

Multidisciplinary Research Program in Medicine Project: *Quantitative localization of autonomic neurons activated by exercise and neuromodulation after spinal cord injury*

Hypothesis or Research Question(s): Spinal cord injury (SCI) disrupts blood pressure (BP) control leading to dangerous BP spikes, a condition called autonomic dysreflexia (AD). Both passive exercise (PE) and spinal cord stimulation (SCS) are promising treatments for AD. However, the underlying mechanisms remain unknown. This proposal will quantify the neuronal subpopulations activated by PE (DISCIPLINE 1) and SCS (DISCIPLINE 2) in a rodent model.

PROJECT BACKGROUND & SUMMARY

Spinal cord injury at cervical or upper thoracic levels can result in drastic impairment of BP control, resulting in a condition called AD, where systolic blood pressure can abruptly rise by more than 20 mmHg above resting levels, in response to peripheral stimuli such as full bladder or bowel routine. The BP during AD can often reach beyond 300 mmHg, with potential implications of seizures, stroke or even death. Our studies have reported that AD episodes can occur as high as 41 times per day, negatively impacting the quality of life. Not surprisingly, recovery of AD is among the highest priorities of recovery in individuals with SCI. Many studies have proposed treatments for AD but they are often unsuccessful or with significant limitations such as surgical invasiveness.

Recent studies have demonstrated that PE (using custom-made motorized bikes) and SCS (electrodes placed in skin) are noninvasive approaches that can mitigate AD episodes. However, the exact mechanisms of PE and SCS effects on AD are only speculated. Application of SCS triggers local large-diameter afferents, thereby closing the gates for noxious stimulus and thus interrupting the hypertensive response from resulting in severe vasoconstriction and AD (gate-control theory). PE has demonstrated benefit on reducing painful fibers in the spinal cord but its effects on activation of spinal interneurons is not understood. In our proposed project, we will aim to fill this knowledge gap and analyze neuronal response patterns triggered by PE (DISCIPLINE 1) and SCS (DISCIPLINE 2) targeted to mitigate AD, using rats with high thoracic SCI (T3), an established model that reproducibly results in AD. In this project, putative neurons will be activated by colorectal distention (CRD; noxious peripheral stimulus) to trigger experimental AD. Rats will be divided into 5 groups after SCI. Group 1 will receive CRD (AD only), group 2 will receive PE alone and group 3 will receive SCS alone. Groups 4 and 5 will receive CRD paired with PE and SCS respectively. Following euthanasia and tissue dissection spinal cords will be made transparent using "clean, unobstructed brain/body imaging cocktails and computational analysis (CUBIC)", a method of tissue clearing. CUBIC extracts lipids and homogenizes the refractive indices in the tissue, rendering it visually transparent. Cleared spinal cords will be immunostained for c-Fos, an immediate early gene used as a marker and a powerful tool to identify the number and location of activated neurons. The number and location of activated neurons will be compared across groups. The proposed project will have three main deliverable goals for TS.

- 1) Establish and perform CUBIC tissue clearing protocol for different animal groups.
- 2) Analyze animal groups to quantitatively compare the activated neuronal response patterns across the groups.
- 3) Present results to national and international research conferences.
- 4) Contribute data to a research manuscript with potential co-authorship.

BENEFIT TO THE STUDENTS

Multidisciplinary Research Program in Medicine Project: *Quantitative localization of autonomic neurons activated by exercise and neuromodulation after spinal cord injury*

ICORD is an internationally recognized research institute committed to prevention, functional recovery and improved quality of life following spinal cord injury (SCI). TS will be able to receive collaborative support and mentorship opportunities from various experts in a multidisciplinary environment consisting of clinicians, trainees, clinical-researchers, post-doctoral fellows, and graduate students. Throughout the duration of the project, TS will acquire knowledge that will help them understand each gear of the machine known as SCI research. With their hands on practical knowledge and information, TS will learn the thorough process of pre-clinical research trouble shooting errors and difficulties that might arise during the research project. Furthermore, they will learn to appreciate the effort that goes in animal care and running a preclinical survival study. The TS will read many scholarly articles and literature pertaining to their research which will improve their experience in translating their knowledge gained from academia to actual field work. Not only will this improve their knowledge in the topic of research but also make them adept at writing and presenting their research to a broad audience. Furthermore, TS will learn about the social, psychological, and physiological consequences that patients with SCI endure on a daily basis, thus creating empathy for their community members. The project provides TS an opportunity to integrate pre-clinical and clinical research and facilitate an increasingly more well-rounded knowledge of SCI pathology and management. At ICORD they will also receive opportunities to interact with their fellow community members suffering from SCI, and help create their day to day lives easier.

Lastly, TS will have the opportunity to prepare and submit abstracts for presentation at local, national, and international conferences as well as prepare manuscripts for submission to academic journals. The TS will receive materials and encouragement to increase their theoretical knowledge of nervous system anatomy in tandem with their lab skills. Periodically, supervisors will conduct a review of TS' knowledge of the field and experimental rationale, to ensure TS' understanding of experimental design and hypothesis formulation. It is expected that the TS have high proficiency in their knowledge about SCI pathology and research. These one-on-one experiences will help the TS gauge their progress and improve over their research period.

Effective speech delivery and presentation of conducted research can be as valuable as conducting the research itself. Since the main goal is for the TS to become a well-rounded scientist, they will receive ample opportunities to present their research within lab meetings. Presenting to familiar faces will help them overcome their fear of presenting scientific literature to an audience. They will also receive feedback thus allowing them to improve every time and deliver a great presentation at national and international conferences.

The learning objectives for the non-MD undergraduate students are to establish a CUBIC tissue clearing protocol. TS will conduct a literature review on the TCS parameters in able-bodied and SCI individuals and succinctly compile the information in preparation for manuscript writing and begin submitting by the end of week eight of the funding period. Throughout this process, TS will critically appraise existing literature and data to identify research gaps and shortcomings of existing research. TS will also have the opportunity to take part in data collection, data analysis and conducting appropriate statistical analyses. TS will be expected to perform all work objectively to uphold a high standard of scientific integrity to ensure confidence and credibility in their findings.

If online instead: During the previous cycle of MRPM, we were able to obtain research exemptions and continue the proposed research project physically as needed. If the project cannot take place physically

Multidisciplinary Research Program in Medicine Project: *Quantitative localization of autonomic neurons activated by exercise and neuromodulation after spinal cord injury*

this year, we will be able to modify the project in several ways. Firstly, if research fellows (Postgraduate Student Advisor or Postdoctoral Fellow) is able to resume researching physically, they will conduct research in the lab as required and forward the data files to TS. At home, TS will be able to process and analyze the data, and contact their supervisors and advisors virtually for updates and guidance. Secondly, if all physical research is fully suspended, we will alter the project and TS will conduct a systematic review on a similar topic. They will learn to conduct a comprehensive review using online search engines, and will take part in abstract screening, result reviewing, and manuscript writing. These skills are fundamental for success in research-based academia and thus will greatly benefit the TS.