

# My Itinerary

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**SATURDAY, NOV. 11, 2017**

## ☆ **Session 065** CPGs: Neuromodulation, Sensory Input, and Descending Control

1:00 - 5:00 PM

Halls A-C

### ☆ **Presentation 65.15 / GG20** Chemogenetic activation of parapyramidal brainstem neurons to evaluate motor consequences

1:00 - 5:00 PM

\*K. E. ARMSTRONG<sup>1</sup>, M. NAZZAL<sup>2</sup>, X. CHEN<sup>3</sup>, K. STECINA<sup>3</sup>, L. M. JORDAN<sup>4</sup>;

<sup>1</sup>Physiol. and Pathophysiology, Spinal Cord Res. Ctr., <sup>2</sup>Physiol. and Pathophysiology, <sup>3</sup>Physiol., Univ. of Manitoba, Winnipeg, MB, Canada; <sup>4</sup>Dept Physiol., Univ. Manitoba, Winnipeg, MB, Canada

#### Abstract

**Purpose:** Several brainstem regions are necessary to provide transmission of a descending signal to motor networks within the lower limbs. The parapyramidal region (PPR) appears to play an important role in the transmission of a descending signal in the neonatal rat. However, the importance of this region in adult rats has not been studied in detail. The recent advent of DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) has allowed for non-invasive in vivo manipulation of neurons. Using DREADDs, the PPR can be selectively activated its designer drug, clozapine-n-oxide (CNO) to investigate its role in motor activity.

**Methods:** DREADDs were stereotaxically injected in the PPR using adeno-associated viral vectors - AAV-hSyn-DIO-hM3Dq (mutated human muscarinic G-protein coupled receptor-Gq) with an m-Cherry reporter protein. After a recovery period to allow transfection, rats were tested in both voluntary (open field) and fictive locomotion to evaluate motor activation after administration of CNO. In acute experiments, rodents received surgery under isoflurane anesthesia to allow for recording of electroneurograms (ENGs) of the tibial (Tib) and the common peroneal (CP) nerves. The preparation was decerebrated to permit the study of direct effects of PPR neurons without contribution of higher brain centers. To reliably evoke fictive locomotion, metal electrodes were stereotaxically targeted in the MLR for electrical stimulation (10-100  $\mu$ A, 10-40 Hz). **Results:** Immunohistochemical detection and fluorescence microscopy revealed the presence mCherry protein within the PPR, providing evidence for the feasibility of transfection of these rodents with the selected AAV viral vector. Open field showed increased instances of freezing and grooming after CNO. Locomotor parameters did not change significantly after CNO compared to saline injected controls. In acute experiments, changes were observed in MLR-induced fictive locomotion and spontaneous ENG activity. In four animals, there was an increase in ENG activity occurring between 3-20 minutes after CNO injection (1 mg/kg). These effects decreased, and a recovery condition similar to control was observed after 40 minutes. **Conclusions:** DREADDs were successfully transfected within the PPR and confirmed by the presence of the mCherry reporter. Activation of these neurons was inconclusive in open field measures but may be due to confounding effects from higher brain centers. The acute decerebrate experiment demonstrates that DREADD activation of the PPR increases MLR-induced activity and spontaneous activity in all nerves recorded.

## ☆ **Session 091** Modeling

1:00 - 5:00 PM

Halls A-C

### ☆ **Presentation 91.07 / UU44** A Hodgkin-Huxley type model of subfornical organ neurons

1:00 - 5:00 PM

\*L. MEDLOCK<sup>1</sup>, W. M. FRY<sup>2</sup>, D. STANDAGE<sup>1</sup>, A. V. FERGUSON<sup>1</sup>;

<sup>1</sup>Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada; <sup>2</sup>Univ. of Manitoba, Winnipeg, MB, Canada

