Electrophysiological and anatomical assessment of the neuroplastic auditory neurons of the Mediterranean field cricket (*Gryllus bimaculatus*)

Brooke Asherman, Class of 2023

Thanks to the Kufe Family Fellowship, this summer I have had the pleasure of working in the Horch lab. The Horch lab studies neuroplasticity using the Mediterranean field cricket (*Gryllus bimaculatus*) as a model organism. Neuroplasticity refers to the reorganization of neurons and synapses. This reorganization is why organisms can respond to injury. In the Horch lab previous research has explored the cricket response to injury to the auditory system. In the cricket their ears are found on both of their forelimbs. There are neurons that pickup information from each side of the body. Typically, these neurons do not crossover the midline to the opposing side of the body. We can investigate their neuroplastic ability by deafferenting (cutting off) a limb, thereby severing the auditory neurons on one side of the body. When this happens we observe a unique response where the crickets auditory neurons from the deafferented side of the body cross the midline to the opposite side of the body and account for the hearing impairment. This is an evolutionarily advantageous response that allows the cricket to react to the sounds of their predators and other crickets, even when they are missing one of their ears.

Moving forward in the Horch lab the overarching goal is to connect a single organism's traits, behavior, physiology, and neuronal structure to better understand the neuroplastic ability of the cricket. What my partner and I have been working on this summer is to backfill the auditory neurons of the cricket using electrophysiology to visualize the neuronal structure. Backfilling involves injecting a fluorescent dye into the auditory neurons so we can visualize their arrangement in the central nervous system. To do this we immobilize the cricket on a ball joint platform and expose the brain while the cricket is still alive. We then use a suction electrode filled with our fluorescent dye and place it on the brain in the location of the auditory neurons. Using electrophysiology we play the frequency of sounds of bat ultrasound to emulate their predator and other cricket chirps. This activates the auditory neurons. We use signal from the electrode to look for auditory action potentials on the computer. Once we see auditory action potentials we inject the dye into the brain (via iontophoresis). The goal of this procedure is that the dye is injected into the auditory neurons in the brain and travels down the central nervous system to the auditory neurons in the prothoracic ganglion. Auditory information is initially sent to the prothoracic ganglion so it is important and valuable to be able to visualize the neuronal structure and see the connections between neurons.

It is valuable to be able to visualize the auditory neurons in the prothoracic ganglion of the cricket because it gives us a piece of the big puzzle which is to understand the neuroplastic ability of the cricket. Moving forward my goals are to inject double-stranded RNA into crickets and combine the backfilling technique with the lab's cricket behavior specialists to understand the effects of traits and proteins on the cricket's neuroplastic ability. Double-stranded RNA knockdowns proteins so we can see the effects of low expression of those proteins on the cricket auditory system. The "behavior specialists" analyze the cricket's flying behavior while their predator's (bats) ultrasound is played from one direction. Using that knowledge of how the crickets will respond in flight behavior when certain proteins are suppressed. We can backfill the auditory neurons and visualize the structure of the neurons. By doing this we can connect an organism's traits, to their behavior, and to the internal structure of their neurons: thereby giving us a wholistic picture of neuroplasticity.

I am grateful to the Kufe Family Fellowship, my faculty mentor Hadley Horch, and the rest of my supporters in the lab for an amazing summer opportunity. I learned so much about lab procedure and protocol, how to successfully work as a group, how to present my research, and problem solve when things are not working. I have enjoyed my time in the Horch lab and this summer has encouraged me to continue doing research and pursue an Honors Project in the upcoming academic year.

Faculty Mentor: Hadley Horch
Funded by the Kufe Family Fellowship