Molecules, Cells & Systems Research Group

1st Annual Research Symposium

Tuesday, April 25, 2023

Peterborough, Ontario

Program

The MCS Research Group would like to thank the *Office of Research and Innovation* and the *School of Graduate Studies* for their generous financial support

The Molecules to Systems Approach

The life sciences are a broad grouping of disciplines that study how organisms function. All organisms, whether unicellular (e.g., bacteria) or multicellular (e.g., humans), require fundamental building blocks. These building blocks facilitate the formation of a cell, which can then associate with other cells to form a multicellular organism. Through evolution, multicellular organisms have become more complex, facilitating the formation of specialized multicellular structures (e.g., tissues and organs). The Molecules to Systems Approach seeks to understand the mechanisms underlying each stage of this biological hierarchy.

The Vision

Trent University has a wealth of expertise in various disciplines within the life sciences including cell biology, molecular biology, biochemistry, microbiology, developmental biology, physiology, psychology, and neuroscience. To exploit this competitive advantage, the Molecules, Cells & Systems (MCS) Research Group brings together researchers from the Departments of Biology, Chemistry, Forensic Science, Kinesiology, and Psychology who work together to apply the Molecules to Systems Approach in research.

Organizing Committee

Dr. Robert Huber
Dr. Carolyn Kapron
Dr. Stephanie Tobin
Tracy Ross

The Organizing Committee would like to thank Adam Remtulla for leading the creation of the MCS Research Group logo, Gillian Ferguson-Martin and Morgan Wilson-Smillie for assisting with catering, and Samantha Logan, William Kim, and Daniel Palberg for evaluating the student talks.

Schedule

*Note: All talks will take place in the Trent University Student Centre (TSC 1.20)

Time	**Activity**
8:45 AM - 9:00 AM	Registration
9:00 AM - 9:05 AM	Introductory remarks
9:05 AM - 9:45 AM	Session 1: Dr. Ina Anreiter, University of Toronto Scarborough
9:45 AM - 10:45 AM	Session 2: Student presentations
10:45 AM - 11:00 AM	Break
11:00 AM - 12:15 PM	Session 3: Student presentations
12:15 PM - 1:00 PM	Lunch
1:00 PM - 1:45 PM	Session 4: Dr. Craig Brunetti, Trent University
1:45 PM – 2:45 PM	Session 5: Student presentations
2:45 PM – 3:00 PM	Break
3:00 PM - 4:00 PM	Session 6: Student presentations
4:00 PM – 4:30 PM	Awards and wrap up

Session 1 (Moderator: Dr. Stephanie Tobin)

9:05 AM – 9:45 PM:
Deciphering pleiotropy: How complex genes are regulated to affect behaviour
Dr. Ina Anreiter, University of Toronto Scarborough

Session 2 (Moderator: Dr. Stephanie Tobin)

9:45 AM – 9:57 AM:
Phytohormones as signaling molecules in fungal (Sordaria macrospora) development
Kimberly Molina-Bean (Graduate student, Environmental & Life Sciences)

9:57 AM – 10:09 AM:
Comparison of the stabilities of bacterial and protozoan flavohemoglobins
Echo Terrell (Undergraduate student, Biology)

10:09 AM – 10:21 AM:
Characterization of gelsolin wildtype and mutant peptides and potential use of cytokinins as aggregation inhibitors
Dev Seneviratne (Graduate student, Environmental & Life Sciences)

10:21 AM – 10:33 AM:
The role of a heme protein in the nitrosative stress response in Giardia intestinalis – a protozoan parasite of humans
Anasofia Vargas (Undergraduate student, Biology)

10:33 AM – 10:45 AM:
Identifying therapeutic avenues for juvenile Batten disease using D. discoideum as a model cell system
Adam Remtulla (Graduate student, Environmental & Life Sciences)

Session 3 (Moderator: Dr. Leslie Kerr)

11:00 AM – 11:12 AM:
Dose-dependent effects of cadmium on angiogenesis signalling pathways
Caitlyn Knight (Graduate student, Environmental & Life Sciences)

11:12 AM – 11:24 AM:
Investigating the functions of lipopolysaccharide induced tumour necrosis factor alpha-factor
Kyra Ball (Graduate student, Environmental & Life Sciences)

11:24 AM – 11:36 PM:
Endoplasmic reticulum stress in Dictyostelium models of Batten disease
Aruban Thanabalasingam (Graduate student, Environmental & Life Sciences)

11:36 PM – 11:48 PM:
Cytokinins reduce in vitro viral production and increase viral spread in frog virus 3
Mark Seegobin (Graduate student, Environmental & Life Sciences)

11:48 PM – 12:00 PM:
Using metabolomics to understand the role of cytokinin hormones in Giardia intestinalis
Vedanti Ghatwala (Graduate student, Environmental & Life Sciences)

12:00 PM – 12:12 PM:
Assessing cytokinin inhibition of large double stranded DNA virus replication
Galair Prevost (Graduate student, Environmental & Life Sciences)

Session 4 (Moderator: Dr. Robert Huber)

1:00 PM – 1:45 PM:
How the butterfly got its spots
Dr. Craig Brunetti, Trent University

Session 5 (Moderator: Dr. Robert Huber)

1:45 PM – 1:57 PM:
Effects of mfsd8 knockout on the Dictyostelium discoideum transcriptome
Joshua Gray (Graduate student, Environmental & Life Sciences)

