

## Explore the space: the behavioral effects of semaphorin-induced neuroplasticity in the nervous system of *Gryllus bimaculatus*

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Scholars have long examined the phonotactic behavioral response of crickets induced by sudden neurological changes resulting from sound stimuli. This research has led neuroethologists (scientists studying animal behavior) to an intensive exploration of the varying functions of the cricket auditory system. The relevant literature has elucidated the two integral functions of crickets' acoustic response necessary for their survival: sexual partner identification and avoidance of predators (Huber et al., 1989). The predator-prey interactions between bats and crickets have shaped the evolutionary adaptations forming the unique auditory system of crickets. Observational studies have concluded that crickets typically initiate flying activity at night and are preyed upon by bats (Ulagaraj, 1975; Jacobs & Bastian, 2016). While hunting, bats utilize echolocation via high-frequency ultrasonic sounds to identify the location of crickets in complete darkness (Jacobs & Bastian, 2016). In response to the high-frequency pulses generated by bats, crickets turn away from the sound stimuli in an attempt to fly away from predators (Moiseff et al., 1978). This behavioral response is known to be negative phonotaxis.

To produce effective behavior, the cricket auditory system displays rare neuronal regeneration in adults that allows them to recover their behavioral response post-injury. Prior to injury, cricket auditory neurons are confined to one side of the prothoracic ganglia (PTG) midline, sending auditory signals from one side of the body to the same side of the brain. Crickets that are deafferented—removing the foreleg containing the cricket auditory organs—lose synaptic connections that allow for the relay of signals from the ear to the brain (Horch et al., 2011). This neural degeneration significantly impairs neural communication, affecting crickets' behavioral response and survival. However, past research in the Horch lab has identified the *semaphorin1a.2* (*sema1a.2*) protein as a possible molecule involved in the regrowth of neurons across the midline post-injury, which in turn helps crickets recover their behavioral response.

This project sought to correlate the axon guidance nature of *sema1a.2* with a unique cricket behavioral response to predatorial sound stimuli. Over the course of this summer, I worked to analyze extensive past data obtained in the Horch Lab. Crickets analyzed in this data were 7<sup>th</sup> instar larval stage crickets injected with double-stranded RNA (dsRNA) targeted to eliminate *sema1a.2* mRNA transcripts. Following injection, crickets were isolated until reaching adulthood, where they were then flown and presented with five sound stimuli between 7 and 10 days later. Each of the stimuli differed in sound frequency, sound intensity, and temporal pattern, attempting to reach the boundaries of the cricket behavioral feature space. Each cricket was presented with all five sound stimuli in random order, and their behavior was tracked using DeepLabCut (DLC) machine learning software. Finally, this summer, I analyzed nearly 500 novel videos using a developed ResNet-50 trained DLC network, and the results were computed by a modified R pipeline to visualize and categorize cricket movement.

Based on prior anatomical research suggesting increased sprouting of the Ascending Neuron-2 dendrites across the PTG midline in the presence of *sema1a.2* dsRNA knockdowns, I hypothesized that the injection of these same knockdowns would result in a more precise behavioral response to predatorial sound stimuli. While the analysis of this project is currently ongoing, preliminary results show the presence of particular behavioral trends of crickets in response to each sound stimulus individually. For instance, there are ample crickets displaying precise and acute negative phonotactic behavior in response to stimulus01, stimulus02, stimulus04, and stimulus05. These crickets display high variability of their tracked body parts and a change in the mean abdominal position only when the sound stimulus is present. On the contrary, these same stimuli produce more sporadic cricket movement where there is high variability and unidentifiable mean abdominal angle trends throughout a particular experiment. Moving forward, I hope to compare *sema1a.2* dsRNA injected crickets to un-injected crickets, within and between each sound stimulus. This analysis will help us understand the involvement of *sema1a.2* in cricket behavior as well as developing a 3-dimensional feature space showing the boundaries of sound stimulus parameters that elicit negative phonotaxis.

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## Literature Cited

- Jacobs DS, Bastian A. Chapter 2; Bat Echolocation: Adaptations for Prey Detection and Capture. In: Predator-prey interactions: Co-evolution between bats and their prey. Cham: Springer International Publishing; 2016. p. 13–29.
- Horch, H.W., et al., *Bilateral consequences of chronic unilateral deafferentation in the auditory system of the cricket Gryllus bimaculatus*. Dev Neurosci, 2011. **33**(1): p. 21-37.
- Huber F, M.T., Loher W (eds), *Cricket behavior and neurobiology*. Comstock Pub Associates, Ithaca, 1989.
- Moiseff A, Pollack GS, Hoy RR. Steering responses of flying crickets to sound and ultrasound: Mate attraction and predator avoidance. Proceedings of the National Academy of Sciences. 1978;75(8):4052–4056. doi:10.1073/pnas.75.8.4052
- Ulagaraj SM. Mole crickets: Ecology, behavior, and dispersal flight (Orthoptera: Gryllotalpidae:Scapteriscus)23. Environmental Entomology. 1975;4(2):265–273. doi:10.1093/ee/4.2.265