Physiological implications of sexually dimorphic auditory interneuron recovery in *Gryllus bimaculatus*

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Abstract

After injury, the auditory system of the cricket undergoes a compensatory growth process in which neurons cross the midline of the cricket's body and form synapses onto the auditory neuron of the undamaged ear. Morphologically, this process has been well studied and shown to be sexually dimorphic, but it has yet to be established if these morphological changes have functional implications. Preliminary tests of function show that there are no functional differences at 3-days post deafferentation between male and female crickets. Differences 18-20 days post deafferentation have yet to be assessed due to female unresponsiveness to all sounds stimuli. Using an electrophysiological approach, I aim to further examine the functional implications of morphological differences seen in previous research by characterizing the neuronal response of female crickets 15 days post deafferentation who have been raised in isolation from males. Through this I hope to avoid any hormonal changes that occur in the female cricket with fertilization of their eggs as well as test their responses earlier in their life, hoping to increases the amount of time females respond to sound.

Project Objectives

Neuroplasticity is lasting change in the nervous system in response to injury or damage. This process is crucial in development; however, with age most species' central nervous systems lose this incredible ability to adapt. The cricket species, *Gryllus Bimaculatus,* is unique because it's nervous system exhibits an uncommon level of plasticity independent of age. The sensory nervous system maintains a high level of structural plasticity, visible in response to auditory system injury.

The high level of plasticity in adult cricket auditory systems reflects how crucial it is for survival. Crickets rely on their auditory systems for predator avoidance and mating (Pfister et al. 2013). This system begins at the foreleg of a cricket, when sound waves hit the tympanal membrane which is located on the dorsal side of the elbows of each foreleg. When sound waves hit the tympanal membrane, vibrations are transferred into electrical energy and carried up the leg, into the body of the cricket through a bundle of neurons called Nerve 5. Nerve 5 forms connections with AN-2, another auditory nerve that brings auditory information to the brain. The connection between Nerve 5 and AN-2 occurs in a bundle of nerves called the prothoracic ganglion, located in the chest of the cricket. The auditory system of a cricket is a mirror image on both sides of the body; each Nerve 5 and AN-2 adhere to one side. AN-2 often approaches the midline but does not cross over to the contralateral side of the prothoracic ganglion. However, previous research has shown that when the foreleg of a cricket is removed, referred to as deafferentation, AN-2 ignores the midline and crosses to form connections with the intact Nerve 5 on the contralateral side of the prothoracic ganglion. Previous research has quantified structural changes through measurements of AN-2 growth across the midline after ear removal (Pfister et al. 2013. As expected, there is increased dendritic sprouting with more recovery time. Males and females have increase dendrite lengths as time increase following auditory system injury (Pfister et al. 2013). In addition to changes that occur with increased recovery time, this process is sexually dimorphic meaning there is a different rate of recovery for males and females. AN-2 sprouting across the midline occurs rapidly in female crickets and then plateaus after about 3 days. In males however, AN-2 growth follows a linear pattern, eventually reaching lengths double the length of female AN-2 dendrites (Pfister et al. 2013). While this process is shown to have structural sexual dimorphism, preliminary evidence shows that there is no functional sexual dimorphism at three days post-deafferentaion (Amano, 2017). Given structural data, increased recovery time should not only lead to increased response but male crickets should respond much better than female crickets. However, the sexual dimorphism of older

crickets has yet to be examined. Amano (2017) demonstrated no difference in AN-2 response at 3-days post deafferntaion but could not examine the difference between males and females at 18-20 days post deafferentaion due to an absence of response of AN-2 to all sound stimuli in females. Amano (2017) noted swollen abdomens, indicative of egg storage, in the 18-20 post deaffferentation females and suggested a potential role of hormones in female unresponsiveness. Hormonal changes within the female occur as she undergoes a life history trade off and begins to focus energy on reproduction over mate finding or predator avoidance (Zera and Cisper, 2001; Zera, Sall, and Grudzinski, 1997). For this reason, less time is spent in the air flying around leaving her exposed to predators and thus the importance of auditory input decreases.

The objectives of this project are to use an electrophysiological approach to further characterize the functional implications of the morphological differences seen in previous research. After learning the dissection, we examined the function of morphological changes by recording from the AN-2 nerves of male and female crickets 15- days post-deafferentation who were raised in isolation from the opposite sex. The aim of this project was to examine the role of hormones in female deafness, explored by raising crickets in isolation from the opposite sex and testing the responsivity of AN-2 at an earlier time point post deafferentaion. The number of action potentials produced in response to the stimulus and the timing of the action potentials were used to quantify the recovery and functionality of AN-2.