1:57 PM – 2:09 PM:
Optimal cytokinin detection in epithelioma papulosum cyprini, a model for evaluating ranavirus infection
Angela Schincaglia (Undergraduate student, Biology)

2:09 PM – 2:21 PM:
The effects of trait alexithymia on the mnemonic similarity task: How is memory discrimination affected by a lack of emotion?: A research study
Haida Mustansir (Graduate student, Psychology)

2:21 PM – 2:33 PM:
Effects of oncology camp on the psychosocial health of childhood cancer patients
Sarah O'Connell (Graduate student, Environmental & Life Sciences)

2:33 PM – 2:45 PM:
Effect of running exercise on retention of spatial memory in rats
Eileen Bolton (Undergraduate student, Psychology)

Session 6 (Moderator: Dr. Neil Fournier)

3:00 PM – 3:12 PM:
Exploring the impact of acute stress on previously acquired fear memory in rats
Javishaa Thiyagarajah (Graduate student, Psychology)

3:12 PM – 3:24 PM:
Influence of biological sex on gene expression and muscle atrophy in a heart failure model of cardiac cachexia
Alexander Rico (Undergraduate student, Biology)

3:24 PM – 3:36 PM:
Effect of chronic stress on kindling-induced emotionality in male and female rats
Faith Tucker (Undergraduate student, Psychology)

3:36 PM – 3:48 PM:
Understanding metabolic health status in a cohort of Nursing students as compared to Biology students
Shanna Lowes (Graduate student, Environmental & Life Sciences)

3:48 PM – 4:00 PM:
Postictal changes in somatosensory and affective components of pain following electrical amygdala kindling in rats
Evana Xiao (Graduate student, Psychology)

Phytohormones as signaling molecules in fungal (*Sordaria macrospora*) development

Kimberly Molina-Bean[1] and Neil Emery[1,2]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada
[2]Department of Biology, Trent University, Peterborough, Ontario, Canada

Fungi, a kingdom of eukaryotic organisms, are widely used for enhancement in medicine, agriculture, sustainability, and industry, either through the production of secondary metabolites or acting in a symbiotic relationship with other organisms. Understanding fungal biology and the signals involved in their growth and development is essential to further utilize fungi in outcomes that benefit society. Hormones are signaling molecules known to bind to specific cell surface receptors followed by an amplified cellular reaction. For instance, classic phytohormones are divided into six main groups: cytokinins (CK), auxins, jasmonic acid (JA), ethylene (Et), gibberellic acid (GA), Abscisic acid (ABA), and Salicylic acid (SA). These phytohormones are known to upregulate plant growth and development depending on biotic and abiotic factors and have been found across many kingdoms. Fungi produce phytohormones and it has been traditionally assumed this is to interact with their host plants, but few researchers have tested the effects of these compounds on the development of the fungus itself. Using liquid chromatography and mass spectrometry phytohormones were analyzed in the model fungus *Sordaria macrospora* wild-type and mutants at key developmental stages in the fungal life cycle. The analysis revealed that active CKs (cZ and iP) and the auxin IAA increase, while less-active CKs (cZR and iPR) decrease across the different stages to form fruiting bodies in *S. macrospora*. Therefore, the analysis supports the hypothesis that CKs and auxin act as signaling molecules during *S. macrospora* development.

Comparison of the stabilities of bacterial and protozoan flavohemoglobins

Echo Terrell[1] and Steven Rafferty[2]

[1]Department of Biology, Trent University, Peterborough, Ontario, Canada
[2]Department of Chemistry, Trent University, Peterborough, Ontario, Canada

Flavohemoglobins (FlHbs) are nitric oxide dioxygenases, used to catalyse the reaction of nitric oxide (NO·) with O_2 and an electron to form nitrate. They consist of an N-terminal heme-containing globin domain and a C-terminal flavin domain. The protist parasite, Giardia intestinalis, encodes a FlHb (gFlHb), despite not containing heme production machinery. gFlHb shares approximately 40% sequence identity with other enterobacteria FlHbs such as HMP, E.coli's hemoglobin-like protein. Distinctly, the gFlHb sequence contains two unique inserts, one in each domain, both approximately 25 amino acids in length. These inserts seem to lay at the interface of the globin and flavin domains, likely contacting each other. It is hypothesised that these inserts increase the intermolecular forces between the subunits, providing additional stability to gFlHb. To compare the conformational stabilities of gFlHb and HMP, chemical denaturation with increasing concentrations of urea (0M-9M) and thermal ramping were utilised. The extent of denaturation was examined by the changes in the Soret band absorbance at 410nm. The resulting ratio of natured protein to denatured protein was used to calculate the free energy of folding (ΔG_{H2O}) for both proteins. Preliminary denaturation curves have been constructed; however, the analysis of these results will be presented at the conference.

