The Physiological Implications of Sexually Dimorphic Auditory Interneuron Recovery In *Gryllus Bimaculatus*

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Abstract

After loss of an ear, a cricket's auditory cells sprout across the midline of its body and form synapses with the auditory neurons from the uninjured ear. The extent of this growth is sexually dimorphic, but whether there are functional implications of these differences is unknown. After unilateral deafferentation (the removal of one ear) female crickets structurally express rapid dendritic sprouting that plateaus after three days, whereas in males dendritic sprouting occurs at a linear rate, reaching lengths double those found in females on average. In order to functionally characterize this sexual dimorphism, an electrophysiological approach was used. Hook electrodes were positioned around the neck connectives of the cricket, a sound sweep that included sounds of different frequencies and intensities was played, and neurological responses from the cricket's auditory neuron (AN-2) were recorded. Preliminary tests of function have shown there is no sexual dimorphism three days after deafferentation. Dimorphic differences from 18-20 days post deafferentation have not yet been assessed. Female unresponsiveness to sound stimuli has prompted focus on reducing the effects hormones may have on female AN-2. Female crickets were thus raised in isolation 15 days after the loss of an ear, to lengthen the period of time in which females are responsive to sound. Female crickets 15 days post deafferentation were still responsive, however, slightly less responsive than an intact female cricket. Generally, the deafferented side of a cricket had less intense responses than the intact side. Further data needs to be collected in order to characterize the female cricket's response.

Project Objectives

Neuroplasticity is the brains unique ability to physically change. This can be crucial to the organism's ability to retain information, learn new concepts, and most importantly repair the nervous system if an injury occurs. In most adult species neuroplasticity does not occur, however, the adult cricket *(Gryllus bimaculatus)* is one exception.

Cricket's ears are located on their elbows. Beyond this, a cricket's auditory system functions similarly to a human's. A sound stimulus hits their eardrums and is converted into electrical signals that are sent through a group of auditory neurons that travel up through the leg. These neurons then pass the signals to an auditory interneuron (a part of the CNS located in the thorax). This auditory interneuron (AN-2) then passes the electrical signals to the cricket's brain, where the cricket decides how to respond. This can be a matter of life or death for the cricket, as they could be avoiding the call of a predator, or following the call of a mate.

When a cricket loses one of its ears due to injury, the neurons in charge of receiving and passing along auditory information will cross the midline of the cricket's body to form new connections with the neurons from the uninjured ear (Schmitz, 1989; Brodfuehrer and Hoy, 1988; Pfister et al., 2013). This is called compensatory growth. This compensatory growth has structurally been well characterized as sexually dimorphic (Pfister et al., 2013). It has been discovered that in female crickets interneurons grow rapidly, plateau after 3 days, and then stop growing. However, in males this regrowth occurs at a more linear rate until the end of the cricket's life. Little is known about how this recovery occurs, why it occurs, and how it functionally impacts male and female crickets.

The focus of this project is to answer the question: What are the functional implications of the compensatory growth of auditory interneurons following injury to one ear and Is there a difference between sexes?

One theory from Pfister et al. (2013) proposes that females are spending more time in flight using their hearing to avoid predators while trying to find their mates who are singing mating calls on the ground. Therefore, in order to survive and reproduce females may need to recover faster. Later in life, female crickets may be more focused on egg production and therefore hearing may not be a necessity.

Previous work from the Horch lab showed that 18-20 day old female crickets across all conditions (injured and uninjured) were unresponsive to sound stimuli. Since these female crickets all had swollen abdomens, indicative of egg storage it is assumed that hormones could be playing a role in female unresponsiveness and AN-2 sensitivity. Using an electrophysiological approach, we aim to examine responses of female crickets raised in isolation 15 days after the loss of an ear. We hope the isolation will lengthen the period of time over which females are responsive to sound.

