



COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR
AND CELLULAR BIOLOGY

Announcement:

*All interested members of the university community are invited to attend the Final Oral Examination for the degree of **Doctor of Philosophy** of*

TRISTEN HEWITT

on Wednesday, September 7, 2022 at 1:30 p.m. (SSC 2315)

Thesis Title: **The role of store-operated calcium entry in transcription factor activation and in accelerated differentiation of induced pluripotent stem cell-neural progenitor cells derived from bipolar disorder patients**

Examination Committee:

Dr. Matthew Kimber, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Jasmin Lalonde, Dept. of Molecular and Cellular Biology
Dr. Melissa Perreault, Dept. of Biomedical Sciences
Dr. Shaun Sanders, Dept. of Molecular and Cellular Biology
Dr. Jean Martin Beaulieu, Dept. of Pharmacology and Toxicology,
University of Toronto (External Examiner)

Advisory Committee:

Dr. Jasmin Lalonde (Advisor)
Dr. Melissa Perreault
Dr. Neil MacLusky

Abstract: Calcium (Ca^{2+}) is a pivotal signaling molecule in non-excitabile and excitable cells. In neurons, it regulates processes from transcription to excitability. Though they have specialized Ca^{2+} channels that influx massive amounts of this cation during activity, these channels are closed during rest. So, to regulate smaller oscillations, neurons use store-operated Ca^{2+} entry (SOCE). This mechanism utilizes the ER Ca^{2+} sensor STIM and the Ca^{2+} channel ORAI for influx after Ca^{2+} stores in the ER are depleted. This influx is known to regulate transcription, differentiation, survivability, and excitability, among other processes. Since the various roles SOCE contributes to are cell type-specific, we wanted to assess its impact on transcription and differentiation with a model seldom used for SOCE-specific research: human-derived neural progenitor cells (NPCs). SOCE is known to modulate activity of transcription factors such as NFAT and Sp4. We sought to test whether CREB, a crucial transcription factor in neurogenesis, could be modulated by SOCE. We found that CREB was phosphorylated as soon as five minutes after SOCE activation, though not by one of its canonical pathways. We also found that SOCE activation contributed broadly to transcription as its activation upregulated several immediate early genes. If SOCE indeed influences neurogenesis in this way, dysregulation in the brain could contribute to neurodevelopmental disease. Though Ca^{2+} signaling is known to be dysregulated in bipolar disorder (BD), few studies have explored potential SOCE dysregulation in cells derived from these patients. We uncovered an attenuation in SOCE-specific Ca^{2+} influx in BD-derived NPCs (BD-NPCs) that was dependent on differentiation. From this we explored neurodevelopmental deficits in these cells, which

included a unique transcriptome, upregulated microRNAs, reduced rate of proliferation, increased migration, longer neurite outgrowth, and abnormal subventricular structures after generating BD organoids. This all suggests that BD pathophysiology may begin long before symptoms of the disease present in early adulthood. Continuing to study the impact SOCE has on NPCs and how dysregulated neurodevelopmental processes in BD-NPCs contribute to disease could uncover new cell signaling pathways and potential therapeutics for the disease.

Curriculum Vitae: Tristen completed his Bachelor of Science (Hons.) in Molecular Biology and Genetics with a minor in Zoology at the University of Guelph in April 2018. He then started his Master of Science in Molecular and Cellular Biology with a specialization in Neuroscience under the supervision of Dr. Jasmin Lalonde at the University of Guelph in May 2018. He transferred to the Doctor of Philosophy program in September 2019.

Awards: Pharmacia Molecular and Cellular Biology Graduate Prize (2021); Best Trainee Talk – Ontario Cell Biology Symposium (2021).

Publications: Thériault RK, St-Denis M, **Hewitt T**, Khokhar JY, Lalonde J, Perreault ML. (2021). Sex-Specific Cannabidiol- and Iloperidone-Induced Neuronal Activity Changes in an In Vitro MAM Model System of Schizophrenia. *International Journal of Molecular Sciences*. 22(11):5511. doi: 10.3390/ijms22115511.

Smith B, **Hewitt T**, Bakovic M, Lu R. ER stress-associated transcription factor CREB3 is essential for normal ROS, Ca²⁺, and ATP homeostasis. (Submitted to *Mitochondrion*).

Hewitt T, Alural B, Tilak M, Wang J, Perreault M, Sheridan SD, Perlis RH, Jones N, Mellios N, Lalonde J. Bipolar disorder-iPSC derived neural progenitor cells exhibit dysregulation of store-operated Ca²⁺ entry and accelerated differentiation. (In preparation for submission to *Molecular Psychiatry*).

Hewitt T, Proud E, Brind'Amour J, Sheridan SD, Perlis RH, Lalonde J. Store-operated Ca²⁺ entry phosphorylates CREB at S133 and upregulates immediate early genes responsible for neurodevelopment. (In preparation for submission to *Experimental Brain Research*).