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 ***Doctor of Philosophy*** *of*

BRODIE BUCHNER-DUBY

on Tuesday, May 14th, 2024 at 1:00p.m. (SSC 1511)

Thesis Title: Accumulation of aggregated alpha-synuclein promotes S-nitrosylation of MAP1A leading to synaptic dysfunction mitigated via targeted degradation utilizing ubiquitin variant induced proximity

Examination Committee:

Dr. Angela Scott, Molecular and Cellular Biology (Exam Chair)
Dr. Scott Ryan, Dept. of Clinical Neuroscience, University of Calgary
Dr. Shaun Sanders, Dept. of Molecular and Cellular Biology
Dr. Ray Lu, Dept. of Molecular and Cellular Biology
Dr. Shawn Hayley, Dept. of Neuroscience, Carleton University (External Examiner)

Advisory Committee:
Dr. Scott Ryan (Co-Advisor)
Dr. Shaun Sanders (Co-Advisor)
Dr. Melissa Perrault
Dr. Wei Zhang

Abstract: Synucleinopathies are neurodegenerative disorders characterized by the accumulation of misfolded α-synuclein (α-syn) protein. Under patho-physiological conditions α-syn aggregates to form oligomers, a toxic precursor of the insoluble Lewy bodies, that deposit in neurons. These toxic forms of α-syn are thought to cause decreased synaptic transmission and impaired proteolysis, resulting in the perpetuation of misfolding. However, the mechanisms linking α-syn aggregation to synaptic dysfunction are yet to be fully elucidated. Herein, synucleinopathies were modelled through the addition of pre-formed fibrils (PFFs) to primary cortical neurons. To study the synaptic dysfunction, electrophysiological analysis of network activity, high resolution microscopy of dendritic arborization and dendritic spines, and immunoblotting were conducted. In this system, there was an observed synaptic dysfunction associated with the N-methyl D-aspartate receptor (NMDAR) including decreases in number and maturity of dendritic spines, impaired synaptic connectivity, reduced neuritic arborization, and S-nitrosylation (SNO) of the dendritic scaffold protein microtubule-associated protein1A (MAP1A). Recent evidence from our lab implicated increased nitric oxide levels as a mechanism for this NMDAR associated synaptic dysfunction. Using an inhibitor of nitric oxide synthase, PFF induced SNO-MAP1A and impaired maturation of spines were ameliorated. This suggests MAP1A is at least, in part, implicated in the tethering of NMDARs in the PSD and therefore synaptic connectivity under physiological conditions. However, under pathological conditions SNO-MAP1A may reduce stability of NMDARs in the PSD, causing them to be internalized, and subsequently, synaptic dysfunction. This thesis also aimed to generate a rescue of synaptic dysfunction (UbVIP). Importantly, expression of the UbVIP exposed neurons mitigated the

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synaptic dysfunction caused by PFFs. This investigation offers novel insight into the molecular basis of synaptic dysfunction in synucleinopathies and determined that preventing oligomeric α-syn accumulation through targeted degradation has the potential to rescue this synaptic dysfunction associated with synucleinopathies.

Curriculum Vitae: Brodie obtained her Bachelor of Science (Hons.) in Biochemistry and Molecular Biology from Trent University in 2019. In the fall of 2019, she entered into the M.Sc program under the supervision of Dr. Ryan. She transferred to the Ph.D. program in the fall of 2020 and became Co-supervised by Dr. Sanders in spring 2023.

Publications: Parmasad J-LA, Ricke KM, Nguyen B, Stykel MG, Buchner-Duby B, Bruce A, Geertsma HM, Lian E, Lengacher NA, Callaghan SM, Joselin A, Tomlinson JJ, Schlossmacher MG, Stanford WL, Ma J, Brundin P, Ryan SD, Rousseaux MWC. (2024). Genetic and pharmacological reduction of CDK14 mitigates synucleinopathy. Cell Death Dis. 15(4): 246. PMID: 38575601

Hallam RD*, **Buchner-Duby B***, Stykel MG, Coakley CL, Ryan SD. (2022). Intracellular accumulation of α-synuclein aggregates promotes s-nitrosylation of MP1A leading to decreased NMDAR-evoked calcium influx and loss of mature synaptic spines. JNeurosci. 42 (50); 9473-9487. PMID: 36414406

* Both authors contributed equally to this work

Stykel MG, Humphries KM, Kamski-Hennekam E, **Buchner-Duby** B, Porte-Trachsel N, Ryan T, Coackley CL, Bamm VV, Harauz G, and Ryan SD. (2021). α-synuclein mutation impairs processing of endomembrane compartments and promotes exocytosis and seeing of α-synuclein pathology. Cell Reports. 35 (6); 109099. PMID: **33979611**

Drolet J, **Buchner-Duby B**, Stykel MG, Coackley C, Kang JX, Ma DWL, and Ryan SD. (2021). Docosahexanoic acid signals through the Nrf2-Nqo1 pathway to maintain redox balance and promotes neurite outgrowth. Mol Biol Cell. 32(7); 511-520. PMID: 33502893

Awards: Parkinson's Society of Southwestern Ontario Graduate Student Scholarship (2021); Ontario Graduate Scholarship (2022); Ontario Graduate Scholarship (2023); Pharmacia Molecular and Cellular Biology Graduate Prize (2023)