Characterization of gelsolin wildtype and mutant peptides and potential use of cytokinins as aggregation inhibitors

Dev Seneviratne[1], Neil Emery[1,2], and Sanela Martic[1,3]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada
[2]Department of Biology, Trent University, Peterborough, Ontario, Canada
[3]Department of Forensic Science, Trent University, Peterborough, Ontario, Canada

Gelsolin is a plasma protein mainly responsible for the regulation of actin filaments in the bloodstream (1). A recently discovered point mutation at N184K/Y influences the formation of amyloid fibers which are eventually deposited in the kidney (1-2). This condition is commonly known as renal amyloidosis; no cure has been found. The understanding of aggregation propensity and the mechanism of Gelsolin protein aggregation is limited and not clearly elucidated. Herein, we investigate *in vitro* aggregation propensity of gelsolin wild type 184-NNGDCFILDL-193 peptide and associated mutants. To characterize aggregation we employed analytical methods such as fluorescence Proteostats, Thioflavin T, and turbidity assays, and Transmission electron microscopy. The mutual amino acid sequence CFILDL led to the formation of fibrillar aggregates with mutants having a higher tendency to aggregate relative to the wild type (3). In addition, we tested the cytokinin plant hormones, Kinetin, and *trans*-Zeatin, as possible aggregation inhibitors and as potential drug candidates against gelsolin amyloidosis. Applications of both cytokinins inhibited aggregation but *trans*- zeatin was more effective than Kinetin. The knowledge gained in these studies related to aggregation and inhibition can be utilized to find therapeutic treatments for renal amyloidosis and other amyloidogenic diseases.

References

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(2) Srivastava, A.; Singh, J.; Singh Yadav, S. P.; Arya, P.; Kalim, F.; Rose, P.; Ashish; Kundu, B. The Gelsolin Pathogenic D187N Mutant Exhibits Altered Conformational Stability and Forms Amyloidogenic Oligomers. *Biochemistry* 2018, *57* (16), 2359–2372. https://doi.org/10.1021/acs.biochem.8b00039.

(3) Ahmad, M.; Esposto, J.; Golec, C.; Wu, C.; Martic-Milne, S. Aggregation of Gelsolin Wild- Type and G167K/R, N184K, and D187N/Y Mutant Peptides and Inhibition. *Molecular and Cellular Biochemistry* 2021, *476* (6), 2393–2408. https://doi.org/10.1007/s11010-021-04085-6.

The role of a heme protein in the nitrosative stress response in *Giardia intestinalis* – a protozoan parasite of humans

Anasofia Vargas[1], Kaitlyn Mowatt[2], Bayan Sajer[2], and Janet Yee[1,2]

[1]Department of Biology, Trent University, Peterborough, Ontario
[2]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario

Giardia intestinalis is a unicellular parasite associated with the infectious disease known as giardiasis or Beaver Fever. Giardia lacks heme-biosynthetic enzymes and does not possess mitochondria, where heme biosynthesis occurs. Despite this, the Giardia genome encodes five different heme-binding proteins, including flavohemoglobin (gFLHb), and four isotypes or paralogues of cytochrome *b*5 (gCYTB5-I, II, III, and IV). The four gCYTB5 isotypes are each localized to a different subcellular region in Giardia but their functions are unknown. Previous work in our lab identified gCYTB5-I in the nucleolus and a potential role for this protein in mediating nitrosative stress. Our lab observed the movement of gCYTB5-I out of the nucleolus in Giardia subjected to nitrosative stress but another nucleolar protein (CBF5 pseudouridine synthetase) did not. Nitrosative stress for the protist occurs after ingestion of the metabolically inactive cyst form that then undergoes excystation to become the motile trophozoite. The trophozoite attaches itself to the duodenal epithelial lining, which leads to the release of nitric oxide (NO) from the epithelium as the host's immune response. NO is a free radical that can lead to possibly cytotoxic reactive nitrogen species. The goal of my research is to determine if the protein level of gCYTB5-I increases when Giardia trophozoite cultures are subjected to nitrosative stress induced by S-nitrosoglutathione (GSNO). I synthesized GSNO and then tested different concentrations to examine their effect on the growth of Giardia cultures over 24 hours. Next, Giardia cultures were incubated with GSNO, and cell samples were removed at selected time points for protein extraction and subsequent western blot analysis. The evaluation of these results will be presented.

Acknowledgments: This research is supported by an NSERC Discovery Grant awarded to JY

Identifying therapeutic avenues for juvenile Batten disease using *D. discoideum* as a model cell system

Adam A.N. Remtulla[1] and Robert J. Huber[1,2]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada
[2]Department of Biology, Trent University, Peterborough, Ontario, Canada

The neuronal ceroid lipofuscinoses (NCLs), collectively referred to as Batten disease, are a group of fatal neurodegenerative disorders that primarily affect children. The etiology of Batten disease is linked to mutations in 13 genes that encode distinct CLN proteins, whose functions have yet to be elucidated. To understand the mechanisms underlying Batten disease, our research group uses the social amoeba *Dictyostelium discoideum* as an informative and powerful model organism. *D. discoideum* is a convenient, genetically tractable, and durable eukaryotic microbe that undergoes a dynamic developmental life cycle when prompted by nutritional stress. Remarkably, a growing body of literature has highlighted a shared profile of functions between CLN proteins in humans and their respective homologs in *D. discoideum.* For instance, *D. discoideum* cells that lack the CLN3 homolog, Cln3, display elevated levels of storage bodies, decreased intracellular enzyme activity, and reduced ATP production - fittingly, these cellular defects are among the hallmark pathologies that characterize Batten disease in humans. Accordingly, studies with *D. discoideum* may offer unique insights into the fundamental cellular processes that are at play in the NCLs. Using a battery of biochemical and developmental phenotype assays, our group aims to identify compounds that rescue defects in autophagy, a self-degradative cellular pathway that is typically disturbed in lysosomal storage disorders such as Batten disease. More specifically, we screened an autophagy-focused drug library in high-throughput fluorometric and luminescent assays that allowed us to compare the viability and levels of storage bodies in wild-type and *cln3*-deficient *D. discoideum* cells under autophagy-stimulating conditions. We then used this knowledge to further characterize the effect of prospective hit compounds on aberrant enzyme activity and developmental phenotypes in *cln3*$^{-}$ cells. By identifying autophagy-targeting compounds that ameliorate the defects displayed by *D. discoideum* models of Batten disease, our findings may contribute to translational work that could one day benefit patients in the clinic.

