells are as yet unidentified. Assessing the global transcriptome patterns provides a way to unravel the molecular pathobiology of prion diseases by identifying perturbed networks during the illness. We performed a thorough high-throughput microarray screen for genes specifically altered in the hippocampal CA1 region of infected mice and determined a clear temporal genetic response to the challenge of replicating prions.
The earliest transcriptional changes within neurons were of particular interest and we identified a subset of ~ 400 genes dysregulated in CA1 neurons during pre-clinical disease. Many of these genes were novel to prion disease, and their biological functions in healthy and/or diseased neurons not well annotated. Comparison with published studies did however reveal striking similarities to transcriptional profiles generated from neurons in response to N-methyl-D-aspartate receptor (NMDAR) stimulation. In particular the strongest correlation in early stages of disease was to a genetic profile triggered by nuclear calcium signaling; we identified 97/185 of these genes as being altered over 2-fold during prion disease. Of the remainder, 22 were below the detection threshold and 21 were not represented on our array. These included genes with putative functions in death/survival such as GADD45β, GADD45γ and NR4A1, genes involved in nuclear calcium signaling such as BTG2, BCL6 and CAMKIV and novel genes such as HOMER1, a scaffolding protein that interacts with post-synaptic density proteins, and TRIB1 also proposed to be an adaptor or scaffold protein.
Synaptic NMDARs are believed primarily to be transducers of signals promoting a neuroprotective genetic cascade, whereas Ca++ influx through extra-synaptic localized NMDARs appears to directly oppose these effects, and to promote cell death. The transcriptional changes we have identified within neurons appear to reflect the early induction of a genomic survival program. This genetic program appears to be lost later in disease, prior to the onset of clinical symptoms.

P-19 Acetylcholine induces a hyperpolarization of voltage threshold in neonatal rat spinal motoneurons

E.E. Vasquez-Dominguez*, B. Fedirchuk.
Department of Physiology, University of Manitoba. Email: brent@scrc.umanitoba.ca

Previous work has established that there is a dramatic increase in the excitability of mammalian spinal motoneurons prior to, and throughout motor activity. One property that is rapidly changed is the voltage threshold for action potential initiation (Vth), which is hyperpolarized (i.e. lowered), thereby facilitating motoneuron firing. Lowering of Vth has been observed in motoneurons during brainstem-induced fictive locomotion, and descending monoaminergic systems have been implicated in this effect. Recently however, we found Vth lowering also occurs during fictive scratch activity in acutely spinalized preparations. This suggests that in addition to descending systems, there is an intrinsic spinal neuromodulatory system able to regulate motoneuron Vth. Others have shown that cholinergic inputs to spinal motoneurons can alter their properties, but it is not clear whether they are able to modulate motoneuron Vth.
The aim of this study is to test the hypothesis that the cholinergic system can modulate motoneuron Vth. Whole-cell patch clamp recordings in either voltage or current-clamp configuration were obtained from lumbar motoneurons in in vitro neonatal rat spinal cord preparations (P0-4). Acetylcholine chloride (ACh; 10-80 µM) or muscarine (10-40 µM) were applied to the extracellular solution in increasing concentrations and the motor output monitored using suction electrodes recording the L2 and L5 ventral roots bilaterally. In some experiments, synaptic blockers (AP5 20 µM, CNQX 10 µM, bicuculline 10 µM, strychnine 10 µM) were used to synaptically isolate the recorded motoneuron.
ACh induced Vth lowering in 7/11 motoneurons and ranged from a 4-26 mV effect. Muscarine induced Vth lowering in 4/8 motoneurons and ranged from 4-6 mV effect. This robust Vth lowering occurred in both L2 and L5 motoneurons, was detectable in either voltage or current clamp, and it was not associated with any evident facilitation of persistent inward currents. Vth lowering persisted with synaptic blockers in the bath and could occur in the absence of discernable network motor output.
Overall, these results suggest that in addition to descending monoaminergic systems, a cholinergic system can lower Vth and thereby enhance motoneuron excitability. Future studies will determine the relative contributions of this spinal system and descending monoaminergic systems in enhancing motoneuron excitability during motor outputs.

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P-20 A TALE of Homeobox Transcription Factors; Their Role in Neural Regulation of Stem Cells

Brendan Olynik*[1, 2], Robby Zachariah[1, 2], Vichithra Liyange[1, 2], Carl Olson[1, 2] and Mojgan Rastegar[1,2]
[1]Regenerative Medicine Program, Faculty of Medicine, University of Manitoba, [2]Department of Biochemistry and Medical Genetics, University of Manitoba Email: Rastegar@cc.umanitoba.ca

Introduction/Objective of Investigation: Homeobox transcription factors are highly conserved across species, well known for dictating the positional identity of bodily segments throughout development. There exists a homeobox subclass known as TALE (Three Amino Acid Loop Extension) proteins, which are essential for normal development; being expressed throughout the nervous system. We are interested in understanding the role of TALE proteins in neural development that can be studied using an in vitro system of neural stem cell differentiation.