#### Abstract

The subfornical organ (SFO), a circumventricular organ that lacks the blood brain barrier (BBB), plays an important role in sensing various blood-borne signals from the peripheral circulation. SFO neurons integrate these signals and transmit them across the BBB to regulate critical autonomic functions, including cardiovascular and energy homeostasis. Previous findings from *in vitro* studies have established that SFO neurons exhibit a heterogeneity in their expression of ionic currents and consequent spiking behaviour,

as well as their response to circulating peptides. Insight into the mechanisms behind this heterogeneity is critical for understanding how the SFO integrates and regulates autonomic function, but is currently lacking due to the limitations of patch-clamp techniques. To address this limitation, we developed a Hodgkin-Huxley style (HH) model of an SFO neuron, searching biophysical parameter values to match *in vitro* spike train data. The resulting HH model demonstrated the two major spiking behaviours exhibited by SFO neurons: tonic firing and bursting, where bursting is characterized by robust membrane potential bistability. These spiking behaviours were produced under different parameter values for a non-selective cation current, transient potassium current, persistent sodium current, and current noise. Established methods for neuronal spike train analysis were then used to classify SFO neurons based on their spiking behaviour, e.g. the coefficient of variation and distribution of interspike intervals, as well as their membrane potentials. Analysis of membrane dynamics characterized the neuronal mechanisms supporting these spiking regimes. These methods were further used to predict the behaviour of SFO neurons in response to the binding of angiotensin-II (ANG), a peptide hormone that acts within the SFO to influence various functions including blood pressure and fluid balance. Future use of this model will allow us to study the integration of ANG and other autonomic signals within the SFO.

**SUNDAY, NOV. 12, 2017**

☆ **Session 157 Communicating Vocally in Non-Avian Model Systems** 8:00 - 12:00 PM

Halls A-C

☆ **Presentation 157.09 / MM8 c-Fos expression following alarm call perception by Richardson's ground squirrel** 8:00 - 12:00 PM

A. FREEMAN<sup>1</sup>, J. F. HARE<sup>2</sup>, \*H. K. CALDWELL<sup>3</sup>;

<sup>1</sup>Cornell Univ., Ithaca, NY; <sup>2</sup>Dept. of Biol. Sci., Univ. of Manitoba, Winnipeg, MB, Canada; <sup>3</sup>Dept. of Biol. Sci., Kent State Univ., Kent, OH

**Abstract**

While the neural mechanisms that underlie communication in birds are well studied, much less is known about how the brain works to interpret vocal signals in non-human mammals. In an attempt to address this deficit, we utilized female Richardson's ground squirrels (*Urocitellus richardsonii*) to determine what neural circuits were activated with vocal signals that are known to convey different types of information. Specifically, we played back chirp calls, which are emitted in response to airborne threats, or whistles, which are emitted in response to terrestrial threats. We then quantified immediate early gene activation (i.e. c-Fos) during the perception of these vocalizations and compared them to a no-vocalization control condition. Our analysis revealed that vocalization type affected the distribution and amount of c-Fos labelling. The majority of c-Fos activation was observed in the lateral septum, the nucleus accumbens, the thalamic nuclei, and the paraventricular nucleus of the hypothalamus (PVN). Modest expression was also detected in the ventromedial nucleus of the hypothalamus, the periaqueductal grey, the amygdalar nuclei, and the auditory cortex. Further, chirp and whistle calls had differential effects on c-Fos activation in the PVN, amygdalar nuclei, and periaqueductal grey, with chirps producing more activation than whistles and/or controls. We hypothesize that these differences may reflect the greater response-urgency associated with chirp vocalizations, which are emitted in the context of highly threatening avian predators. The extensive c-Fos labelling observed within the lateral septum of all groups in response to alarm calls may be due to the ability of these squirrels to recognize alarm callers and hence represent social recognition memory, as demonstrated for other rodents, or may reflect stress and anxiety during alarm call perception. Taken together, our findings identify neural correlates of alarm call perception consistent with the purported function of those brain regions in rodents, and with the documented responses of alarm call receivers in nature.