Dose-dependent effects of cadmium on angiogenesis signalling pathways

Caitlyn Knight, Ju Liu, and Carolyn Kapron

Cadmium is a toxic metal that has detrimental effects on blood vessel development and function. At different concentrations, it has both enhancing and inhibiting effects on angiogenesis, the development of capillaries from pre-existing blood vessels. As cadmium bioaccumulates, endothelial cells produce pro-angiogenic proteins such as AKT and its downstream effector, VEGF. Conversely, cadmium can also cause cell dysfunction and death by dysregulating tumour suppressor proteins, such as PTEN. To examine the effect of cadmium on angiogenic signalling pathways, the expression of PTEN, AKT and VEGF was investigated using the mouse aortic ring assay. Aortic rings, 1 mm in width, were harvested from mice between the ages of 8 to 12 weeks. Subsequently, the rings were incubated with 0, 0.5, 1, or 10 µM cadmium chloride for 10 days, and protein expression was examined through western blotting. Initial results demonstrate that cadmium-induced cytotoxicity appears to occur at high cadmium concentrations and is accompanied by increased PTEN expression. In addition, low cadmium levels stimulated AKT and VEGF activation, which could permit blood vessel expansion. Thus, the divergent effects of cadmium on blood vessels are accompanied by divergent effects on related protein expression. The industrial use of cadmium continues to threaten global health, and a thorough understanding of these effects is imperative to developing successful therapeutic interventions for cadmium toxicity.

Investigating the functions of Lipopolysaccharide Induced Tumour Necrosis Factor Alpha-Factor

Kyra Ball[1], William D. Kim[1], Robert J. Huber[1,2], and Craig Brunetti[1,2]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada
[2]Department of Biology, Trent University, Peterborough, Ontario, Canada

Lipopolysaccharide induced tumour necrosis factor alpha-factor (LITAF), is a gene that plays important roles in the transportation and delivery of proteins throughout the cell. In its wildtype form LITAF is localized to the endosomes/ lysosomes where it can easily carry out endosomal trafficking activities. Although it is known that LITAF plays roles in endosomal trafficking, many of the gene's underlying molecular mechanisms are largely unknown. This project aims to gain insight into how LITAF functions by comparing wildtype and overexpression cell lines in the model organism *Dictyostellium discoideum*. *D. discoideum* is an amoeba that undergoes both unicellular and multicellular development. This unique organism encodes a homolog mammalian LITAF known as Litaf. Gene overexpression lines can give insight into how a gene of interest directly impacts cellular function, localization, and phenotypic changes. By creating and comparing wildtype Litaf to a Litaf overexpression cell line, we can develop an understanding of the expression of Litaf throughout the 24-hour life cycle of *D. discoideum*. We can then compare differences of Litaf expression to disparities in cellular development. By investigating the molecular mechanisms of LITAF/Litaf we can further our understanding of the impacts mutated forms of this gene play in disease and the role LITAF plays in viral-host interactions.

Endoplasmic reticulum stress in *Dictyostelium* models of Batten disease

Aruban Thanabalasingam[1] and Robert J. Huber[1,2]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada
[2]Department of Biology, Trent University, Peterborough, Ontario, Canada

The neuronal ceroid lipofuscinoses (NCLs), commonly known as Batten disease, are a family of fatal neurodegenerative disorders that primarily affect children. Several subtypes of NCL have been reported and each is caused by a mutation in a distinct ceroid lipofuscinosis neuronal (*CLN*) gene (e.g., mutations in *CLN3* cause CLN3 disease, mutations in *MFSD8* cause CLN7 disease), which results in aberrant lysosome function and the accumulation of lipo-protein aggregates within cells. There are several cellular pathways that can alleviate the stress caused by the buildup of this material such as those mediated by the endoplasmic reticulum (ER). The ER maintains cellular homeostasis through protein production, quality control, and regulating several signalling pathways. The unfolded protein response (UPR) consists of several conserved pathways devoted to attenuating ER stress induced by an accumulation of misfolded proteins. At the center of this stress response is GRP78, a molecular chaperone that binds to misfolded proteins to facilitate proper folding. Previous work showed increased ER stress and altered amounts of GRP78 protein in several neurodegenerative diseases, due to an accumulation of misfolded proteins. Here, we used the social amoeba *Dictyostelium discoideum* as a model system to examine ER stress in gene knockout models of CLN3, CLN5, and CLN7 disease. *D. discoideum* is an excellent model system that encodes more CLN-like proteins than other classical model organisms (e.g., yeast, worm, fruit fly) and is a powerful model system for studying ER stress. Our work revealed that the morphology of the ER is altered in *D. discoideum cln3*$^{-}$, *cln5*$^{-}$, and *mfsd8*$^{-}$ cells and that gene loss increases the intracellular and extracellular amounts of GRP78. Taken together, this study provides new insight into the role of ER stress in Batten disease.