Methods: We study neural stem cells (NSC) that are isolated from the forebrains of embryonic day 14 mice. Neural stem cells are expanded for 7 days in vitro, meanwhile, NSC conglomerates called neurospheres that will form after 7 days will be broken apart and subjected to neural differentiation conditions. Cell lysates are harvested at various stages of differentiation to analyze proteins via Western Blotting, as well as other methods (i.e. RT-PCR for RNA samples). Additionally, cells are fixed and analyzed, using an immunocytochemistry system of fluorescently labeling biomarkers of interest. Forebrain sections are also used for comparative immunohistochemistry studies.

Results/Conclusion: Our collected data implies that certain TALE proteins may be important for regulation of neural system development. Further experiments are in progress to corroborate our preliminary findings.

Supporting Agencies: Canadian Foundation for Innovation, Health Sciences Center Foundation, Manitoba Health Research Council, Natural Sciences and Engineering Research Council of Canada, Scottish Rite Charitable Foundation of Canada

P-21 BNIP3 and OGD-induced oligodendrocyte death: Role in white matter injury in cerebral ischemia

Teng Guan*, Chengren Li, Jiming Kong.
Human Anatomy and Cell Science, University of Manitoba. Email: kongj@cc.umanitoba.ca

Oligodendrocytes (OLs), the major myelin-forming cells in the central nervous system and are highly vulnerable to ischemic insult. OLs undergo a complex pattern of death in cerebral ischemia with an early loss from excitotoxicity via the glutamate AMPA receptors, and die at more delayed time points via apoptosis. Since OLs death and white matter damage contribute to functional deficits following cerebral ischemia, enhancing the long-term survival of mature and newly born OLs could promote white matter function at delayed time points following ischemic injury. We sought to identify potential role of BNIP3, a proapoptotic member of the Bcl-2 family of death-regulating proteins that promote the intrinsic pathway of programmed cell death in cerebral ischemic white matter damage. Primary cultures of rat OLs were subject to oxygen-glucose deprivation and reoxygenation. Middle cerebral artery occlusion (MCAO) was induced in BNIP3 knockout (KO) and wild type (WT) mice with 1, 3 or 7 d of reperfusion. Cell death was quantitatively assessed by LIVE/DEAD viability assay and TUNEL assay. Stage-specific OL cultures were identified by immunocytochemical characterization. Expression and level of proteins were examined using immunohistochemistry and immunoblotting. Survival assays revealed that oligodendrocyte precursor cells (OPCs) and later-stage precursors were highly vulnerable. Exposure of OLs to OGD resulted in a significant increase in BNIP3 expression. Down-regulation of BNIP3 with shRNA or pharmacological inhibition with necrostatin-1 reduced OGD-triggered OL apoptosis. After tMCAO/R, loss of OLs was detected at ipsilateral external capsule (EC) concurrent with highly BNIP3 expression. TUNEL showed greater cell death in EC in WT mice when compare with KO mice. Both PDGFR-α and GalC level was significantly increased in WT and KO ipsilateral white matter. Moreover, the increase in KO mice was significantly higher than WT. Our results suggested BNIP3 deficiency may promote OPCs proliferation and protect the immature OLs from ischemic injury. These studies unveil BNIP3 as an important mediator in OLs death, which may contribute to a better understanding of white matter injury in stroke.

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P-22 CD73 is not required for hypoxic inhibition of synaptic activity in hippocampus

Dali Zhang[1]*, Wei Xiong[1], Stephanie Chu[1], Chao Sun[1], Benedict C. Albensi[1, 2], Fiona E. Parkinson[1]
[1]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, R3E 0W3, Canada, [2]Division of Neurodegenerative Disorders, St Boniface Hospital Research Centre, Winnipeg, R2H 2A6, Canada Email: zhang7@cc.umanitoba.ca

Adenosine, through activation of its A1 receptors, has neuroprotective effects during hypoxia and ischemia. The present study was performed to test the importance of CD73 (ecto-5'-nucleotidase) for basal and hypoxic/ischemic adenosine production. Hippocampal slice electrophysiology was performed with CD73 +/+ and CD73 -/- mice. Adenosine and ATP had similar inhibitory effects in both genotypes, with IC50 values of approximately 25 µM. The inhibitory effects of ATP in CD73 +/+ and CD73 -/- slices were blocked by the adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and were enhanced by the nucleoside transport inhibitor S-(4-nitrobenzyl)-6-thioinosine (NBTI), consistent with effects that are mediated by adenosine after metabolism of ATP. AMP showed a similar inhibitory effect to ATP and adenosine, indicating that the response to ATP was not mediated by P2 receptors. In comparing CD73 -/- and CD73 +/+ slices, hypoxia and oxygen-glucose deprivation produced similar depression of synaptic transmission in both genotypes. An inhibitor of tissue non-specific alkaline phosphatase (TNAP) was found to attenuate the inhibitory effects of AMP and ATP, increase basal synaptic activity and reduce responses to oxygen-glucose deprivation selectively in slices from CD73 -/- mice. These results do not support an important role for CD73 in the formation of adenosine in the CA1 area of the hippocampus during basal, hypoxic or ischemic conditions, but instead point to TNAP as a potential source of extracellular adenosine when CD73 is absent. Supported by Canadian Institutes of Health Research.

