Analyzing new experimental methods to quantify negative phonotactic responses before and after injury to *Gryllus bimaculatus* CNS

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Abstract

Plasticity in the central nervous system (CNS) is presumed to be crucial for recovery after injury to the system. Adult Mediterranean field crickets, *Gryllus bimaculatus,* have shown unique evidence of compensatory growth after injury to the auditory system. As a result, this model system is ideal for understanding regenerative mechanisms that can be adapted to other species. Our research aims to construct and modify testing methods to better analyze the negative phonotactic response of adult crickets before and after deafferentation of auditory nerves. By comparing two video-tracking software, Tracker and Ethovision XT 14, we are closer to finetuning our experimental design. We also developed a new assay to quantify the percentage of correct turns away from bat ultrasounds. This experiment elucidates distinct behavioral patterns of good turning crickets after deafferentation. Furthermore, these results help us identify a model for testing negative phonotactic responses that we can apply to single-cricket analysis in the future.

Project Goals

The prothoracic ganglion, a cluster of cells, abides by a chemical midline in the *G. bimaculatus* CNS. This midline restricts auditory signals from the ear to the same side of the midline. After deafferentation of auditory nerves in the ganglion, compensatory growth across the midline is observed (Pfister et al., 2012). Although the physiological and anatomical underpinnings of said compensatory growth are being investigated, the behavioral consequences are still largely unknown. Research has shown that acoustically competent crickets are more likely to survive than deaf crickets (Hoy et al., 1985). One-eared crickets that have regenerated their legs have shown more accurate tracking than animals with immediate foreleg amputation (Schildberger et al., 1986). This suggests that the compensatory growth yields functional synapses and could be advantageous to phonotactic behaviors.

Previously, the Horch lab has developed a unique experimental setup to record cricket's orientation in flight. Using this design, the main goals of our project are to (1) continue to construct and modify the assays for testing negative phonotactic response in flying crickets (2) to analyze the negative phonotactic behaviors observed in the summer. To achieve our first goal, we compare two video-tracking software. Our second analysis uses a new assay to quantify phonotactic response after foreleg amputation.

Materials and Methods

Animals

Gryllus bimaculatus obtained from an inbred colony at the Hoy Lab (Cornell U, Ithaca, NY) were raised in the laboratory at 28°C with 60-70% humidity, on a 12hr-on, 12hr-off light cycle, with bountiful access to cat food and water. We isolated 7th and 8th instar adults, females and males in separate cohort bins. Crickets are isolated into individual cages with food, water, and hiding crates after becoming adults Crickets were tested 7-15 days after becoming adults. Crickets tested in the sochophase are indicated in the results.

Behavioral Set Up

When in flight position, crickets contract their abdomen. We observed (via video recording) the abdominal alignment as a phonotactic response. The crickets were tested in a wooden box with speakers. Crickets were attached to a flexible swivel with wax on the thorax and placed proximal side uppermost and the lateral facing a fan simulating wind at 2.4m/s. Sound stimuli were introduced to the speakers positioned 90 degrees on right and 180 degrees in front of the suspended crickets. Speakers independently receive signals from amplifiers connected to a computer source. Using SVL, an ultrasound detecting software, sound stimuli ranged from 65dB to 70dB.

Deafferentation Experiments

Baseline (before deafferentation) negative phonotactic responses of eight adult crickets were measured, males and females alike. After baseline tests, crickets undergo right foreleg amputation. Negative phonotactic responses are measured 5 minutes after acute deafferentation. Same phonotactic responses were measured 3 days post- deafferentation. All experiments were conducted using a sound file of 50ms pulses with 2s inter-pulse silence repeated 15 times. Sound stimuli were played from one speaker at a time.

Assay

To quantify the behavioral tests from the summer, we created an assay with hopes of recreating Nolen and Hoy., 1986 results. For each cricket, we marked the first turn after the introduction of ultrasound. Consequent turns must reach the initial turn bounds to be considered a phonotactic response. We considered “out of bounds” to be double the distance of the first turn on cricket’s y-axis. The orientation was adjusted to make sure the tip of the abdomen was at the center of initial turn bounds.