☆ **Session 234 Spinal Cord Injury: Posture and Locomotion** 1:00 - 5:00 PM

Halls A-C

☆ **Presentation 234.04 / GG13 Locomotor activity facilitated by chemogenetic activation of grafted serotonergic neurons in paraplegic rats** 1:00 - 5:00 PM

\*M. NAZZAL<sup>1,2</sup>, U. SLAWINSKA<sup>2</sup>, L. M. JORDAN<sup>1</sup>;

<sup>1</sup>Dept. Physiol. and Pathophysiology, Univ. of Manitoba, Winnipeg, MB, Canada; <sup>2</sup>Dept. Neurophysiol., Nencki Inst. of Exptl. Biol., Warsaw, Poland

**Abstract**

**Purpose:** Cell replacement therapy to recover locomotor capability is a promising avenue of treatment for spinal cord injury. Grafting embryonic serotonergic neurons below the lesion of spinal cord injury in a paraplegic rat has been successful in the recovery of locomotion, yet needed additional exteroceptive stimulation to observe the effect. A method to regulate the activity of

the grafted cells is still lacking. We applied chemogenetic technology through the use Designer Receptors exclusively Activated by Designer Drugs, (DREADDs) that are only expressed in specific cell types to selectively activate the grafted serotonergic (5HT) neurons. **Methods:** A complete transection was done at the level of T9/T10 in Sprague Dawley rats. One week after the transection a graft was placed below the level of the lesion at T12/T13. The grafts were derived from embryonic (E14) neurons from the B1-B3 area of the brainstem of the offspring of floxed DREADD mice (CAG-LSL-Gq-DREADD -acquired from Jackson labs) crossed with ePet-Cre mice, so that the CAG promotor-driven excitatory DREADD (HA-hM3Dq-pta-mCitrine) was expressed in all 5HT neurons. EMG electrodes were implanted into the soleus and tibialis anterior muscles of right and left hind limbs 7-8 weeks after spinalization. Locomotion was observed on a treadmill with EMG and video recordings. The pharmacologically inert DREADD ligand, Clozapine N-oxide (CNO), was given i.p in doses from 0.05 - 1 mg/kg and locomotor behavioral changes were monitored at different intervals from 2 minutes to 4 hours after injection. In a stereotaxic frame the rats were immobilized and decerebrated to allow for monitoring of fictive locomotion without afferent input and to observe changes in reflexes before and after CNO administration. Bipolar electrodes were used to monitor activity from the common peroneal and tibial nerves as well as from the dorsal surface of the spinal cord near the area of the graft. The rats were transcardially perfused and fixed, their spinal cords harvested for immunohistochemistry processing. **Results:** Behavioral experiments on the treadmill showed an improvement in locomotor ability after the administration of CNO. Spontaneous air stepping appeared, spontaneous stepping on the treadmill occurred, and the intensity of the exteroceptive stimulation required to elicit plantar stepping was decreased. In the decerebrate preparation periodic episodes of spontaneous fictive locomotion were observed after CNO administration. **Conclusion:** Locomotor ability can be facilitated in paraplegic rats by chemogenetic activation of grafted DREADD-bearing 5-HT neurons.

**MONDAY, NOV. 13, 2017**

☆ **Session 351 Neuroethology of Listening: Learning, Perception, and Preference in Female Songbirds - Leslie Phillmore**

1:30 - 4:00 PM

146A

☆ **Presentation 351.07 Are there sex differences in the use of spatial cues for reorientation by birds?**

3:15 - 3:35 PM

D. Kelly;  
Psychology, University of Manitoba, Winnipeg, MB, CANADA.

**Abstract**

Songbirds are an excellent system to study everything from behaviour to cellular change, extending research beyond rodent models, and allowing consideration of convergent evolution and comparative research. While early focus was on male song learning and production, the importance of research on female songbirds is now being recognized beyond response to male vocalizing and as a comparator for sex differences. The speakers in this minisymposium will present data on their current research on female behaviour and neurobiology. Researchers in this symposium investigate wide ranging topics: how female Clark's nutcrackers encode and use spatial cues for relocation, how environmental factors alter neural mechanisms underlying reward and motivation in female European Starlings, the neuromodulators that work together to fully transform a non-breeding female into a completely reproductive female, neuromodulation of song preference in females, the neural basis of memory formation in females, and how early experience affects the behavioural and neural responses to father's song in zebra finches. Our goal is to show how the field of songbird research is progressing beyond song learning and its neural correlates to understanding the neuroethology of females.

