AN IMMUNOHISTOCHEMICAL STUDY OF THE SENSORY INNERVATION IN THE MOUSE SKELETAL MUSCLE
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Recent developments in novel antigen markers, antibodies, fluorochromes, and the utilization of confocal microscopy have not only enriched our understanding in the structure and function of cells and tissues, but also prompted novel drug development targeting novel pathways.

In the periphery (e.g., skin and muscle tissues), pain is sensed by a group of specialized afferent nerves, termed nociceptors. Currently, the project I am participating in the Light Lab aims to determine the profiles of the nociceptors that innervate the skeletal muscles to better understand the mechanisms of muscle fatigue and pain. To do this, we used a group of polyclonal and monoclonal antibodies to label the molecular receptors and other biomarkers that are specifically expressed in different nociceptors. To investigate the termination of these nerve fibers in the muscle, we used LYVE-1 and CD-31 antibodies to label muscular blood and lymphatic vessels. Secondary antibodies conjugated with fluorescent dyes are then applied to visualize these structures. By performing this experiment the results produce will allows us to retrieve more information on the receptors that are associated with pain and eventually lead to an altered and improved treatment opportunity for conditions like chronic fatigue syndrome, multiple sclerosis, and fibromyalgia.

The gastrocnemius muscle tissues are collected from 2% paraformaldehyde-fixed adult mice, embedded into agar and sectioned into 50-100µm thick slices on a Vibratome, or 10-20µm thick on a cryostat. Samples are first dried and washed with PBS then permeabilized with 1% PBST. The sections are washed again with PBS three times to remove the 1% PBST. Primary antibodies (generated from rabbit, mouse, or guinea pig) are diluted in 1% NGS+BSA. After adding the primary antibody mix, the sections are incubated at room temperature for 2 hours, or at 4°C overnight, washed three times with PBS, then incubated in secondary antibody mix for one hour. The sections are washed again and mounted on glass slides using Fluoromount-G, which preserves the fluorescent chromes from quick photo-bleaching. Samples are imaged using a Nikon confocal microscope to evaluate the fluorescent patterns of the nerve endings, blood vessels, and lymphatic vessels in muscle tissues.