Effects of Compensatory Neuroplasticity on the Negative Phonotactic Capabilities of the Adult Cricket, *Gryllus Bimaculatus*

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The auditory system of crickets is particularly important for their survival, as it plays a central role in the avoidance of predators and identification of sexual partners. While most crickets move towards a mating call, consisting of a low-frequency tone (3-10 kHz) produced by a male cricket, all crickets will turn away from a predatory call from bats, a high-frequency chirp (>18 kHz). This turning away is known as negative phonotaxis, and is characterized by a shift of the head, antennae, legs, and abdomen away from the stimulus (May et al., 1988). The need for effective identification and localization of auditory stimuli has led to the development of a highly sensitive auditory nervous system, which displays a remarkable capacity to respond to injury.

A normally functioning auditory system in the cricket is constructed to allow for the precise analysis of the sounds the cricket encounters in its environment, delineating the sounds by frequency, timing, pattern, and source location. To identify the location of the sound, crickets compare the intensity of the auditory stimulus between their auditory organs on opposite forelegs, and thus both ears are necessary for the accurate localization of sound (Moiseff et al., 1978). The auditory stimuli are received by tympanal membranes, converted into electrical signals by the auditory organs, and then conveyed to the central nervous system (CNS). In a normal cricket, the auditory inputs originating from the right foreleg will arrive at CNS neurons which remain on the right side of the midline, and vice versa. When an adult cricket's leg is cut off and auditory input is lost for half the auditory system, the central auditory neurons which no longer receive input (deafferented) cross the midline of the CNS in an organized manner and form novel connections with the auditory neurons from the opposite ear (Hoy & Nolen, 1985). This level of compensatory neuronal plasticity is unusual at an adult level. While molecular and physiological research in the Horch Lab seeks to elucidate the mechanisms of this plasticity (the "how"), projects exploring cricket behavior via their negative phonotactic responses seek to understand the function of this plasticity and its implications for cricket survival (the "why").

One project explored this summer sought to apply modern machine learning tools to an experiment conducted from 2015-2016 in the Horch Lab. This experiment sought to elucidate the extent to which the cricket can restore its sound localization capabilities post-deafferentation by measuring the turn angle of the cricket before deafferentation and at various stages after deafferentation. Crickets were tethered to a screw by the back of their neck (pronotum), and a gentle airflow was blown onto them to induce flight. Ultrasonic stimuli were then presented to the cricket from either side, and their responses were videoed. These "flights" were conducted before deafferenting the cricket, immediately after deafferentation, and periodically for up to 20 days post-deafferentation. While initial data analysis was conducted in 2016, the method employed at the time was very inefficient as it required manually tracking the turn angle of crickets in each frame. We trained a deep neural network, DeepLabCut (DLC), to track the position of the cricket, and employed an R pipeline created in conjunction with Professor Jack O'Brien to identify the turn angle. Results from this experiment indicated that some female crickets (insignificantly but observably) recovered the ability to respond appropriately to an ultrasonic stimulus, while male crickets did not.

An additional experiment we conducted this summer involved presenting the auditory stimuli to the crickets from different angles. Past research had indicated that normal crickets are unable to localize sound within ±20° (angle of uncertainty), and thus within that range they consistently turn away from their "dominant ear" (Nolen & Hoy, 1986). Over the course of one trial with each cricket, the speakers were placed in 11 different locations. The locations were in the cricket's horizontal plane (level with the cricket) at angles of 0° (straight ahead) to ±90° with respect to the longitudinal body axis. We again recorded the responses of the cricket before deafferentation and periodically for up to 20 days post-deafferentation. We anticipated that in normal crickets, the angle of sound origin would impact the turn angle of the cricket (a graded response). We also anticipated that, as time increased post-deafferentation, within the angle of uncertainty the crickets would turn away from their deafferented side, where their dominant ear was. The complete analysis is ongoing, but qualitatively the recovery of the turn towards the dominant ear did not occur.

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