P-23 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 R. Smith*[1,2], Subir K. Roy Chowdhury[2], Katie Frizzi[3], Lakshmi Kotra[4], Nigel A. Calcutt[3] and Paul Fernyhough[1,2]
[1]Dept of Pharmacology & Therapeutics, University of Manitoba, Winnipeg. [2]Division of Neurodegenerative Disorders, St Boniface Hospital Research Centre, Winnipeg. [3]Dept of Pathology, UCSD, La Jolla. [4]Center for Molecular Design and Preformulations, University Health Network, Toronto and Dept of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto. Email: dsmith@sbrc.ca

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-1a 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.

P-24 NMDA receptor activation induces amphiregulin, a potential survival factor production from CD4+ T cells

Kanami Orihara*, Sonia Charran, Redwan Moqbel
Department of Immunology, University of Manitoba, Winnipeg, MB EDmail: oriharak@cc.umanitoba.ca

Background: Increase in epidermal growth factor receptor (EGFR) expression in the airways is associated with severe and corticosteroid-resistant asthma. Recently, several reports showed that amphiregulin, a member of the epithelial growth factor (EGF) family, might be closely associated with airway remodeling in corticosteroid resistance. The impact of NMDA receptors in neurodegenerative conditions is convincing in a number of neurodegenerative conditions including Alzheimer's disease, HIV dementia, epilepsy and Huntington's disease. However, the current knowledge of NMDA receptors in immune regulation is very limited. Previously, we have shown that activated human CD4+ T cells express NMDA receptors.

Objective: We hypothesize that signaling via NMDA receptors, enhances amphiregulin expression and secretion from CD4+ T cells, and contribute for the survival of the CD4+ T cells.

Materials and Methods: CD4+ T cells were isolated from PBMC from consenting healthy donors, using a routine magnetic-activated cell sorting method, and activated with anti-CD2/3/28 antibodies. Expression of amphiregulin mRNA and protein was determined using quantitative real-time PCR and ELISA, respectively. Cell viability was detected by MTT assay. Promoter analysis was performed in silico, using the online software, Transfac, Matrix Search for Transcription Factor Binding Sites provided by Biobase.
Results: NMDA receptor activation treatment in vitro significantly enhanced amphiregulin levels from activated CD4+ T cells. Also, exogenous amphiregulin treatment promoted CD4+ cells survival. In silico promoter analysis indicated that amphiregulin gene sequence has the highest theoretical number of GATA-3 binding sites in its promoter sequences than other EGFR agonists. Real-time PCR data showed that amphiregulin expression level correlates positively with IL-4 expression levels in activated CD4+ T cells.
Conclusion: We detected amphiregulin production from CD4+ T cells, which is enhanced through NMDA receptor activation. Evidence about GATA-3 and IL-4 indicated that amphiregulin may be differentially expressed in association with Th2, but not Th1 cells. Thus, we suggest that amphiregulin may be a potential survival factor for Th2 cells, regardless of cytokine signaling.

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P-25 Lysophosphatidic acid (LPA)-induced enhancement of blood-brain barrier (BBB) permeability as a potential method for enhancing drug delivery to the brain.

Ngoc H. On*, and Donald W. Miller.
Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, Canada

Background: Delivery of drugs to the CNS is limited due to the restrictive nature of the BBB. Transient modulation of BBB permeability is one method for enhancing drug delivery to the brain and may have potential CNS drug delivery applications.

Objectives: Characterize the extent of LPA-induced modulation of BBB permeability and provide initial proof-of-concept for use of LPA to enhance drug delivery to the brain.

Methods: Alterations in BBB permeability were characterized in Balb/C mice using a small (gadolinium contrast-enhanced agent (Gad), and large (IRdye800cw PEG) vascular permeability imaging agent. In addition, Rhodamine 800 (R800) imaging agent was used to monitor changes in P-glycoprotein-mediated BBB permeability. Mice were also administered 3H-methotrexate, either alone or in the presence of LPA to determine improvements in brain delivery of chemotherapeutic agent.

Results: The magnitude of BBB disruption was greatest for Gad with increases of 20-fold. Macromolecule marker, IRdye 800cw PEG, showed approximately 3-fold enhancement in brain accumulation following LPA. Increased brain penetration of R800 was observed following LPA exposure. The brain accumulation of methotrexate was increased 17-fold in LPA treated mice compared to vehicle.

Conclusions: LPA produced a rapid and reversible increase in BBB permeability to a wide variety of agents. Use of LPA in combination with therapeutic agents may be an effective strategy to increase drug delivery to the brain.

Supporting Agency: Manitoba Health Research Councils (MHRC)

P-26 Effect of human equlibrative nucleoside transporter[1] and ecto-5'nucleotidase (eN) in adenosine formation by astrocytes under ischemic conditions.

Stephanie Chu*, Fiona E. Parkinson
Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada Contact: umchu2@cc.umanitoba.ca

Objectives: Under ischemic conditions, levels of adenosine (ADO) increase up to 100-fold in brain. Intracellular or extracellular pathways of ADO formation from neurons or astrocytes could contribute to these rising levels of ADO. The present study examined release of ADO from primary cultures of cortical astrocytes from wild type C57bl6 (wt) or CD73 knock out (KO) mice, under basal or ischemia-like conditions.