Cytokinins reduce in vitro viral production and increase viral spread in frog virus 3

Mark Seegobin[1], Samantha Logan[1], RJ Neil Emery[1,2], and Craig Brunetti[1,2]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada
[2]Department of Biology, Trent University, Peterborough, Ontario, Canada

Cytokinins (CKs) are a group of N6-substituted adenylate derivatives most widely known as plant signaling molecules. Although the presence, uptake, and interconversion of CKs has also been documented in vertebrate tissues and cells, their role remains elusive. In contrast to the few studies including endogenous detection, there have been many reports outlining the therapeutic properties of CKs in exogenous applications. Here, we evaluate the virostatic potential of CKs in vitro against frog virus 3 (FV3), the ranavirus type species. Four CKs were screened; including, N6-(Δ2-isopentenyl) adenine (iP), N6-(Δ2-isopentenyl) adenine-9-riboside (iPR), N6-(Δ2-isopentenyl) adenine-9-riboside-5'-monophosphate (iPMP), and 2-methylthio-N6-isopentenyladenosine (2MeSiPR). Antiviral activity was evaluated by plaque formation assays, plaque morphology, and single step growth curves. Treatment with iP and iPR significantly reduced viral replication indicated by a reduction in plaque formation (33.8% and 59.6%) and a decrease in viral load measured 72 hours post infection (48% and 60%). Interestingly, while plaque counts and virus titres decreased, changes in plaque morphology suggest an increase in cell-to-cell spread. Average plaque area increased subsequent to iP (83%) or iPR (112%) treatment and lytic zone formation was more prevalent in groups treated with iP, iPR, and iPMP relative to control groups. These results provide further evidence of CK antiviral activity and suggest that iP and iPR significantly reduce frog virus 3 replication but may aid in the cell-to-cell transmission, in vitro.

Using metabolomics to understand the role of cytokinin hormones in *Giardia intestinalis*

Vedanti Ghatwala[1], Anna Kisiala[2], Neil Emery[2], and Janet Yee[2]

[1]Environmental and Life Science Graduate Program, Trent University, Peterborough, Ontario
[2]Department of Biology, Trent University, Peterborough, Ontario

Giardia intestinalis is a protozoan parasite responsible for the diarrheal disease in mammals called 'beaver fever', but the mechanisms of disease pathogenesis are unclear. While proteins secreted by *Giardia* can affect the physiology and response of host cells, the impact of any hormone-like molecules secreted by *Giardia* has not been studied. Cytokinins (CKs) are adenine-derived compounds that were first identified as signalling molecules involved in the regulation of plant growth and development. More recently, CKs have been detected across multiple kingdoms of life, including protists; however, little is known about their roles in organisms beyond plants. Preliminary experiments show that Giardia trophozoites are capable of converting CK intermediates (ribosides) into their presumed-active CK (free base) forms during growth. Moreover, Giardia trophozoites thrive in a CK-rich environment but can not grow in CK-deprived media. Thus, to test the hypothesis that CKs stimulate trophozoite growth, selected exogenous CKs were added to a minimal CK medium that does not support Giardia growth. Moreover, to look for impacts of exogenous CKs beyond macro-changes (*i.e.* growth rates, visible phenotypes), a metabolomic assessment was performed on culture supernatants using UHPLC-HRMS/MS. This study shows that the CK interconversion by Giardia is possible irrespective of growth, however, CKs do not seem to act as trophozoite growth promoters. Furthermore, along with confirmation of some known Giardia metabolic pathways, the results of this study add new insights to the nucleoside salvage mechanisms used by Giardia.

Acknowledgments: This research is supported by NSERC Discovery Grants awarded to NE and JY

Assessing Cytokinin Inhibition of Large Double Stranded DNA Virus Replication

Galair Prevost, Shishir Suresh, Mark Seegobin, Samantha Logan, and Craig Brunetti

Cytokinins, N6 -adenine derivatives, are a group of small signalling molecules that involved in cell differentiation and proliferation in plants. Previous studies have shown that cytokinins, N6 -(Δ2-isopentenyl) adenine (iP) and N6 -(Δ2-isopentenyl) adenine-9-riboside (iPR), inhibited frog virus 3 (FV3) infection. To determine if iP and iPR can inhibit other viruses, we will infect mammalian cell lines such as HeLa or BGMK cells with vaccinia virus in the presence or absence of iP and IPR. After 24 hours, the virus will be collected and tittered to determine if iP and iP inhibit vaccinia virus infection. We predict that iP and iPR would reduce Vaccinia virus replication, in a similar manner to inhibition seen in FV3. The cells with also be pretreated with iP and iPR at various time points to evaluate their virostatic potential. Both FV3 and Vaccinia virus are large double stranded DNA viruses. We hope to determine whether the inhibition of FV3 by iP or iPR is a specific phenomenon to FV3 or is it a more general phenomenon shared by other viral families.