**TUESDAY, NOV. 14, 2017**

☆ **Session 462 Autism: Environment and Pathology**

8:00 - 12:00 PM

Halls A-C

☆ **Presentation 462.02 / B45 Epigenetics, DNA methylation, and potential biomarkers for fetal alcohol spectrum disorders**

8:00 - 12:00 PM

S. AMIRI, C. OLSON, W. XU, G. HICKS, J. R. DAVIE, \*M. RASTEGAR;  
Dept. of Biochem. and Med. Genet., Univ. of Manitoba, Winnipeg, MB, Canada

**Abstract**

Epigenetics control gene expression and cellular identity through orchestrated molecular events that are not directly reflected by the genomic DNA sequences. Recent discoveries have highlighted the importance of epigenetic modifications in brain development, neuroscience, and mental health. Such mechanisms include histone post-translational modifications (PTMs), DNA methylation, the action of regulatory RNA molecules, and chromatin remodelling, among others. While histone PTMs constitute the most diverse type of epigenetic modifications, DNA methylation is perhaps the best-studied epigenetic modification that links environmental factors to neuroscience and mental health.

Fetal Alcohol Spectrum Disorders (FASD) refer to a broad spectrum of neurodevelopmental disorders of the brain that are caused by prenatal alcohol exposure. FASD is a life-long disorder that is associated with mental disability, facial abnormalities, impaired cognitive and behavioural symptoms. Through a collaborative team effort, we aim for a combination of genome-wide and candidate gene approach to study the role of DNA methylation in deregulated gene expression program of brain-derived neural stem cells along with *in vivo* studies in mice, in order to identify potential biomarkers for FASD. Such biomarkers are critically important for the diagnosis of FASD cases, where the patient does not show any facial characteristics of FASD.

FASD is one the most common neurodevelopmental disorders in the Western World with a frequency of 1-2% and over 6 billion dollars spending per year (only in Canada) for FASD-associated health-related cost and productivity-loss in the affected individuals. Currently, FASD has no cure or effective therapy strategy. Identification of potential FASD biomarkers is critically important for early detection of the disease for intervention strategies during the time period that the brain is still under development.

## ☆ Session 495 Visually-Guided Reaching

8:00 - 12:00 PM

Halls A-C

### ☆ Presentation 495.1 / GG9 Grasping 2D targets: The influence of shape and position on gaze and grasp accuracy

8:00 - 12:00 PM

\*R. W. LANGRIDGE, J. J. MAROTTA;  
Psychology, Univ. of Manitoba, Winnipeg, MB, Canada

#### Abstract

A stable grasp is one in which a grasp line connecting the index finger and thumb would bisect the target object's center of mass. When grasping either 2D or 3D stationary rectangles, our lab has seen this achieved by placing the index finger on the top edge, close to the horizontal center. However, when grasping horizontally translating targets with the right hand, we see a final index finger position that lands *to the left* of the target's horizontal center (Bulloch, Prime, & Marotta, 2015; Langridge & Marotta, 2016), regardless of direction. Individuals may be unconsciously adjusting their reaches and grasping ahead of a leftward moving target's center to account for the increased mechanical risk present when executing a grasp 'across the body' for a target moving away from the hand. This study investigated how target shape and position influence preferred grasp location. **Methods:** Participants executed right handed reach-to-grasp movements for stationary 2D shapes positioned on the left, right, or in the center of a computer screen. Shapes were either square (Fig. 1), or were missing part of the top and bottom edges, intended to force a choice between grasping the left or right side of the target (Fig. 2). **Results:** Final gaze and index placement was positioned further rightward when grasping 'complex' shapes compared to controls. Participants fixated on the right side of targets positioned on the left, the left side of targets presented on the right, and the center of central targets, regardless of shape. Index finger placement corresponded with gaze: A rightward bias was observed when grasping left and central targets, whereas a leftward bias was observed when grasping targets on the right. **Conclusions:** When reaching for 2D targets, individuals prefer to choose grasp locations that require the least amount of mechanical effort. Both the shape of the target and its position in relation to the reaching hand influences where gaze is directed and the preferred index finger placement when grasping.



Figure 1.

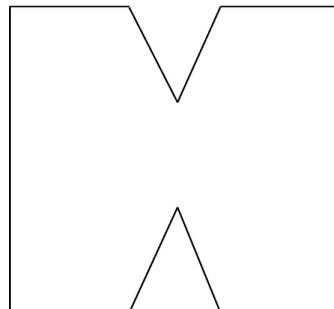


Figure 2.

