Exploring the role of Semaphorins on the maintenance of AN physiology and morphology

Jada Scotland, 2023

Neural plasticity, or the ability of neurons in the central nervous system (CNS) to adapt in response to environmental changes, is extremely important to development in organisms. The auditory system of *Gryllus bimaculatus*, exhibits an unusual compensatory plasticity. Amputating the cricket's foreleg severs the connection between afferent auditory nerves and ascending neurons in the prothoracic ganglion. Following deafferentation, dendrites grow across the midline, a typically respected boundary. This plasticity allows for an organism to respond to environmental changes through the flexibility of synapses, axons, and dendrites. The morphology and physiology of cricket ANs were explored to provide better understanding of this phenomenon.

Three conditions of crickets were used to preform backfills to visualize morphology: uninjected, Sema dsRNA-injected, and GFP dsRNA-injected. The control, uninjected cricket helped create a baseline and were not as carefully controlled for age as the injected species. The altered animals were injected with either Sema dsRNA or GFP dsRNA. The GFP test controls for the injection, as crickets that have been injected tend to reach adulthood later than uninjected species. The protein knockdown injections imitate deafferentation.

A suction electrode was used to locate the auditory neuropil on the exposed brain. Guidance sound stimuli (70dB) at frequencies 5 kHz, 10 kHz, 18 kHz, and white noise (20 kHz) played from two speakers approximately seven inches from the cricket's head until action potentials confirmed that the electrode contacted AN-1 and AN-2. A test stimulus further confirmed position. A stimulator iontophoresed neurobiotin, a neuroanatomical tracer, into AN-1 and AN-2 using positive, alternating electrical pulse for ten minutes. The cricket was then stored at 4˚C for ~24 hrs. to allow the dye to travel into the prothoracic ganglion. The prothoracic ganglion was then extracted from the cricket, fixed, reacted with Streptavidin 594, and then dehydrated. Finally, the tissue was visualized under a fluorescent microscope.

Analysis of the physiological activity of a cricket injected with dsRNA targeting Sema 2a, compared to those injected with dsRNA against GFP, showed decreased responses to the guidance stimulus and test stimulus. Compared to all control crickets, the Sema 2a dsRNA injected cricket had the smallest burst duration in response to the guidance and test stimulus. This delayed burst response could potentially be a result of auditory responses from the contralateral side of the prothoracic ganglion, due to the downregulation of the Semaphorin chemorepellent. Technological difficulties hindered the ability to collect sufficient morphological data. While the sound stimuli confirmed position over the auditory neuropil, dye was not being driven into the cells. This could point to issues with the stimulator or electrode. In the future, the amplitude of the electrical pulse could be increased to ensure dye is being pulsed into the brain. Another potential solution to this issue would be to replace the platinum electrode within the electrode holder that was used. Lastly, potential rewiring or recalibration of the stimulator and amplifier apparatus could potentially allow neuroanatomical tracer to be pushed out of the suction electrode and into the auditory neuropil.

This summer was largely focused on building a strong foundation for the future. Subsequent studies will explore the role of different protein families, particularly the Toll receptors, in this neural plasticity. Vertebrates have a similar receptor in their central nervous systems called the Toll-like receptor. One study suggests that these receptors' signaling pathway plays a role in axonal growth and plasticity in adult mammals. If Toll receptors show evidence of being involved in this plasticity in the cricket, it could help create better understanding about plasticity in humans.

Faculty Mentor: Hadley Wilson Horch

Funded by: Student Faculty Research Fellowship through the Maine INBRE Network