Results

Comparing Tracker and Ethovision

In the previous years, Horch lab has used Tracker as the primary video-analysis software. Tracker is open software that can track an individual point throughout an entire video. In Tracker, we can measure the maximum angle of turn by tracking a point on the head (center of the pronotum) of the cricket and another point on the abdomen. In order to account for slight movements, these points are tracked to be at the exact same position on the cricket using the “autotracker” setting in the application. The protractor tool calculated the angular values of the cricket’s abdomen in relation to the pronotum from the starting position till the end. Although Tracker can produce very precise traces, it is a very time consuming and unreliable process.

On the other hand, Ethovision XT 14 is a video -tracking software designed to analyze behavioral activity of larger animals like rodents and goldfish. The user can change experimental settings like testing arena, detection settings, collection criteria etc. In the detection settings, we select module more suitable for other animals like crickets. We can attempt to track the whole cricket during flight by designing an optimum detection criterion. The software uses the contours of the animal to define the head and tail which is the cricket’s pronotum and abdomen tip respectively. We conducted an experiment with the same cricket and compared the data produced by Ethovision XT 14 and Tracker. We hoped that data collected from Ethovision XT 14 would be similar to the “ideal” trace from Tracker, figure 3A. However, while collecting data, the head and tail point does not remain fixed. This is a problem because the software is not tracking the necessary points on the cricket during the entire duration of the flight. This is evident in figure 3B with a jumbled trace of the phonotactic response. Our results show no statistical significance to the turn angles detected by Ethovision XT 14. However, support from Ethovision XT 14 creators and the adaptability of the program holds promise.

Deafferentation Tests

These experiments aim to grasp a general understanding of the patterns of negative phonotactic response after deafferentation. Figure 4 shows the percentage of turns away from the sound stimuli. During baseline testing, crickets with >70% correct turns were classified as “good turners.” Out of the eight crickets tested only three were good turners. After foreleg amputation, response to stimuli varied. However, in the acute and 3-day post experiments, the crickets always turned toward stimuli- if they turned at all. Figure 4B shows one pattern of response where there is no response to ultrasound even days after deafferentation (data not shown). Figure 4A & C show that there is a preference for position of the speakers after deafferentation. Figure 4C shows an increase in incorrect turns 3-days after deafferentation. Although the information received from this qualitative assay is worth exploring, we need to develop a way to better quantify the different phonotactic responses our results show.

In the future, we hope to begin automated analysis of single crickets in order to fully understand the compensatory responses in an individual cricket. This analysis can elucidate plasticity mechanisms in adult CNS that could be applied to other species including humans.

Figures and Charts

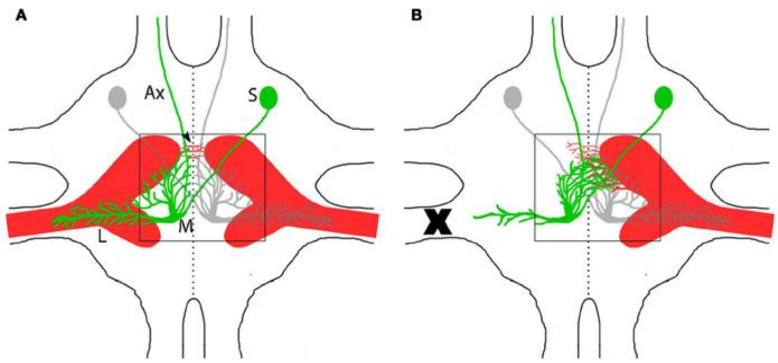


Figure 1: Anatomical evidence of novel midline crossing days after nerve injury in CNS of the adult Mediterranean field cricket, *Gryllus bimaculatus.* Adapted from Pfister et al., 2012.