Methods: Astrocytes cultured from wt or CD73 KO mice were incubated with 3H-adenine to radiolabel intracellular ATP. Astrocytes were then subjected to glucose deprivation (GD) or oxygen-glucose deprivation (OGD) conditions by treatment with 2-deoxyglucose (10mM) in glucose-free buffer for 30 min (37oC) in a humidified chamber or for 1 hour (37oC) in 95% N2 and 5% CO2. The effects of dipyridamole (DPR; 30 µM), an inhibitor of ENT1 and ENT2, or α,β-methylene ADP (AOPCP; 50 µM), an inhibitor of CD73 on [3H]purine release from astrocytes was tested.
Results: CD73 KO astrocytes produced less ADO under GD and OGD conditions ($p < 0.001$) than wt astrocytes; inosine (INO) levels did not differ between wt and CD73 KO cells. Under GD and OGD conditions, ADO levels were significantly higher in wt cultures ($P < 0.001$).

Conclusions: Astrocytes produce ADO, but not INO, via an extracellular pathway that requires CD73. These data confirm the role of CD73 in the extracellular pathway contributing to rising levels of ADO formation under ischemia like conditions.

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P-27 Prevalence of SNPs in the miRNA-binding regions of genes implicated in neurodegeneration

Reuben Saba[1], Sarah Medina*[1] and Stephanie A. Booth[1,2]
[1.] Molecular Pathobiology, Public Health Agency of Canada; [2.] Medical Microbiology and Infectious Diseases, University of Manitoba. Reuben.Saba@phac-aspc.gc.ca

Objective: We aimed to identify genetic variability of miRNA target sites in a catalogue of genes differentially expressed in prion-induced neurodegeneration. We were specifically interested in determining whether SNPs at these sites could potentially represent a starting point for the discovery of novel germline markers of susceptibility to conditions that may contribute to neurodegeneration in humans.

Methods: In silico approaches were used to screen for the presence of SNPs in the 3'UTR of 125 genes. The potency of the SNPs in altering miRNA::mRNA interaction was evaluated by performing in silico hybridization of the miRNA with the 3'UTR of the mRNA in the presence of the common or variant allele. Difference in the free energies of binding between the two alleles was computed as variation of ΔG (i.e. ΔΔG) and a possible indication of whether the occurrence of an SNP could possibly impact on the interaction of that particular miRNA with the mRNA.

Results: We documented 119 SNPs in 53 of these genes. Characterization of the potency of the SNPs in altering miRNA::mRNA interaction through evaluation of the thermodynamics of RNA::RNA binding revealed that 42 SNPs increased the binding potential of miRNAs, while 57 decreased the binding potential, and 14 had neutral effects on the interaction. We identified an abundance of SNPs in neuronal receptors, including genes coding for GABA-receptors which bind the main inhibitory neurotransmitter in the vertebrate CNS.

Conclusion: We have identified SNPs in the miRNA binding sites in a set of genes differentially expressed in prion-induced neurodegeneration and also implicated in other neuropathological conditions. Difference in the ΔG of binding calculated for miRNA::mRNA interaction in which the variant or common allele is considered, suggests that certain sets of SNPs may influence the interaction and also the possible regulatory function of miRNAs with their target mRNA. Our study is beneficial for investigating the involvement of miRNAs in neuropathalogical conditions and could therefore represent a starting point for the exploration and/or development of novel disease-associated germ line markers of susceptibility to human neurodegenerative conditions.

P-28 Effect of neuronal expression of human equilibrative nucleoside transporter (hENT1) and caffeine on cerebral blood flow and cortical stroke in mice.

Hanifi Soylu*, Dali Zhang, Richard Buist, Melanie Martin, Benedict C Albensi, Fiona E Parkinson.
Pharmacology and Therapeutics, University of Manitoba. parkins@cc.umanitoba.ca

Adenosine, through activation of its receptors, has neuromodulatory effects in animal stroke models. Our previous invitro studies showed that neuron specific expression of human equilibrative nucleoside transporter 1 (hENT1) increases ENT1 activity and decreases extracellular adenosine action. Caffeine is a non-selective Adenosine A1, A2 receptor antagonist. Our objectives were to compare the effect of ENT1 overexpression on cerebral blood flow (CBF) and subsequent ischemic damage in hENT1 transgenic (Tg) and wild type (Wt) mice following intra-cortical injection of endothelin-1 (ET-1) and to compare that effect by giving intraperitoneal (IP) caffeine prior to the ischemic stroke event. For this purpose, 8-10 week old (35-40 g) CD1 and Tg mice were stratified into 2 groups: Group A (n = 25), received unilateral single cortical injection of ET-1 (400 pmol, 1ul); and Group B (n=20) received 25 mg/kg IP caffeine prior to the same dose of intracortical ET-1 injection. CBF was measured by perfusion MRI at 4 hours and 48 hours after the injections and stroke size was measured on T2-weighted MRI at 48 hours. Our result showed that at 4 hours following unilateral ET-1 injection, ipsilateral CBF decreased significantly than contralateral hemisphere ($p<0.01$), which was more prominent in Tg mice in all groups. At 48 hours after injection of ET-1, a decrease in ipsilateral CBF was still evident. ET-1 injections produced infarct sizes that were significantly greater in Tg (9 ± 1.1 mm3) than Wt (5.4 ± 0.8 mm3) mice without given Caffeine. However, there was no difference in infarct size between Tg (6.2 ± 1 mm3)and Wt (6.7 ± 1 mm3) mice in Caffeine injected group. In conclusion, this study showed that neuronal expression of hENT1 was associated with an increased cerebral infarct size following ET-1 injection, relative to Wt mice. This genotype difference was not observed in mice that had received the adenosine receptor antagonist caffeine. These data are consistent with our previous study using hippocampal slices, in which we found that hENT1 Tg mice have reduced basal adenosine levels and reduced ischemia evoked increases in adenosine as compared to Wt mice.

