

Behavioral analysis of negative phonotaxis in *Gryllus bimaculatus* following injury-induced deafferentation

Josh Goldstein, Class of 2025

The cricket auditory system has been of interest to neuroethologists for over 40 years. Previous research has demonstrated the auditory system is set up in a way that the cricket is sensitive to two main types of stimuli, sounds at approximately 5 kHz and those above 16 kHz. Upon hearing mating calls (5kHz), female crickets will perform positive phonotaxis, turning towards the sound source. Conversely, literature indicates that bats (a natural predator of crickets) emit echolocation signals at frequencies around 18 kHz (Moiseff et al., 1978). Night time induces flying behavior in crickets, and when hearing predatorial bat ultrasounds, crickets perform negative phonotaxis and fly away from the sound (Moiseff et al., 1978). Past experiments indicate that crickets have a dominant ear. When bat ultrasounds are presented between 20 degrees to the left or right either directly in front of the cricket or behind it, the animal is not able to localize the direction of the stimulus. Instead, the cricket will consistently turn to either the left or right direction, meaning that crickets have a dominant ear (Nolen and Hoy 1986).

The auditory organs of the crickets are located on either foreleg, and auditory afferents protrude into a central nervous system hub located inferior to the brain called the prothoracic ganglion. In healthy crickets, auditory neurons from both sides of the body stay on their respective side within the prothoracic ganglion as they extend their projections into the brain. When a cricket is deafferented (meaning it's foreleg is cut off, and thus its auditory organs as well), however, a compensatory neuroplasticity phenomenon occurs. Neuroplasticity refers to the reorganization of neurons, in this case after injury. Acutely post deafferentation, the cricket loses the bilateral connection from the ear to the brain, losing the ability to localize sound sources. Through an unknown mechanism, the auditory neurons from the deafferented side are able to cross the previously respected midline in the prothoracic ganglion and make synapses with the contralateral side (Fischer et al., 2018). Thus, while auditory inputs are only received from the intact ear, axonal projections proceed up both sides of the prothoracic ganglion into the brain for processing and potentially localization.

Experiments this summer aimed to figure out why the compensatory plasticity occurs and how it is beneficial to the survival of crickets. The experimental question was studied through a behavioral lens, characterizing the flight behavior of crickets in novel ways and evaluating whether the cricket regains localization abilities after the auditory system neuroplasticity occurs. I started analysis on past behavior experiments done in the Horch Lab using DeepLabCut machine learning software. I also began a novel experiment analyzing how the negative phonotactic behavior of crickets changed after they were deafferented while simultaneously comparing behavior in response to bat echolocation calls from different source locations. Crickets were isolated as 7th instars and flown 5-10 days into adulthood. They were flown once to find their dominant ear, which was then deafferented and crickets were flown every couple days after. We have started analyzing these videos with DeepLabCut, and this quantitative analysis is still ongoing.

Preliminary results from the past experiments have suggested that crickets might be regaining their localization abilities, as turn angles in response to stimuli have shown to be less extreme post deafferentation, especially in female crickets. This analysis will help us to further characterize and understand the special plasticity which crickets undergo.

Faculty Mentor: Hadley Horch, Ph.D.

Funded by the Student Faculty Research Grant Fellowship supported by the IDeA Networks of Biomedical Research Excellence (INBRE) National Institutes of Health award

References:

- Fischer, H. P., Pascual, M. G., Jimenez, S. I., Michaelson, D. A., Joncas, C. T., Quenzer, E. D., Christie, A. E., & Horch, H. W. (2018). De novo assembly of a transcriptome for the cricket *Gryllus bimaculatus* prothoracic ganglion: An invertebrate model for investigating adult central nervous system compensatory plasticity. *PloS one*, *13*(7), e0199070.
- Moiseff A, Pollack GS, Hoy RR. 1978. Steering responses of flying crickets to sound and ultrasound: Mate attraction and predator avoidance. *Proc Natl Acad Sci U S A*. *75*(8):4052-4056.
- Nolen TG, Hoy RR. 1986. Phonotaxis in flying crickets. I. Attraction to the calling song and avoidance of bat-like ultrasound are discrete behaviors. *J Comp Physiol A*. *159*(4):423- 439.