A past lab member also found that the best electrophysiological recordings were taken in the middle of the night. In order to explore if there could be a relationship between the cricket circadian rhythm and AN-2 responsiveness a second cricket room was created where an opposite day/night cycle was occurring. In our original cricket room when it is the middle of the day for us it is the middle of the day for the crickets, in this new room when it is the middle of the day for us it is the middle of the night for the crickets. Therefore, crickets from both rooms we recorded from in order to see if there were any significant differences in the clarity of the recordings.

It was also an important objective to further troubleshoot a protocol for electrophysiological recordings. Although AN-2 has been described as easily identifiable in another species of cricket, this was not the case in our recordings from *G. bimaculatus* (Brodfeuhrer and Hoy, 1988; Friedlander, 2014). Electrical noise as well as spontaneous activity affects the clarity of recordings. Our focus has been on reducing this electrical noise and increasing response signal amplitude in order to achieve the best possible recordings.

Methodology Used

Animals

Male and female *G. bimaculatus* (Mediterranean field crickets) obtained from a colony at the Hoy Lab (Cornell U, Ithaca, NY) were kept on a 12l:12D cycle at 70-80% humidity and 20-25°C and fed dry cat food and water as needed. To prepare for dissection, crickets in each room were isolated by sex in cages before their final adult molt and deafferented between 2 days after molting to ensure their auditory system had developed. The deafferentation process was done by removing each cricket from their cage and severing their right prothoracic leg directly above the tympanal membrane and returning them to their respective cages. The primary dataset was obtained from crickets at fifteen days following deafferentation.

Dissection

Animals were anesthetized in ice for approximately 20 minutes in order to stop them from moving. The wings and all of the legs except for the two foremost ones were removed. The cricket was then placed ventral side up on a magnetic block using wax (50% cello rosin, 50% beeswax). In order to prep for electrophysiological recording the cricket was waxed into a position that is adopted by crickets during flight. This is due to the possibility that sound stimulus can be processed depending on the context of a scenario (Nolen and Hoy, 1984; Hofstede et al.,2009). Once the cricket's legs are waxed so that the tibia is touching the femur and a well is formed between the coxa and the cricket's mouth parts, the cricket's mouth is then covered in wax to prevent air consumption. Lastly, the cricket is checked to ensure there is no damage to any auditory organs. After waxing is complete the cricket's neck is opened to remove the esophagus and trachea and expose the neck connectives. O'Shea-Adams Ringers saline was used to keep the connectives clean and easy to see (140 mM NaCl, 5mM KCl, 7 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM TES, 4 mM $NaHCO_3$, 5mM Trehalose, pH 7.3). The thin sheath surrounding the connectives

was also removed in order to achieve better electrophysiological recordings. This was done by peeling the sheath from the top of the neck to the bottom using a desheathing tool.

Physiological Recording

Electrophysiological recordings were obtained by placing two silver hook electrodes beneath the neck connectives and then isolating them in petroleum jelly. After placement of the electrodes the cervical and promesothoracic connectives were cut in order to minimize electrical activity that was not from AN-2. Responses were amplified with an A-M Systems differential amplifier Model 1700 and recorded on a PC using a CED Micro 1401 board and the computer software Spike 2, version 7.17.

Sound Stimuli

During recordings, a sound sweep was played in order to elicit an auditory response from the cricket. This sweep was generated using a custom SciLab4.1 script (Digiteo; Le Chesnay Cedex, France). This sweep contained frequency pulses at 3, 5, 7, 8, 10, 12, 15, 18, 20, and 22kHz. To encompass both the frequencies of mating calls and bat calls. Each of these frequency pulses consisted of sound intensities from 40-95db in 5db increments. Each pulse lasted 35 ms, with a rise/fall time of 5ms and an interpulse period of one second, and each intensity was played in triplicate. The sound sweep was played through a speaker (Motorola/CTS piezoelectric tweeters, KSN1165A; frequency response 2-30khz). The area in which recordings were performed was lined with egg carton mattress foam to prevent interfering noise and bouncing sound waves.