P-29 Modulatory effects of Neuregulin-1 on oligodendrocytes differenciation and preservation after spinal cord injury

Marie-Krystel Gauthier*, Kamilla Kosciuczyk, Laura Tapley and Soheila Karimi
Department of Physiology, Regenerative Medicine Program and Spinal Cord Research Center, University of Manitoba, karimis@cc.umanitoba.ca

Spinal cord injury (SCI) is a leading cause of permanent disability worldwide. After SCI, loss of oligodendrocytes and demyelination contribute to neurological deficits. We have recently found a marked and long-lasting down-regulation of Neureguline-1 (Nrg-1) after SCI that correlates with the poor capacity of spinal neural precursor cells (NPCs) in replenishing oligodendrocytes. Nrg-1 with its ErbB tyrosine kinase receptors (ErbB 2, 3, 4) represents a key endogenous growth factor network involved in the establishment and maturation of oligodendrocytes in the developing nervous system. Our objective was to examine whether restoring Nrg-1 level after SCI promotes the differentiation of NPCs into oligodendrocytes. In rats SCI, we delivered vehicle, rhNrg-1β or rhNrg-1β+ErbB inhibitor to the spinal cord intrathecally for two weeks. We used confocal immunohistochemistry and western blotting to analyze the spinal cord tissue. We also used primary cultures of spinal cord-derived NPCs to directly investigate the effects of Nrg-1 on their proliferation and differentiation. Our results demonstate a signifcant increase in the number of newly generated oligodendrocytes and preservation of existing oligodendrocytes after SCI under rhNrg-1β treatment. Moreover, rhNrg-1β increased axonal preservation, attenuated inflamation and reduced tissue degeneration after SCI. The positive effects of rhNrg-1β were diminished after co-administration of ErbB inhibitor suggesting its specificity. In vitro, Nrg-1 treatment also stimulated oligodendrocyte differentiation in spinal cord NPCs. For the first time, we provide evidence for the impact of rhNrg-1 on modulating oligodendroglial cell replacement after SCI and its potential as a novel therapy for SCI repair.

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P-30 Connexin36 at nerve terminals in the vestibular, cochlear and hippocampal systems: mixed chemical/electrical transmission in mammalian CNS

James Nagy, Wendy Bautista*.
Department of Physiology, University of Manitoba. Email: nagyji@ms.umanitoba.ca

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.

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P-31 Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy Identify Abnormalities in the Development of a Rodent Model of Covert Stroke

Sheryl L. Herrera*[1], Krista Hewlett[2], Jonathan D. Thiessen[1], Richard Buist3, James Peeling[3], Dale Corbett[2, 4], Christopher Bidinosti[1, 5], Melanie Martin[1, 3, 5]
[1]Physics & Astronomy, University of Manitoba; [2]Biomedical Sciences, Faculty of Medicine, Memorial University of Newfoundland; [3]Radiology, University of Manitoba; [4]Cellular & Molecular Medicine, Faculty of Medicine, University of Ottawa; [5]Physics, University of Winnipeg *sherylherrera@gmail.com

Vascular cognitive impairment (VCI) is characterized by a progressive decline in executive functions (e.g. complex planning, decision making) as a result of small lacunar infarcts in the thalamus. Evidence suggests a sedentary lifestyle as well as a diet high in fat, salt, cholesterol and sugar (HFSCS) contributes to the VCI epidemic. Developing a rodent model for covert stroke incorporating both the cerebrovascular disease (CVD) dietary risk factors and small infarcts will be useful for determining if declining executive function can be attenuated/delayed by different interventions. One aim of the study was to determine if MRI and MRS, done both in vivo and ex vivo, are able to detect any changes in brain morphology and lesion development, thus confirming that this new rodent model will have the desired anatomical effects. Male Sprague-Dawley rats were subjected to permanent bilateral occlusion of the carotid arteries (sham surgery consists of artery exposure only). Simultaneously, small unilateral lacunar infarcts were induced in the medialis dorsalis nucleus of the thalamus by stereotaxic injections of 0.25 µl of Endothelin-1 (ET-1,400pmol). Rats were either fed a HFSCS diet or a control(REG) diet. 3D T2-weighted MR Images were acquired on the central region of the brain of live rats from control and experimental groups. From the in vivo images, the brain was segmented manually from the rest of the anatomy using MatLab and Amira software. Further manual segmentation was performed on the bright regions found in these brains, which correspond to the presence of cerebral-spinal fluid in the ventricles of the brain and lesions in the white matter. These segmented regions were sorted and distinguished as either ventricle or brain volume, and voxel counting was used to determine the volume sizes of the brain and the ventricles. 3D T2-weighted MR images and MR Spectroscopy measurements were also acquired on the fixed brains and livers of rats from both groups. Fat content in the excised liver was calculated in two regions of interest (4mm)3 by the integration of the areas under fat peak and water peaks. Based on the ex vivo brain images, abnormalities were detected in rats fed a HFSCS diet; located in the medial aspect of the dorsal hippocampus and dorsomedial thalamus, both important regions for learning and memory. These ex vivo measurements showed an increase in fat content for the rats fed the HFSCS diet, with the average percent area integrated under the fat peak relative to the water peak, to be (2.3±2.3)% in normal rats compared to that of (20.0±4.2)% of those fed the HFSCS diet. The in-vivo brain images show a trend [$F(5, 53) = 1.73$, $p<0.15$] of larger ventricle sizes between covert stroke model rats with HFSCS diets when compared to those on REG chow. The HFSCS diet led to a significant executive impairment following a thalamic stroke, while the REG diet did not. Higher resolution anatomical images, slice-by-slice image analysis as well as a measure of blood flow can also be studied for more detailed anatomical information. Future experiments will involve pre-ischemia imaging so that any natural variations in ventricle sizes could be ascertained in advance. These results show that applying MRI and MRS is important in the development of this covert stroke rodent model. Our custom diet results in the development of CVD risk factors in rats and thereby provides a better approximation of human disease.
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P-32 Endogenous D-serine and NMDA receptor activation mediate astrocyte-induced cerebral vasodilation.