Effects of *mfsd8* knockout on the *Dictyostelium discoideum* transcriptome

Joshua Gray[1], William D. Kim[1], and Robert J. Huber[1,2]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada
[2]Department of Biology, Trent University, Peterborough, Ontario, Canada

Major facilitator superfamily domain containing protein 8 (MFSD8) is a ubiquitous integral membrane protein that localizes to the lysosome where it functions as a chloride channel. Humans with homozygous mutations in *MFSD8* develop a severe form of neurodegeneration called ceroid lipofuscinosis neuronal 7 (CLN7) disease, which is a late-infantile onset form of Batten disease. Individuals with Batten disease suffer from vision loss, seizures, reduced motor and cognitive capabilities, and a shortened lifespan. The social amoeba *Dictyostelium discoideum* encodes a homolog of human MFSD8, Mfsd8, that plays an essential role in several conserved cellular processes such as proliferation, pinocytosis, cytokinesis, adhesion, delayed aggregation, and protein secretion. In this study, we used comparative transcriptomics to explore the molecular mechanisms underlying *mfsd8*-deficiency phenotypes during growth and the early stages of multicellular development (when *mfsd8* expression is maximal). RNA sequencing, followed by bioinformatic analysis, identified 445 and 4954 genes that were differentially expressed during growth and the early stages of development. GO term enrichment analyses revealed that genes associated with protein tagging, catalytic activity, and transporter activity were affected during growth. During early development, genes linked to cellular movement and localization were affected. Finally, western blotting was performed to determine if proteins associated with the affected processes were also impacted. In total, this work provides insight into the mechanisms underlying *mfsd8*-deficiency phenotypes in *D. discoideum* and sheds light on the function of Mfsd8 in *D. discoideum* and humans.

Optimal cytokinin detection in *epithelioma papulosum cyprini*, a model for evaluating *ranavirus* infection

Angela Schincaglia[1], Mark Seegobin[2], Craig Brunetti[1,2], and Neil Emery[1,2]

[1]Department of Biology, Trent University, Peterborough, Ontario, Canada
[2]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, Canada

Frog virus 3 (FV3) is the type of species of the genus *Ranavirus*, which results in edema, hemorrhage, and necrosis of cold blooded vertebrate tissues. To date, there is no known treatment for *Ranavirus*, for which the mortality rate is over 90%. A well researched group of adenine-derived molecules known as cytokinins, have been shown to have therapeutic potential towards combating the replication of FV3 during infection. Cytokinins are small signaling molecules present in all kingdoms of life; however, their biological relevance outside of plants is poorly characterized. Cytokinin detection across kingdoms has been shown to be inconsistent, and is often organism or cell line dependent. In order to ensure optimal detection in *epithelioma papulosum cyprini* (EPC), a model cell line for studying FV3, a range of biomasses were evaluated. Cytokinins were extracted by solid-phase extraction and then detected using high performance liquid chromatography and tandem mass spectrometry. Five forms of cytokinins were detected overall within the samples analyzed, including, N6-(Δ2-isopentenyl) adenine-9-riboside (iPR), N6-(Δ2-isopentenyl) adenine-9-riboside-5' (mono/di/tri)phosphate (iPNT), trans-zeatin-9-riboside-5' (mono/di/tri)phosphate and cis-zeatin-9-riboside-5' (mono/di/tri)phosphate (tzNT, cisZNT) and 2-methylthio-zeatin (2MeSZ). The total cytokinin concentrations ranged from 0.03 pmol/g to 758.59 pmol/g. The results from this study provided evidence that EPC cells can process and synthesize cytokinins. In addition, 100 mg of biomass is optimal for consistent detection, and will be used to evaluate the role of cytokinins during FV3 infection.

Acknowledgments: NSERC Discovery Grants of CB and RJNE supported the project

The Effects of Trait Alexithymia on the Mnemonic Similarity Task: How is Memory Discrimination Affected By A Lack of Emotion?: A research study

Haida Mustansir and Neil M. Fournier

Department of Psychology, Trent University, Peterborough, ON, Canada K9L 0G2

Introduction: Emotions can significantly impact many cognitive functions, such as attention, problem-solving, learning and memory. Different emotional states can influence the content of learned or recalled information. Individuals with Alexithymia cannot link emotions and feelings with their memories, performing worse than non-alexithymic individuals on associative memory tasks. I will examine the impact of trait-related deficits in alexithymia on emotional recognition memory in a non-clinical sample of university students.

Methods: Students will be recruited through local campus announcements. They will take the Toronto Alexithymia Scale to be categorized into non-alexithymic and alexithymic groups. The Mnemonic Similarity Task (MST) will be used. It provides a measure of memory discrimination or the ability to correctly identify a new ("lure") stimulus as distinct from a set of previously studied, highly similar, old stimuli. The MST evaluates behaviours consistent with pattern separation, a process that allows for distinct memory representations for stimuli and events.

Results: I hypothesize that participants who endorse more alexithymic traits should present greater difficulties discriminating "lure" items from previously encountered items on the MST. In addition, I also expect to find an association between lure identification accuracy and the severity of depressive symptoms and alexithymic traits.

Conclusion: The results of this study will add to a growing literature investigating the impact of emotion on cognitive functions and demonstrate the negative effects of reduced emotional processing on mnemonic discrimination abilities. It may lead towards development of novel psychoeducational interventions that can help improve emotional processing that impact school performance.