(<http://files.abstractsonline.com/CTRL/be/a/d53/5b9/c33>)

## ☆ Session 579 Headache and Migraine

1:00 - 5:00 PM

Halls A-C

### ☆ Presentation 579.06 / Z4 Intrinsic brain network abnormalities in chronic migraine are reversed following Onabotulinum toxin-A

1:00 - 5:00 PM

\*A. K. KANUNGO<sup>1</sup>, D. TURCOTTE<sup>2</sup>, J. KORNELSON<sup>2</sup>, T. KOLESAR<sup>2</sup>, B. MANSOURI<sup>1</sup>;

<sup>1</sup>Neurol., <sup>2</sup>Univ. of Manitoba, Winnipeg, MB, Canada

#### Abstract

**Objective:** The objective of this study was to compare functional connectivity patterns across cortical brain regions utilizing resting state functional magnetic resonance imaging (rsfMRI) in patients with chronic migraine (CM) before and after treatment with onabotulinum toxin-A (BoNT-A).

**Methods:** An open-label, prospective interventional pilot study was conducted involving a total of 12 patients with confirmed CM. Patients followed a structured timeline consisting of follow-up with headache logbooks, well-validated migraine significance assessments and psychological inventories along with two cycles of BoNT-A injections on Day 0 and 84. Patients underwent rsfMRI scanning protocol at baseline and on Days 56, and 140 to monitor for differences in brain activity. Psychological and pain changes were analyzed by ANOVA, and rsfMRI data via independent component analysis.

**Results:** After the first treatment of BoNT-A, a change in brain connectivity was observed in the default mode network (DMN), right central executive network (RCEN) and salience network (SN), that most prominently involved the left superior parietal gyrus and the bilateral extrastriate gyri, which correspond to components of the lateral pain system and the visual association areas, respectively. Changes to the pattern of functional connectivity within these brain regions was correlated with clinical improvement of migraine frequency and severity (i.e. transformation of CM into episodic migraine (EM)), as well as associated anxiety. Additionally, we observed at baseline and following BoNT-A administration, no significant functional brain connectivity within the left central executive network in our cohort with CM.

**Conclusions:** CM patients demonstrate an abnormal pattern of functional connectivity in the DMN, RCEN and SN, corresponding to brain regions involved with higher cortical pain and visual processing. This pattern of connectivity is altered by BoNT-A administration. The functional activity differences between CM and EM have been described in subcortical and brainstem areas in the past. However, to the best of our knowledge, our study demonstrated cortical connectivity changes after transforming CM to EM for the first time. This novel finding may have significant application in the clinical approach for management of CM in future. Furthermore, the absence of a left executive network in CM patients, which did not change after BoNT-A treatment, was unexpected and may provide a mechanistic explanation for cognitive abnormalities seen in this disorder. To confirm these findings, further research with larger number of patients are needed, in conjunction with placebo control.

WEDNESDAY, NOV. 15, 2017

## ☆ Session 666 Alzheimer's Disease: Biomarkers, Metabolism, and Proteomics

8:00 - 12:00 PM

Halls A-C

### ☆ Presentation 666.09 / P2 Using Nilotinib to improve mitochondrial function in Alzheimer's disease

8:00 - 12:00 PM

\*B. C. ALBENSI<sup>1</sup>, R. S. TURNER<sup>2</sup>, A. ADLIMOGHADDAM<sup>1</sup>;

<sup>1</sup>Pharmacol. & Therapeut., Univ. of Manitoba, Winnipeg, MB, Canada; <sup>2</sup>Neurol., Georgetown Univ., Washington, DC