Jillian LeMaistre*, Lingling Lu, Hope Anderson, Christopher Anderson.
Department of Pharmacology and Therapeutics, University of Manitoba. jlemaistre@sbrc.ca

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. The objective of the present study was to characterize the role of astrocyte D-serine in 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.

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P-33 The role of FoxO3a in PARP-1-induced Bnip3 signaling pathway during hypoxia in cortical neurons.

Ping Lu*, Amit Kamboj, Christopher M. Anderson.
Division of Neurodegenerative Disorders, St. Boniface Hospital Research Center. plu@sbrc.ca

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.

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P-34 Bnip3 up-regulation and mitochondrial dysfunction in PARP-1 induced neurotoxicity.

Amit Kamboj*,[1] Ping Lu,[1] Spencer B. Gibson,[2] Christopher M. Anderson[1].
[1]Department of Pharmacology and Therapeutics, University of Manitoba and Division of Neurodegenerative Disorders, St. Boniface Hospital Research Center, Winnipeg, Manitoba Canada. [2]Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba Canada. amitkamboj11@gmail.com

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 depolarizing and 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; 50 µM, 30 min), which is a direct normoxic PARP-1 activator. Hypoxic neuron survival (48 hours) 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 and mitochondrial integration was enhanced by normoxic PARP-1 activity. This directly implicates a role for PARP-1 in Bnip3 expression and mitochondrial activity. MitoTracker/calcein and JC-1 were used to measure mitochondrial permeability transition (MPT) and membrane potential (ΔΨm) respectively. MNNG (PARP-1)-induced neuron death was accompanied by MPT and ΔΨm collapse, which was significantly attenuated by deletion of Bnip3. Furthermore, MNNG caused NAD+ dependent mitochondrial Bnip3 integration and nuclear AIF translocation eventually causing neuron death. These results show that PARP-1 activation leads to increased Bnip3 expression and Bnip3-mediated mitochondrial dysfunction and neuron death.
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P-35 Impaired AMP-activated protein kinase signaling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes

Subir K. Roy Chowdhury [(1)] *, Darrell R. Smith [(1,2)], Jason Schapansky [(1,2)], Ali Saleh [(1)], Suzanne Gomes [(1)], Eli Akude [(1,2)], Dwane Morrow [(1)], Nigel A. Calcutt [(3)] and Paul Fernyhough [(1,2)].
Division of Neurodegenerative Disorders, St Boniface Hospital Research Centre (1), Department of Pharmacology & Therapeutics, University of Manitoba (2), Winnipeg, MB, Canada and Department of Pathology (3), University of California, San Diego, CA 92093, USA

Mitochondrial dysfunction occurs in sensory neurons and may contribute to distal axonopathy in animal models of diabetic neuropathy. The AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) signaling axis senses the metabolic demands of cells and regulates mitochondrial function. Studies in muscle, liver and cardiac tissues have shown that the activity of AMPK and PGC-1α is decreased under diabetic conditions. In this study, we tested the hypothesis that deficits in AMPK/PGC-1α signaling in sensory neurons underlie impaired axonal plasticity, sub-optimal mitochondrial function and development of neuropathy in rodent models of type 1 and type 2 diabetes.

Phosphorylation and expression of AMPK/PGC-1α 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 activity using mutant proteins modulated neurotrophin-directed neurite outgrowth in cultures of sensory neurons derived from adult rats. Addition of resveratrol to cultures of sensory neurons derived from rats after 3-5 months of STZ-induced diabetes, significantly elevated AMPK levels, enhanced neurite outgrowth and normalized mitochondrial inner membrane polarization in axons. The bioenergetics profile (maximal oxygen consumption rate, coupling efficiency, respiratory control ratio and spare respiratory capacity) was aberrant in cultured sensory neurons from STZ-diabetic rats and was corrected by resveratrol treatment. 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 in STZ-diabetic rats.

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 the AMPK/PGC-1α pathway.

