Visualizing the role of sema1a in the compensatory growth of *Gryllus bimaculatus*

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The central nervous system is a complex network made up of neurons that have a relatively simple task: send signals from part of the body to the other. However, when this system is damaged or subject to injury, its response anatomically and morphologically is extremely complex. The functional adaptation of neural networks to reorganize or modify their structure is a demonstration of compensatory plasticity. However, injury has also been shown to result in cell death and subsequently, a loss of the system's function. In adults, injury can be fatal to their systems as aging reduces a neuron's ability to demonstrate plasticity (Manini et al., 2013).

An unusual example of compensatory plasticity in adults is found in the cricket species *Gryllus bimaculatus*. *Gryllus* retains its auditory function even in cases of injury and may serve as a model organism for understanding the molecular mechanisms behind adult neuronal plasticity (Horch et al., 2011). The tympanic membrane, which is located on each foreleg of the cricket serves as the 'ear' of the cricket. Auditory input travels through the tympanic membrane to Nerve 5 in the prothoracic ganglion. It then meets its postsynaptic partners AN-1 and AN-2. These Ascending Interneurons (AN-1, 2) are symmetrically bilateral on either side of the cricket and their dendrites respect the midline of the ganglion. Their axons then extend up to the brain where they innervate the auditory neuropil (Schildberger, 1984; Kostarakos and Hedwig, 2017). When one 'ear' is deafferented, or in crickets, their leg is severed, Nerve 5 retracts. Yet, *Gryllus* demonstrates dendritic growth **across** the midline of the ganglion to compensate for this loss, forming functional synapses with Nerve 5 on the opposite side of the body (Brodfuehrer and Hoy, 1988; Hoy et al., 1985).

The Horch lab previously isolated molecular candidates that may be responsible for this growth. One class of molecules that plays a role in guiding axonal and dendritic growth is sempahorins. Semaphorin1a was previously characterized *in situ* in the cricket brain and demonstrated a significant drop in expression levels after cricket deafferentation (Chong, 2015). This correlated with the dendritic-crossing anatomical changes observed. Using a modified non-invasive electrophoretic staining technique (Kostarakos & Hadewig, 2017) and dsRNA targeting sema1a, dendritic crossing is able to be visualized and quantified. By experimentally "knocking down" or decreasing levels of sema1a expression in uninjured crickets and visualizing and quantifying growth using confocal microscopy, causation may be confirmed.

This summer I continued to visualize the effects of Sema1a knockdown on crickets. Prior research found knockdown of sema1a resulted in increased dendritic sprouting across the midline (Moynihan, 2019); however, the sample size was an n=11 and needed to be increased to confirm its causation. The majority of the summer was spent mastering backfill and extracellular recording techniques. Additionally, more double stranded RNA was synthesized in lab in preparation for future injections of sema1a and green fluorescent protein (GFP) that acted as a control. While no samples were visualized, we expect to see increased dendritic sprouting in sema1a injected crickets. Work will be continued this autumn to further increase the sample size and visualize the injected cricket's dendritic morphology.

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