



**Biomechanics of Painful Injury**  
**Harrison Troche (EAS 2019)**  
**Advisor: Beth Winkelstein**

Under the Penn Undergraduate Mentorship Program, I worked in the Spine Pain Research Lab under Dr. Beth Winkelstein. The aim of my research this summer was to develop a reliable method to quantify microglial infiltration in dorsal root ganglion (DRG), and to utilize it in a painful nerve root compression rodent model.

The spinal nerve root is a common source of pain and can be directly injured via compression, often by disc herniation, spondylosis, or through other spinal traumas. Previous studies using this rodent model have shown upregulation of microglia in the spinal cord as early as one day after injury and up to seven days after injury. However, microglial infiltration in the DRG, another site known to modulate pain responses, after painful nerve root compression has previously not been studied. Based on the findings in the spinal cord, we hypothesized that there will be increased infiltration of microglia in the DRG after a painful neuropathic injury. Nevertheless, to test our hypothesis we first needed to develop a method to reliably quantify microglia within the DRG.

In order to evaluate the density of the microglia in the dorsal root ganglion, two identically sized regions of interest were chosen for each serial DRG section (4-6 sections per rat.) Iba1 and MAP2 calcium-binding proteins were used to identify microglia/macrophages and neurons respectively. Each image was analyzed using a custom Matlab script that calculated the percent positive area of each label. The density of iba1 was similar for all the DRG sections of each animal; therefore, moving forward, quantification was performed using one DRG section per rat. To quantify microglial infiltration after nerve root compression, DRG sections were taken from rats that underwent a painful or non-painful compression, as well as a sham and normal group that underwent no compression, at both one day and seven days after injury. For each DRG section, two individual regions of interest were cropped to include ten neurons each, and the percent positive pixels for iba1 and MAP2 were determined using the same custom Matlab script.

The analysis showed that iba1 labeling in the DRG was significantly greater in the painful group over the non-painful, sham, and normal groups at day one. There were also no significant differences between groups at day seven. However, iba1 in the DRG was significantly increased in the painful group at day one compared to day seven. These results supported our hypothesis.

My time in the Spine Pain Research Lab was a great learning experience and opportunity to gain exposure to research. I gained a variety of research skills and techniques, including how to image and analyze tissue, conduct immunohistochemistry, and utilize Matlab. Spending these ten weeks in the lab allowed me to see the research environment for myself and decide how I wanted it to play a role in my next three years at Penn. I will remain working in the SPRL Lab through the school year in hopes of learning more valuable skills and gaining more insight into the Bioengineering field.