#### Abstract

**Objective:** Alzheimer's disease (AD) is a progressive age-related neurodegenerative disorder. AD is also associated with the build-up of amyloid beta (AB) plaques and tau neurofibrillary tangles (NFTs) in brain tissue; however, AD is considered a multifactorial condition. The earliest deficits in the pathological progression of AD actually seem to be caused by impaired mitochondrial function - before the robust appearance of AB and NFTs. In fact, current evidence suggests there are several mechanisms that can affect mitochondrial function that are associated with normal aging and/or linked with AD and other mitochondrial disorders. In this study, we investigated the effect of the FDA-approved anti-cancer drug, Nilotinib, on mitochondrial function and evaluated mitochondrial protein subunit expression. **Methods:** Astroglia cells (microglia and astrocytes) were isolated from the brain cortices of 7 day old C57BL/6 mice (control background strain) and 3xTg mice, a transgenic model of AD. After 2-3 weeks, cells were cultured on Seahorse XF24 analyzer (Seahorse BioSciences) plates at a density of 80,000 cells/well. After 24 hrs., the oxygen consumption rate (OCR) was measured in control vs. 3xTg cells in real time using the XF24 analyzer after dose-dependent treatment with



Nilotinib (10 nm-1 uM). Additionally, Western blots were used to detect expression levels of key proteins involved in mitochondrial function: nuclear factor kappa B (NF-κB) p50/p105/p65/C-rel subunits, and manganese superoxide dismutase (MnSOD), cAMP response element-binding protein (pCREB), and select oxidative phosphorylation (OXPHOS) complex protein subunits in astroglial cells in the presence and absence of Nilotinib treatment. **Results:** Our data show Nilotinib enhances mitochondrial function putatively through the up-regulation of transcription factor NF-κB, and via changes associated with antioxidant MnSOD, pCREB, and OXPHOS signaling. Both basal and maximal respiration levels were significantly increased ( $p < 0.05$ ) after a 24 hr. treatment with 100 nM Nilotinib in astroglial cells from AD mice, but not in control cells. Additionally, we found Nilotinib increased expression of NF-κB p50/p105 subunits, and pCREB, and MnSOD in AD cells and NF-κB p50/p105 subunits and pCREB in control cells. Moreover, Nilotinib increased expression of mitochondrial complex (I-V) protein subunits in 3xTg cells, but not in control cells. **Conclusions:** These results highlight a potential role for Nilotinib in regulating astroglial bioenergetics in early-stage AD and suggest that energy metabolism may be an effective therapeutic target for preventing or treating AD.

## ☆ Session 755 Alzheimer's Disease: APP and Its Processing

1:00 - 5:00 PM

Halls A-C

### ☆ Presentation 755.06 / K6 Testing secreted APP alpha as a therapeutic for diabetic encephalopathy

1:00 - 5:00 PM

\*B. AULSTON, G. ODERO, G. GLAZNER;  
St. Boniface Res. Ctr., Winnipeg, MB, Canada

#### Abstract

Hyperphosphorylation of the microtubule associated protein tau leading to tau aggregation and the formation of toxic neurofibrillary tangles is hypothesized to contribute to cognitive dysfunction and cell death in both Alzheimer's disease (AD) and diabetic encephalopathy. Despite evidence that the APP cleavage product secreted amyloid precursor protein alpha (sAPPα) activates insulin signaling pathways and can prevent tau phosphorylation in the brains of AD mice; the effects of sAPPα on diabetes-induced tau pathology remains unexplored. With this work, we studied the effects of sAPPα on the diabetic brain by rendering Tg, sAPPα-overexpressing mice STZ diabetic. No differences in fasting blood glucose or HbA1c levels were detected between diabetic Wt and Tg mice indicating that sAPPα-overexpression did not affect glucose utilization. However, overexpression of sAPPα blocked diabetes-induced tau phosphorylation in cortical tissue after 16 weeks diabetes which was associated with decreased activation of the unfolded protein response (UPR). Furthermore, we found that sAPPα prevented diabetes-induced phosphorylation of AKT/GSK3. In total, these data show for the first time that sAPPα has a protective effect on diabetic brain tissue and warrants further investigation into the therapeutic potential of sAPPα as a treatment for AD-associated insulin signaling impairment.

