Behavioral Plasticity in the Cricket (*Gryllus bimaculatus*)

Researcher: Rahul Prabhu, Advisor: Hadley Horch
Bowdoin College
Department of Biology

The auditory system of the cricket presents a particularly simple stimulus-response model of phonotaxis – the movement of an organism in response to sound – due to its binary nature (1,2). Cricket phonotaxis may be negative, in response to predatory ultrasonic (high frequency) stimuli by bats or positive, in response to species specific calling songs by male crickets. In terms of neural circuitry, the cricket has a mirror image set of two interneurons to process these sounds, one set to process sounds from the left side and one for the right side. AN1 neurons respond best to low frequency mating calls and AN2 neurons respond best to high frequency predatory sounds (3). Previous work had shown that when one side of the cricket's auditory system is removed, the interneurons on the damaged side re-grow towards the intact side and make specific connections (4,5,6). This ability is known as compensatory plasticity. It is unclear, however, if this ability gives the cricket a behavioral advantage, that is, if it allows for behavioral plasticity.

The specific goal of this research was to characterize the cricket's negative phonotactic flight behavior in response to high frequency predatory ultrasound and the subsequent effect of injury on this behavior. Pure tone sounds varying in frequency (pitch), temporal pattern and amplitude (loudness) are used to trace the full range of behavior in normal crickets and shifts in these parameters are expected in crickets with one side of their auditory systems removed. This work exists within a larger project in the Horch Lab to compare behavioral data, animal-by-animal, to morphology (form, shape, and structure of the nervous system) and physiology (activity of neurons) so as to characterize the cricket's compensatory plasticity as a form of "recovery" post injury.

To conduct our experiments, we tethered adult crickets by their pronotum (anterior thorax) to a metal screw that was suspended from the top of an experimental chamber. The chamber is sound insulated on all sides except the front opening of the box, from which we record videos. Furthermore, the box is not provided with any light apart from the ambient light needed for video recording, as the cricket's bat avoidance behavior is predominantly nocturnal. When the cricket gets into flight position – its forelegs tucked in, abdomen and hindlegs extended – we play a predatory sound stimulus. These sound stimuli were designed on Audacity to capture and mimic the full range of sounds the cricket might respond to – from low intensity to high intensity, a range of ultrasonic frequencies, rapid chirps, and long tones. One sound stimulus played to the cricket even replicates the specific sound pattern of echolocating brown bats (7).

This summer, we recorded the flight of 20 crickets. Additionally, we spent much of our time learning, troubleshooting, and rectifying our approach to the analysis of these videos in DeepLabCut. DeepLabCut is an artificial intelligence program that was first trained to estimate human hand poses. This residual network of intelligence can now be trained to identify and track the movement of organisms and their specific parts. A previous student trained DeepLabCut to correctly identify the cricket's head, thorax, abdomen, and forelegs in the videos as well as a sound bar that tracked the onset of the stimulus within the video itself. We found significant errors and noise in DeepLabCut's tracking of the sound bar and worked towards eliminating this noise, through a process of re-training. This involves the manual labelling of around 300 frames and running these through 200,000 iterations until the network is 'smarter' – the latter done on Bowdoin's High Performance Computing (HPC) grid. The output from the program is a spreadsheet containing cartesian coordinates of each labelled part of the cricket, which can then be analyzed statistically to quantify the behavioral effect of compensatory plasticity.

I thank Dr. Hadley Horch, lab technician Lisa Ledwidge, lab partner José Ávila and the Horch Lab team, Marko Melendy's animal care team, Dj Merrill, NASA Maine Space Grant Consortium for funding.

References

1. Popov, A. V. & Shuvalov, V. F. Phonotactic behavior of crickets. *J. Comp. Physiol.* **119**, 111–126 (1977).
2. Nolen, T. G. & Hoy, R. R. Phonotaxis in flying crickets. *J. Comp. Physiol.* **159**, 423–439 (1986).
3. Wohlers, D. W. & Huber, F. Processing of sound signals by six types of neurons in the prothoracic ganglion of the cricket,Gryllus campestris L. *J. Comp. Physiol.* **146**, 161–173 (1982).
4. Hoy, R. R., Nolen, T. G. & Casaday, G. C. Dendritic sprouting and compensatory synaptogenesis in an identified interneuron follow auditory deprivation in a cricket. *Proc Natl Acad Sci U S A* **82**, 7772–7776 (1985).
5. Brodfuehrer, P. D. & Hoy, R. R. Effect of auditory deafferentation on the synaptic connectivity of a pair of identified interneurons in adult field crickets. *Journal of Neurobiology* **19**, 17–38 (1988).
6. Horch, H. W. *et al.* Bilateral Consequences of Chronic Unilateral Deafferentation in the Auditory System of the Cricket Gryllus bimaculatus. *DNE* **33**, 21–37 (2011).
7. Hiryu, S., Bates, M. E., Simmons, J. A. & Riquimaroux, H. FM echolocating bats shift frequencies to avoid broadcast–echo ambiguity in clutter. *PNAS* **107**, 7048–7053 (2010).