Data Analysis

Analysis was conducted by extracting the number and latency period of action potentials that were produced in response to sound stimuli from Spike2 software (Cambridge electronic Design; Cambridge, UK) and performed analysis using Prism 7 software. The number of action potentials that occurred in response to sound stimuli was used as an indicator of the strength of the response. Responses fired beyond 100ms after stimulus onset were excluded. Strength tuning curves were constructed by previous Horch lab members by averaging the number of action potentials that occurred in response to three 85db pulses at each sound frequency. The intensity of 85db was selected because it was well above the threshold for all conditions and consistently elicited responses from intact connectives. The frequency that produced the strongest response was defined as 15kHz.

Results Obtained

Results indicate that 15 day post deafferentation females that were raised in isolation from males, do respond to sound stimuli at an intensity of 85db and a frequency of 15kHz. However, with a sample size of one, this data cannot be used to form any substantial conclusions. From this we can only predict we are heading in the right direction towards getting a substantial number of responses from 15 day post deafferentation female crickets. The deafferentation side of the female cricket was slightly less responsive than the intact side (Figure 1.).

Significance and Interpretation of Results

Although sample size was small (N=2) and thus unable to provide any accurate conclusions, 15 day females responded to a 15kHz, 85db stimulus. Results show that a 15 day post deafferentation female generally had a lower action potential spike count than a control 15 day female. The deafferented side of the females also displayed a lower spike count compared to the intact side.

Sample size was the biggest limitation, followed by recording quality, and dissection quality. Sample size was so small that no accurate conclusions could be made. This also played a role in limiting analysis of the circadian rhythm on female unresponsiveness. Recording and dissection quality also take a substantial time to perfect in order to ensure human error is not a factor.

Ideally, future directions will be focused towards gathering a larger sample size. Recording and dissection protocol will also be fine-tuned. In order to further characterize a functional sexual dimorphism, female cricket responses will be focused on and then compared to 15 day post deafferentation males.

Figures/Charts

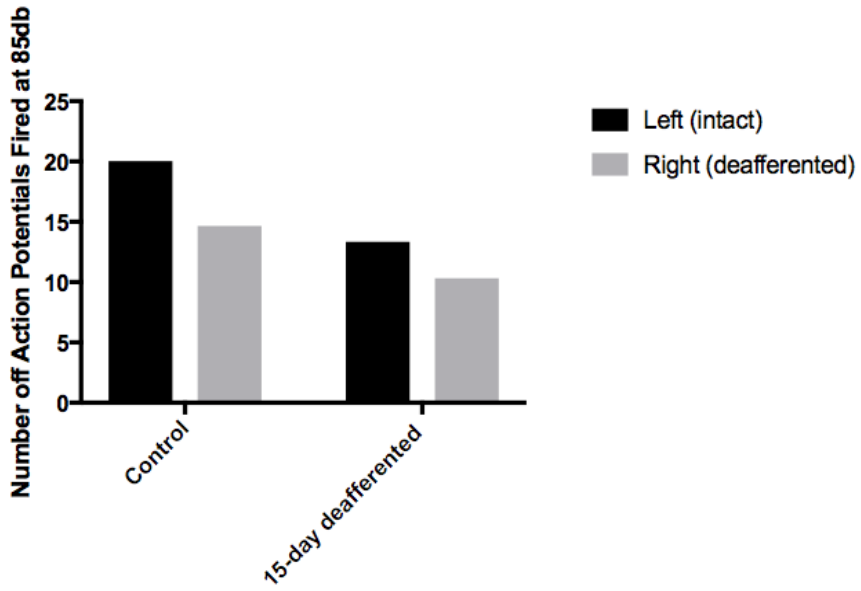

Figure 1. AN-2 Recovery, quantified as the number of action potentials fired in response to a 15kHz, 85db stimulus. Sound stimuli was played in triplicate ipsilateral to the intact tympanal membrane, and responses from AN-2 were averaged in a 15 day, control, female (n=1) as well as a 15 day post deafferentation female (n=1). In each condition, the side with the intact connective displayed a higher spike count than the deafferented side. The control female also exhibited a higher spike count than the 15 day post deafferentation female.

Acknowledgements and References

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