## ☆ Session 761 Neuroinflammation: Disease Models

1:00 - 5:00 PM

Halls A-C

### ☆ Presentation 761.1 / V10 Microglial PARP-1 ablation prevents cognitive impairment in offspring exposure to gestational diabetes mellitus

1:00 - 5:00 PM

\*J. KIM<sup>1,2</sup>, L. TESSLER<sup>1,2</sup>, K. BRAUN<sup>1,2</sup>, A. MEHRABADI<sup>1,2</sup>, T. KAUPPINEN<sup>1,2,3</sup>;  
<sup>1</sup>Pharmacol. and Therapeut., Col. of Medicine, Univ. of Manitoba, Winnipeg, MB, Canada; <sup>2</sup>Neurosci. Res. Program, Kleysen Inst. for Advanced Med., Winnipeg, MB, Canada; <sup>3</sup>The Children's Hosp. Res. Inst. of Manitoba, Winnipeg, MB, Canada

#### Abstract

Gestational diabetes mellitus (GDM) is the most common complication of pregnancy. Population health studies have demonstrated a link between GDM and impaired cognitive abilities in the offspring. GDM and resulting blood glucose elevation promote inflammatory responses. Prolonged inflammation can impair the fetal neuronal circuitry development resulting in lifelong effects on cognitive functions. Our previous work has established that PARP-1 has a key role in regulation of microglia, the resident central nervous system immune cells. We hypothesized that microglial PARP-1 ablation reduces neuroinflammation-driven pathological changes in developing fetal neuronal networks and rescues cognitive abilities of the offspring exposed to GDM. We induced GDM by exposing dams to diets high in fatty acids and sucrose (HFS) 6 weeks prior and throughout their pregnancy. A control dams were kept on healthy diet (Lean). Male offspring (both WT and PARP-1KO), produced by breeding PARP-1-lox<sup>+/+</sup>:CD11d-cre- dams kept on HFS or healthy diet with PARP-1-lox<sup>+/+</sup>:CD11d-cre<sup>+</sup> sires, were examined at 15 weeks of age (=young adult). The cognitive functions were evaluated by Open field, Morris Water Maze and Novel Object Recognition tests, and brains were analyzed by immunohistochemistry. Complementing *in vitro* experiments analyzed how microglia prepared from WT vs. PARP-1 KO offspring from GDM vs. Lean dams responded to TNFα stimulation. Offspring from GDM dams had impaired memory and atypical explorative behaviour, both of which were prevented by microglia-

targeted PARP-1 ablation. Microglial PARP-1 ablation also reduced astroglial GFAP expression, microglial morphological activation and pro-inflammatory cytokine expression, and prevented loss of synaptic vesicle proteins seen in WT offspring exposed to GDM. Microglia cultures prepared from GDM offspring pups demonstrated that GDM exposure primes microglia to be hyper-reactive. This microglial hyperactivity was prevented by PARP-1 depletion and inhibition.

In combine our *in vitro* and *in vivo* data strongly supports the hypothesis that GDM induces PARP-1-driven chronic microglial inflammatory responses resulting in synaptic degradation and cognitive impairments in the offspring brain. Microglia culture experiments confirmed that PARP-1 interference can reduce GDM induced microglial hyperactivity. PARP-1 serves as a therapeutic target in prevention of behavior changes, and memory and learning impairments in GDM offspring.

## ☆ **Presentation 761.11 / V11** PARP-1 driven microglial activation promotes disease progression in 3xTg-AD mouse model of Alzheimer's disease

1:00 - 5:00 PM

A. REZAEIAN MEHRABADI<sup>1</sup>, J. KIM<sup>1</sup>, L. TESSLER<sup>1</sup>, \*T. M. KAUPPINEN<sup>1,2</sup>;

<sup>1</sup>Kleysen Inst. for Advanced Med., Univ. of Manitoba, Winnipeg, MB, Canada; <sup>2</sup>Neurosci. Res. Program, Hlth. Sci. Ctr., Winnipeg, MB, Canada

### Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder with profound chronic neuroinflammation. Microglial pro-inflammatory and neurotoxic release activity and on the other hand impaired release of trophic factors, phagocytoses and synaptic pruning have been suggested to have detrimental effects on brain health, though the direct *in vivo* evidence is missing. Our previous cell culture studies have identified poly(ADP-ribose) polymerase-1 (PARP-1) as a key driver of microglial pro-inflammatory functions. Here we tested a hypothesis that microglial PARP-1 mediated pro-inflammatory responses can directly disrupt blood-brain barrier (BBB) integrity (*in vitro*) and also promote other AD pathological events accelerating disease progression (*in vivo*). *In vitro* evidence for microglial direct effects on BBB was assessed in co-cultures with endothelial cells and astrocytes. The chronic pro-inflammatory microglial activation in 3xTg-AD and WT mice was induced by injecting hippocampus with microglia-directed (Iba-1 promoter) lentiviral construct of mutant PARP-1 (LV-Iba1-mPARP-1-RFP), which transduces constitutively active PARP-1 expression in microglia. The lentivirus transduction was confirmed by IVIS imaging of RFP expression. The effect of mPARP-1 vs. control lentivirus transducing just red fluorescence protein expression (LV-Iba1-RFP) was assessed 2-3 months later at 4 and 5 months of age by open field and novel object recognition (NOR) tests. The pathological progression of AD was further assessed by quantitating expression of hippocampal synaptic proteins, amyloid beta (A $\beta$ ) load and neuroinflammation status. A $\beta$ -stimulated microglial release of NO and TNF $\alpha$  triggered activation of co-cultures astrocytes and reduced tight junction protein expression in co-cultured endothelial cells in a PARP-1 dependent manner. NO and TNF $\alpha$  also increased endothelial paracellular permeability. Microglial pro-inflammatory activation in the hippocampus of 3xTg-AD mice accelerated AD progression. AD-mice injected with LV-Iba1-mPARP-1-RFP had memory deficits identified by NOR test already at 4 months of age, whereas the control LV injected AD mice showed signs of memory deficits only after 5 months of age. Immunostaining of the post-mortem 3xTg-AD mice brains revealed that LV-Iba1-mPARP-1-RFP elevated neuroinflammation status, promoted synaptic protein degradation and slightly increased A $\beta$  accumulation. Our *in vivo* data demonstrates that PARP-1 driven microglial pro-inflammatory responses can directly promote AD pathology and disease progression.

## ☆ **Session 778** Motor Systems: Molecular, Synaptic, and Cellular Mechanisms

1:00 - 5:00 PM

Halls A-C

## ☆ **Presentation 778.04 / GG16** Selective expression of DREADDs in thoraco-lumbar cholinergic interneurons

1:00 - 5:00 PM

\*K. STECINA, X. CHEN, P. B. BHULLAR, K. ARMSTRONG, M. NAZZAL;  
Physiol., Univ. of Manitoba, Winnipeg, MB, Canada

### Abstract

**Purpose:** After spinal cord injury, when the regulation of spinal cholinergic neurons is impaired, their aberrant activity contributes to autonomic dysreflexia. If selective control over the activity level of the cholinergic spinal interneurons could be achieved, it may reduce the severity or the frequency of autonomic dysreflexia. Designer receptors exclusively activated by designer drugs or DREADDs provide novel means for modifying activity in selective neural pools. A commercially available genetically modified mouse line carrying Cre-recombinase in all cholinergic neurons was tested for incorporation of DREADDs in thoracic and lumbar cholinergic neurons. **Methods:** The spinal injection of DREADDs that consists of mutated, human muscarinic G-protein coupled receptors conjugated with adeno-associated viral vectors with a double-floxed inverse open-reading frame - pAAV-hSyn-DIO-hM3D(Gq) and pAAV-hSyn-DIO-hM4D(Gi) were used. Following a minor laminectomy, injections into low thoracic or lumbar segments were done by a device from Neurostar GmbH, DE developed originally for brain stereotaxic work. After recovery (7 to 110 days) mice were transcardially perfused and cords were harvested for immunohistochemical and fluorescent microscopic analysis.

**Results:** Injections of 80 to 250 nL volumes at single sites were found to provide transfection of neurons in a region of at least 400-800  $\mu\text{m}$  in the thoracic and the lumbar spinal cords. Injections at multiple sites tracks have provided transfected cells up to 7 mm. Selective transfection of cholinergic interneurons vs. motoneurons was possible by controlling the depth at which the injections were performed. Expression of constructs in non-cholinergic cells was low, but analysis is still ongoing. **Conclusions:** The use of these constructs is a viable method for cholinergic cell transfection in the spinal cord. By this approach, the contribution spinal cholinergic neurons to various physiological functions in the healthy or the injured spinal cord, will be feasible to evaluate and quantify.