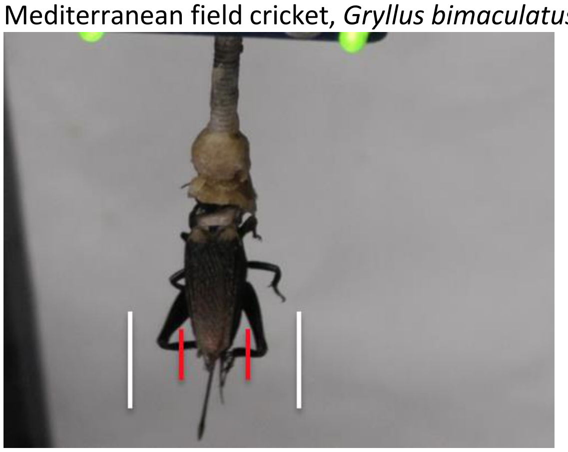
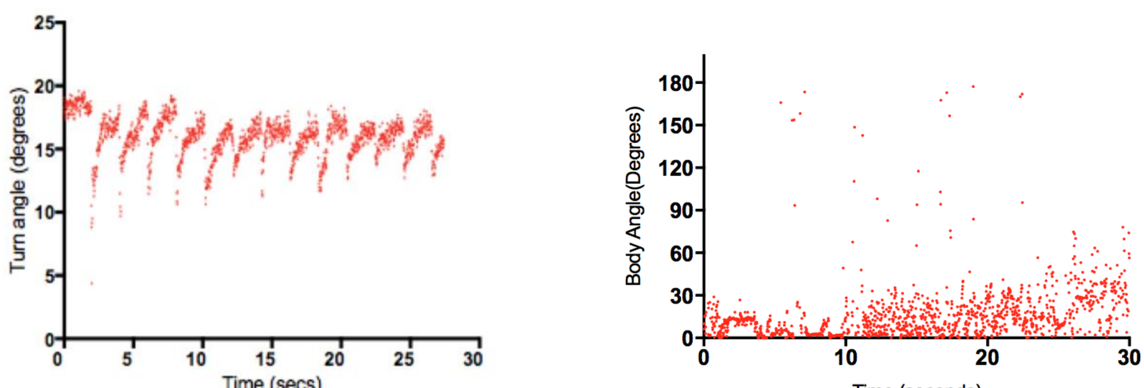
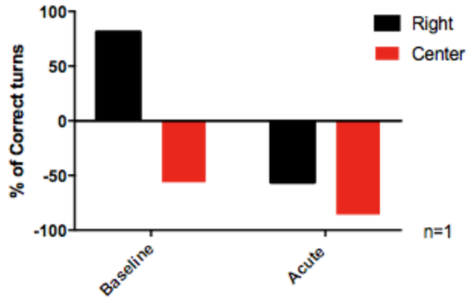


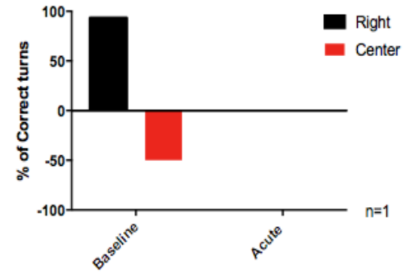
Figure 2. Still frame of a brown morph adult female cricket during flight. Red lines represent the "initial turn bounds." White lines represent "out of bound."



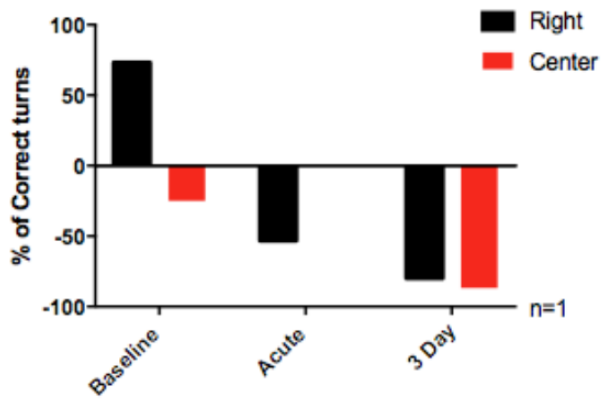
A. B.

Figure 3. (A) Trace of a cricket's turn angle using Tracker, this is an example of ideal negative phonotactic response. **(B)** Trace of a cricket's turn angle using Ethovision XT 14





B.



C

Figure 4. Percentage of correct turns for good turners. Individual variability in negative phonotactic responses.

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