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P-36 The role of receptor for advanced glycation end products (RAGE) in sensory neurons isolated from normal rats

Ali Saleh*(1), Darrell R. Smith(1,2), Lori Dunn(1), Corina Mertens(1), Abigail R Mateo(1), Randy Van der Ploeg (1), Cory Toth (3), Douglas W. Zochodne (3) and Paul Fernyhough (1,2).
(1)Division of Neurodegenerative Disorders, St. Boniface Hospital Research Centre, Winnipeg. (2)Dept of Pharmacology & Therapeutics, University of Manitoba, Winnipeg. (3)Dept of Clinical Neurosciences and the Hotchkiss Brain Institute, University of Calgary, Calgary.
Email: pfernyhough@sbrc.ca

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 signalling 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-1β 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.

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P-37 Orexins are involved in the expression of fear and anxiety in a rat model of post-traumatic stress disorder (PTSD)

Xiaoyu Chen*[1], Yonghui. LI[1], Sa Li[1], Hugo Bergen[2], Gilbert J. Kirouac[1,3]
[1] Department of Oral Biology, [2] Department of Human anatomy and cell biology, [3] Department of Psychiatry, University of Manitoba
Email: kirouac@cc.umanitoba.ca

Post-traumatic stress disorder (PTSD) is a psychiatric disorder that can develop when individuals experience a stressful and life-threatening event. Similarly, rats exposed to an acute episode of footshocks develop long lasting (> 4 wks) fear (defined as immobility to the shock chamber) and anxiety (immobility to a novel chamber). However, little is known about the neural mechanisms that lead to the long-term behavioral changes following exposure to an acute and intensely stressful experience. Recent evidence indicates that orexins, arousal peptides produced by neurons in the hypothalamus, play an important role in stress, fear and anxiety. In this study, we examined whether the expression of the gene involved in the synthesis of orexins is increased in rats that show fear and anxiety at early (Day 3) and late (Day 31) time points following footshock exposure. In addition, we also wanted to evaluate the effect of the orexin receptor antagonist TCS1102 on the expression of fear and anxiety in these rats. Sprague Dawley rats (n = 16) received footshocks (5 x 2s of 1.5 mA shocks over 3 min) while control rats (n = 13) were exposed to the shock chamber but did not receive shocks. The intensity of the fear and anxiety-like behaviors (freezing durations) was examined at different time points during the post-shock period before the rat brains were collected. Brain sections of the hypothalamus were cut and processed for in situ hybridization with oligonucleuotide probe for prepro-orexin mRNA. The hybridized sections along with 14C microscale standard were exposed to a hyperfilm for 10 days and orexin expression on the film was analyzed using densitometry. We found that prepro-orexin mRNA expression was elevated in shock rats both at Day 3 and Day 31 after shock exposure. More importantly, the synthesis of prepro-orexin mRNA at 31 days post-shock was found to be correlated with the level of fear to the shock chamber at Day 24 and the amount of anxiety expressed in a novel chamber at Day 30. For the pharmacological study, the amount of fear and anxiety-like behaviors was evaluated in shock (n = 39) and nonshock rats (n = 38) pretreated with TCS1102 (5, 10 or 20 mg/kg, i.p.) at Day 15 post-shock. We found that TCS1102 treated shock rats at doses of 10 and 20 kg/mg displayed decreased freezing in the shock chamber (index of fear) and in an open field (index of anxiety). Further evidence for anxiolytic effects for TCS1102 is shown by the observation that all doses decreased the latency for shock rats to enter into the center area of the open field while the latency of nonshock rats was not affected. The results indicate that the orexin system may be functionally upregulated in a rat model of PTSD and that administration of an orexin receptor antagonist attenuated fear and anxiety in this model. This study provides the first preclinical evidence in support of the use of orexin antagonists for the treatment of PTSD.

P-38 Characterization of Juvenile Ferrets Following Induction of Hydrocephalus with Kaolin Orexins

Domenico L. Di Curzio*[1,4,5], Terry L. Enno[2,4,5], Richard J. Buist[3], & Marc R. Del Bigio[1,2,4,5]
Department of Human Anatomy & Cell Science[1], Pathology[2], and Radiology[3] University of Manitoba, John Buhler Research Centre[4], Manitoba Institute of Child Health[5], Winnipeg, Manitoba, Canada Email: delbigi@cc.umanitoba.ca

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. Fourteen-day old ferrets were given an injection of kaolin (aluminum silicate) into the cisterna magna, which causes an inflammatory scar that obstructs CSF flow. Two days later, magnetic resonance (MR) 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. Increased 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 (7.1 to 9.3% vs. 6.3 to 2.7%, $F(1,56) = 4.668$, $p = .035$) and in Ki67 staining for cellular proliferation (5.0 to 8.0% vs. 8.5 to 13.9%, ($F(1,56) = 4.964$, $p = .030$) in the subventricular zone and dentate gyrus. Glial fibrillary acidic protein content was significantly higher in hydrocephalic ferrets than controls, but myelin basic protein content was not significantly altered. In conclusion, the hydrocephalus induced periventricular disturbances are similar to those shown by other species; the ferret should prove useful for testing hypotheses about white matter damage and protection.