Effects of oncology camp on the psychosocial health of childhood cancer patients

S. O'Connell[1], N. O'Keeffe[1], L. Kerr[1,2], G.D. Wells[3], and S.L. West[1,2,3,4]

[1]Environmental & Life Sciences, Graduate Studies, Trent University, Peterborough, ON K9L 0G2, Canada
[2]Department of Biology, Trent University, Peterborough, ON K9L 0G2, Canada
[3]Translational Medicine, SickKids Research Institute, Toronto, ON M5G 0A4, Canada
[4]Department of Kinesiology, Trent University, Peterborough, ON K9L 0G2, Canada

Objectives/purpose: Childhood cancer patients encounter significant adversity, therefore experiences promoting psychosocial health are necessary. This study determined the impact of oncology camp (OC) on the resilience, hope, social functioning, mental wellbeing, and stress of childhood cancer patients.
Methods: Childhood cancer patients (6-18 years) enrolled in a 12-day session of OC at Campfire Circle (Muskoka, Ontario, Canada) were invited to participate. Participants completed a survey on the first (T1) and last day (T2) of camp, and 3 months post-camp (T3). This survey included the: Child and Youth Resilience Measure (CYRM-R), Children's Hope Scale (CHS), Social Provisions Scale (SPS-5), and Short Warwick-Edinburgh Mental Wellbeing Scale (SWEMWBS). Afternoon saliva samples were collected at T1 and T2 to determine cortisol (ELISA). Repeated-measures ANOVAs evaluated differences in survey scores between all timepoints. A paired t-test evaluated differences in salivary cortisol (T1 vs. T2).
Results: Ten participants (14.1±2.5 years) were included in the analysis. CYRM-R, SPS-5, and SWEMWBS scores were high but did not differ between timepoints. CHS scores did not differ between T1 and T2; however, T3 (23.70±7.364) was lower compared to T1 (28.30±5.889; p=.004) and T2 (29.30±6.717; p=.018). Salivary cortisol levels were within normal age-based ranges.
Conclusion and clinical implications: While at OC, childhood cancer patients have high levels of resilience, hope, social support, and mental wellbeing, as well as normal stress levels. CHS scores decreased 3 months post-camp suggesting that continued psychosocial interventions may be necessary. Overall, the OC environment is associated with positive psychosocial health.

Effect of running exercise on retention of spatial memory in rats

Eileen Bolton, Hugo Lehmann, and Neil M. Fournier

Department of Psychology, Trent University

New neurons are continuously generated in the hippocampus throughout life. As new neurons are added, they must compete with existing neurons for inputs and outputs which can alter hippocampal circuitry and potentially impact access to information already stored within these circuits. Running exercise is a potent stimulator of hippocampal neurogenesis and is well known to improve enhance new learning. However, running exercise was previously shown to interfere in the recall of a previously acquired spatial memory in mice suggesting that neurogenesis may induce "forgetting" of older memories. In the present study, we set out to replicate and expand on these previous observations. In our study, male rats were trained to learn the location of a hidden platform in the Morris water task (MWT) and were then assigned to either sedentary (SED) or running exercise (RE) groups. The RE group was provided access to a running wheel in their home cages for 4 weeks, whereas rats in the SED were housed in standard cages during this period. At the end of 4 weeks, both groups underwent training in a second MWT before receiving a test for their recall (i.e. memory) of the platform location from the first MWT. The second MWT was conducted using a different a pool with distinct cues from the first to create a unique spatial memory, and RE was maintained for another four weeks before memory of the second MWT was assessed. Our results clearly show a comparable level of memory recall for the SED and RE groups during both tests suggesting that RE did not interfere with the successful remembering of either platform locations (i.e. memories).

Exploring the impact of acute stress on previously acquired fear memory in rats

Javishaa Thiyagarajah, Lexi Thivierge, Eileen Bolton, Hugo Lehmann, and Neil M. Fournier

Department of Psychology, Trent University

Stress exposure can have a profound impact on neurobiological processes necessary for memory consolidation. Several studies have shown that repeated stress can enhance the formation of fear memories but impair the extinction of these memories. However, for many of these studies, stress exposure typically precedes fear and extinction learning. Thus, the impact of stress on already acquired memories formed before the onset of stress is not well understood. In the present study, we trained male Long-Evans rats on a robust context fear learning procedure to create a fear memory that would be resistant to extinction from repeated testing. Following conditioning, a subset of rats was exposed to elevated platform stress (EPS) for 30-60 minutes over two or three consecutive days. Conditioned fear was assessed at multiple time points after exposure to EPS by measuring the amount of defensive freezing the animal exhibited during a 5-minute test session. The results showed that EPS impaired the retrieval of recently acquired context fear memory but not context fear memory formed one week earlier. To explore potential neurobiological mechanisms that might underlie stress-induced memory impairment, we examined the expression of reelin, a large extracellular glycoprotein that is essential for synaptic plasticity. We found that EPS significantly reduced reelin mRNA levels in the medial prefrontal cortex and that a single peripheral injection of recombinant reelin ameliorated stress-induced fear memory impairments. Our findings suggest that stress can enhance the loss of recently formed memories and that enhancing reelin signalling may help alleviate stress-induced forgetting.

Influence of biological sex on gene expression and muscle atrophy in a heart failure model of cardiac cachexia

Alexander Rico and Stephanie W. Tobin

Department of Biology, Trent University, Peterborough, Ontario, Canada

Worldwide it is estimated that there are currently more than 60 million individuals currently living with heart failure (HF), accounting for a third of overall mortality in the United States alone. Attributed to poor disease prognosis, cardiac cachexia effects up to 42% of all heart failure patients, and is indicated by at least 5% edema-free body weight loss in the last year of illness and severe muscle wasting. While several sexual dimorphisms have been linked to HF incidence and severity, sex specific differences in cardiac cachexia remain elusive. In this study, we used muscle mass data and gene expression analysis to investigate the molecular mechanisms that contribute to muscle wasting in cardiac cachexia. To test the hypothesis that a sexual dimorphic phenotype exists in cardiac cachexia, male and female C57BL/6 were injected with either Monocrotaline (MCT) (male, n=7 : female, n=6), or saline (male, n=7 : female, n=7), as well as body weights recorded, for a total of 8 weeks. Upon termination, the tibialis anterior, soleus, gastrocnemius and heart were dissected and weighed to analyze gross muscle mass differences. Total RNA was isolated from Gastrocnemius and heart tissue and subject to RT-qPCR analysis to analyze markers of heart inflammation and muscle atrophy. By further investigating the sex specific differences in cardiac cachexia, it may provide further insight into the severity of this disorder and potential therapeutic interventions.

