UNIVERSITY of GUELPH

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 ***Master of Science*** *of*

RYAN D. HALLAM

on Tuesday, April 7, 2020 at 1:30 p.m. in SSC 2315

Thesis Title: **Alpha-synuclein fibrils impair calcium flux in cortical neurons through aberrant nitric oxide-mediated inhibition of NMDA receptor function**

Examination Committee:

Dr. A. Bendall, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. S. Ryan, Dept. of Molecular and Cellular Biology
Dr. J. Lalonde, Dept. of Molecular and Cellular Biology
Dr. M. Perreault, Dept. of Molecular and Cellular Biology

Advisory Committee:

Dr. S. Ryan (Adv)
Dr. J. Lalonde
Dr. G. Harauz

Abstract: Cortical synucleinopathies, including Dementia with Lewy Bodies (DLB) and Parkinson's Disease Dementia (PDD), are characterized by the aberrant aggregation of misfolded αsynuclein protein into large inclusions in cortical tissue, leading to impairments in proteostasis and synaptic connectivity and eventually resulting in neurodegeneration. However, the mechanisms linking α-synuclein aggregation with synaptic dysfunction have been difficult to ascertain. Here, I show that primary cortical rat neurons exposed to exogenous α-synuclein preformed fibrils (PFFs) accumulate large, insoluble deposits which are resistant to enzymatic digestion, consistent with advanced synucleinopathy. Live-cell imaging of calcium dynamics reveals that aberrant intracellular accumulation of α-synuclein inhibits synaptic response to glutamate through inhibition of calcium intake, but that these deficits manifest slowly over a 7- day period, suggesting that they may be indirect. To further assess this conjecture, I found that extracellular PFFs do not influence calcium dynamics, and that intracellular accumulation of αsynuclein does not reduce synaptic protein expression, suggesting that the observed deficit is not due to a perturbation in synaptogenesis. Further, α-synuclein aggregates reduce the frequency of spontaneous calcium transients, consistent with previous findings of calcium dysregulation in synucleinopathies. Using a combination of selective receptor activation and targeted pharmacological inhibition, I present evidence that the impairment of glutamate-evoked calcium flux is specific to NMDA receptors, as AMPA receptor function remains intact. Moreover, inhibition of nitric oxide synthase results in a rescue of glutamate-evoked calcium flux and neuronal network activity, suggesting that aberrant accumulation of nitric oxide may impair NMDA receptor function in cortical synucleinopathies. Collectively, this data suggests that loss of synaptic function in PDD and DLB may result from synucleinopathy-evoked nitrosative stress and subsequent NMDA receptor dysfunction, and that targeted prevention of nitric oxide accumulation may have therapeutic benefit against α-synuclein induced synaptopathy.

Curriculum Vitae: Ryan completed his Bachelor of Science (Hons.) in Neurobiology at the Brock University in April 2018, and then began his M.Sc. in Dr. Scott Ryan's lab in May of the same year.

Awards:
Canadian Graduate Scholarship (NSERC-CGSM; 2019-2020).
Keystone Symposia Travel Scholarship (NIH; 2019).

Publications: Czaniecki C, Ryan T, Stykel MS, Drolet J, Heide J, **Hallam R**, Wood S, Coackley C, Sherriff K, Bailey CDC and Ryan SD. (2019). Axonal pathology in hPSC-based models of Parkinson's Disease results from loss of Nrf2 transcriptional activity at the Map1b gene locus. Proceedings of the National Academy of Sciences USA, 116(28), 14280-14289.