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P-39 Characterization of Aminosilane Coated Iron Oxide Nanoparticles for Brain Targeted Delivery

Zhizhi Sun*, Vinith Yathindranath, Stephanie Chu, Fiona E. Parkinson, Torsten Hegmann, Donald W. Miller
Department of Pharmacology and Therapeutics, University of Manitoba. miller05@cc.umanitoba.ca

Aminosilane coated iron oxide nanoparticles (AmS-IONPs) have been widely used in constructing complex and multifunctional drug delivery systems. However, suitability of AmS-IONPs for brain related drug delivery applications is unknown. To determine AmS-IONPs toxicity and cell accumulation in brain related cell cultures and assess permeability across cell culture model of the blood-brain barrier (BBB). AmS-IONPs were examined in a mouse brain microvessel endothelial cell line (bEnd.3) and mouse primary neurons and astrocytes. Cell accumulation of IONPs was examined using Ferrozine assay and cytotoxicity was assessed by MTT assay. Permeability of AmS-IONPs in bEnd.3 monolayer grown on PET membrane inserts (1 µm pore) was evaluated. Acute toxicity was observed in neurons and astrocytes above 70 ug/ml. Rank order of accumulation of AmS-IONPs was astrocytes> bEnd.3 cells> neurons. Presence of a magnetic field increased cell uptake but had minimal effect on AmS-IONP toxicity. Negatively charged AmS-IONPs showed 16% flux across bEnd.3 monolayers after 24 hrs with aid of magnetic field. AmS-IONPs were well tolerated by all cells examined. Permeability of positively charged AmS-IONPs across confluent bEnd.3 monolayers was negligible. Modification of surface chemistry of the AmS-IONPs improved the permeability profile in cell culture model of the BBB. Therefore, AmS-IONP is a promising candidate for delivery of drugs into the brain.

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POSTER ABSTRACTS

P-40 NF-kappa B p50 subunit knockout impairs late LTP and alters long term memory.

Eric Platt*, Ken Oikawa, Gary L. Odero, Avril Hatherell, Melanie Neuendorff, Michael Bernstein, Benedict C. Albensi.
St. Boniface Res. Ctr., Winnipeg, MB, Canada; Pennsylvania State Univ., Abington, PA; Pharmacology & Therapeutics, Univ. of Manitoba. Email: balbensi@sbrc.ca

Background: Nuclear factor kappa B (NF-kB) is a transcription factor often expressed with the subunits, p50 and p65, and also IkB. Investigators have reported that NF-kB is activated during the induction of in vitro long term potentiation (LTP), suggesting that NF-kB may be necessary for some aspects of memory encoding. Furthermore, NF-kB has been implicated as a potential requirement in behavioral tests of memory. Little work has been done to explore the effects of deleting specific NF-kB subunits on memory. In particular, some studies have shown that NF-kB p50 subunit deletion (p50-/-) leads to memory deficits, although recent studies suggest the contrary where p50-/- mice show enhanced memory in the Morris water maze (MWM).

Methods: To more critically explore the role of the NF-kB p50 subunit in synaptic plasticity and memory, we assessed long term spatial memory in vivo in mice using the Morris water maze (MWM), and LTP in slices from the hippocampus of NF-κB p50-/- versus their controls (p50+/+).

Results: We found that the lack of the NF-kB p50 subunit led to significant decreases in late LTP and in selective but significant alterations in MWM tests.

Conclusions: These results support the hypothesis that the NF-kB p50 subunit is required in long term spatial memory in the hippocampus.

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P-41 Label-free Imaging of Brain Tissue with High Resolution FTIR-FPA

M. Rak,[1]* M. Kastyak-Ibrahim,[1] C. Liao,[1] M. Unger,[2] C.J. Hirschmugl,[2] B. Albensi,[3] KM Gough[1]
[1]Department of Chemistry, University of Manitoba, Winnipeg, MB, Canada , [2]Department of Physics, University of Wisconsin–Milwaukee, Milwaukee, WI, USA, [3]Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, and Division of Neurodegenerative Disorders, St. Boniface Hospital Research Centre, Winnipeg, MB, Canada Email: kmgough@cc.umanitoba.ca

The critical questions into the cause of neural degeneration, in Alzheimer's disease (AD) and other neurodegenerative disorders, are closely related to the question of why certain neurons survive. Answers require detailed understanding of biochemical changes in single cells. Fourier transform infrared microscopy is already an excellent tool for biomolecular imaging in situ; we have conducted large scale survey imaging of brain tissue sections in our lab at U. Manitoba. The sample is illuminated with infrared light; the transmitted light, carrying biospectroscopic information, is then imaged onto a focal plane array (FPA) detector to produce a spectrochemical image that may be processed to reveal many different tissue components. The new mid-infrared beamline IRENI (InfraRed ENvironmental Imaging) at the Synchrotron Radiation Center, University of Wisconsin-Madison, homogeneously illuminates a sample with 12 brilliant synchrotron beams, again imaged onto a FPA, to create sub-cellular spectrochemical images with a pixel resolution of 0.54×0.54 micron2, an increase of two orders of magnitude over in-house systems. With IRENI's capabilities, it is possible to study changes in individual neurons in situ, and to characterize their surroundings, using only the biochemical signatures of naturally-occurring components in unstained, unfixed tissue. Comparisons among spectra from dried and freshly acquired tissues show that certain restrictions in tissue preparation and storage are critical for preservation of some chemically unstable biomarkers. Recently acquired images of brain tissue from older 3xTg mice, a model for AD, show remarkable compositional similarities to those from TgCRND8, a very different AD model, that suggest similar anatomical changes that lead to plaque formation, regardless of genetic predisposition.

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