Effect of chronic stress on kindling-induced emotionality in male and female rats

Faith V. Tucker, Gillian Ekins, Evana Xiao, and Neil M. Fournier

Department of Psychology, Trent University

It is widely accepted that stress can trigger and worsen seizures in both human patients and animal models of epilepsy. Most studies investigating the relationship between stress and epilepsy have focused on the proconvulsant actions of stress hormones on seizure susceptibility. However, seizures, particularly those that arise from mesial limbic structures, are also commonly associated with a higher occurrence of several neuropsychiatric conditions, such as depression and anxiety, that are known to be worsened by stress. In the present study, we set out to explore the interaction between stress and seizures on the development of interictal emotionality in male and female rats using the amygdala kindling model. In this study, kindled rats received either 40 sham or 40 electrical stimulations to the left basolateral amygdala. Because our prior work found that this form of kindling does not generally affect interictal emotional behaviour, we hypothesized that combining stress with suboptimal kindling should enhance the development of interictal anxiogenic behaviours. To test this, rats were assigned to either stress (comprised of 5 days of repeated elevated platform stress) or non-stress conditions after the completion of kindling and then underwent a series of behavioural tests. We found that combining stress with suboptimal kindling was required to enhance anxiety- and depressive-like behaviours, and that these effects appeared to be largest for male kindled rats compared to female kindled rats. These findings add to a growing literature that stress can significantly worsen behavioural outcome in epilepsy and call attention to the important of stress-management in epilepsy management.

Understanding Metabolic Health Status in a Cohort of Nursing Students as Compared to Biology Students

Shanna L. Lowes[1], Annika F. Tan[2], Ingrid K.M. Brenner[3,4], Holly E. Bates[2], and Sarah L. West[2,3,4*]

[1]Environmental & Life Sciences, Graduate Studies, Trent University, Peterborough, ON
[2]Department of Biology, Trent University, Peterborough, ON
[3]Trent/Fleming School of Nursing, Trent University, Peterborough, ON
[4]Department of Kinesiology, Trent University, Peterborough, ON
[*] Both authors contributed equally to this work

Background: Increasing rates of obesity and poor metabolic health are of growing concern globally. Registered nurses have an important role in managing obesity by counselling patients towards healthier living. However, research shows that nurses tend to be overweight/obese and have poor metabolic health that may be influenced by lifestyle choices. Whether this begins earlier in life during their nursing education is poorly understood.
Methods: Undergraduate nursing and biology (comparison group) students had their metabolic health assessed through measurements of fasting blood glucose and lipids, blood pressure, and waist-to-hip ratio. Additionally, lifestyle factors of physical activity, nutrition, stress, and sleep were assessed both subjectively and objectively using questionnaires, accelerometry, food logs, and heart rate variability.
Results: A total of 42 nursing and 15 biology undergraduate students participated in the study. Statistical analysis is ongoing and aims to characterize metabolic health and lifestyle of undergraduate nursing students in comparison to biology students.
Significance: Results of the current study will aid in determining the metabolic health of nursing and biology students, and what lifestyle factors are most correlated with their metabolic health. Nursing students could then benefit from education or interventions aimed at improving health, which could also be applied to patient care.

Postictal changes in somatosensory and affective components of pain following electrical amygdala kindling in rats

Evana Xiao, Gillian Ekins, Kaylea MacDonald, and Neil M. Fournier

Department of Psychology, Trent University

Pain is a complex experience, which consists not only a sensory/discriminative dimension (e.g., the quality, location and intensity of pain) but also an affective affective/motivational dimension (e.g., unpleasantness or aversiveness to pain). Kindling is the process by which daily administration of electrical stimulations to a particular brain region results in the gradual development and intensification of motor seizures. We have previously shown that amygdala kindling produces long-lasting increases in fear and anxiety-related behaviour in rats. Interestingly, many of the same neural circuits impacted by kindling are also involved in processing pain information. This led us to hypothesize that recurrent seizures might sensitize neural circuits involved in mediating pain responses, which in turn could lead to impairments in the processing of sensory and affective features of pain. In the present study, Long-Evans rats underwent short-term (30 stim) amygdala kindling and at various points after the completion of kindling were examined on a battery of pain-related behaviours. We found that kindling was associated with a delayed development of hypersensitivity to mechanical but not thermal pain stimuli. In addition, kindled rats engaged in greater displays of emotional pain behaviours and showed greater activation of the rostral anterior cingulate cortex—a key structure in affective pain perception in humans and animals—in response to inflammatory pain induced by formalin. Interestingly, chemogenetic (DREADD) inhibition of excitatory neurons in the anterior cingulate cortex alleviated kindling-induced impairments in conditioned pain avoidance learning and promoted pain relief. These results suggest that chronic seizures can alter pain sensitivity and further highlight the involvement of the anterior cingulate in the modulation of pain-induced emotional behaviours.