Methodology Used

Animals

Female and male Common Mediterranean field crickets, *Gryllus bimaculatus*, were acquired from an inbred colony at the Hoy Lab (Cornell U, Ithaca, NY) and housed in rooms at 70-80% humidity and 20-25°C on a 12L:12D cycle and fed commercial cat chow and water *ad libitum*. Housing conditions varied slightly from previous work (Amano, 2017) where crickets were housed at 40-60% humidity and 28°C. Additionally, daylight conditions were reversed so that it was dark for the crickets during the day to change the circadian rhythm of the crickets to increase responsivity of AN-2. Crickets were isolated as 7^{th} and 8^{th} instars until they molted into adults. They were further isolated for 17 days. Two days after their initial molt, crickets were temporarily removed from their cage for a deafferentation procedure where the right foreleg was cut at the tibiofemoral joint. A control procedure was performed, leaving the auditory system intact but subjecting the cricket to the stress of the procedure by severing the leg between the tarsus and claw.

Dissection

15-day post-deafferentation male and female, control and deafferented crickets were cooled for 20 minutes on ice to immobilize them. Following the removal of the cricket's wings, mesothoracic and metathoracic legs, crickets were positioned, using hot wax (50% cello rosin, 50% beeswax), on to a magnetic block ventral side up. Further immobilization was accomplished using wax to ground the coxa to the back of the head and the claw of the intact foreleg to the coxa, insuring the exposure of the tympannal membrane. Crickets were positioned to mimic their body position in flight because of the context dependent response of the auditory system to sound stimulus (Nolen and Hoy, 1984; Hofstede et al., 2009). Wax was then placed over the mouth of the cricket until air consumption was inhibited. After immobilization, the prothoracic plate, esophagus, trachea and gut were removed to expose the neck connectives. The neck cavity was filled with saline (140 mM NaCl, 5mM KCl, 7 mM CaCl2, 1 mM MgCl2, 5 mM TES, 4 mM NaHCO3, 5mM Trehalose, pH 7.3) and each connective was desheathed by peeling back the sheath from the lateral side of each connective to minimize damage to AN-2 located on the ventral medial quadrant of the neck connective.

Physiological recording and sound stimuli

An electrophysiological set up was used to record extracellularly from exposed neck connectives. Silver hook electrodes were placed so that AN-2 rested on each electrode, saline was removed and electrodes were isolated with petroleum jelly. To decrease ascending and descending spontaneous

activity, unrelated to AN-2 activity, neck connectives were cut at the head and between the mesosternite and metesternite chest. All recordings were performed in an egg carton mattress foam lined box to prevent interference of extraneous noise. AN-2 responsivity was measured in response to stimulated sound sweeps, created with SciLab4.1 scripts (Digiteo; Le Chesnay Cedex, France) and consisting of 3, 5, 7, 8, 10, 12, 15, 18, 20, and 22kHz frequencies. For every frequency, intensities of 5 db increments ranging from 40-95db were played in triplicate. Each pulse was 35ms with 1 sec interpulse period in between and increased and fell for 5ms. The sweep was played using speakers (Motorola/CTS piezoelectric tweeters, KSN1165A; frequency response 2-30 kHz). All responses were amplified using an A-M Systems differential AC amplifier Model 1700 (A-M Systems, Inc.; Carlsborg, WA) and recorded with a PC computer using a CED Micro 1401 board and the computer software Spike2, version 7.17 (Cambridge Electronic Design; Cambridge, UK).

Statistical Analysis

All data were recorded using Spike2 software (Cambridge Electronic Design; Cambridge, UK) and analyzed at 15kHz and 85db. Strength of AN-2 response was quantified by counting the number of action potentials that occurred in response to stimulus onset and lasting for no longer than 100ms. Latency was measured by determining the time difference between the onset of the stimulus and the beginning of the response. All data were averaged across the three 85db pulses and graphed using Prism 7 software.

Results Obtained

Our results indicate that 15-day post deafferentaion isolated females may be responsive to a sounds stimulus of 15 kHz and 85db; however, a sample size of one is far too small to make any conclusions regarding response and neuronal function. The response of the intact AN-2 of the deafferented female cricket appears to be generally stronger (Figure 1) with a shorter latency (Figure 2) than the right, deafferented side of the female deafferented cricket.

Significance and Interpretation of Results

A female cricket raised in isolation from males was generally responsive to sounds stimuli. This differed from previous research where female crickets, raised with males, were unresponsive to stimulus (Amano, 2017). However, a sample size of one is not sufficient to draw any conclusions. With a greater sample size that theoretically follows this trend, these results may eventually support the theory that hormones play a role in the absence of auditory system responsiveness of 18-20 day post-deafferentation female crickets.

While the current study may lend circumstantial support for the functional aspects of the compensatory growth process with a greater sample size, our current understanding of this process will be increased. Our study is limited by the sample size. In addition to this, our results are limited by the difficulty of the dissection procedure, specifically desheathing, which may cause damage to AN-2 and thus reduce responsivity. Finally, the noise of the recoding, from other spontaneous activity or electrical activity may limit our ability to exclusively analyze AN-2 response.

In future studies, we aim to increase our sample size in order to better explore the responsivity of isolated 15-day females, the timeline of functional sexual dimorphism and ultimately the functional aspects of compensatory growth. Additionally, we aim to further improve our desheathing technique and continue to troubleshoot ways to decrease extraneous electrical noise and spontaneous activity.

Figures/Charts

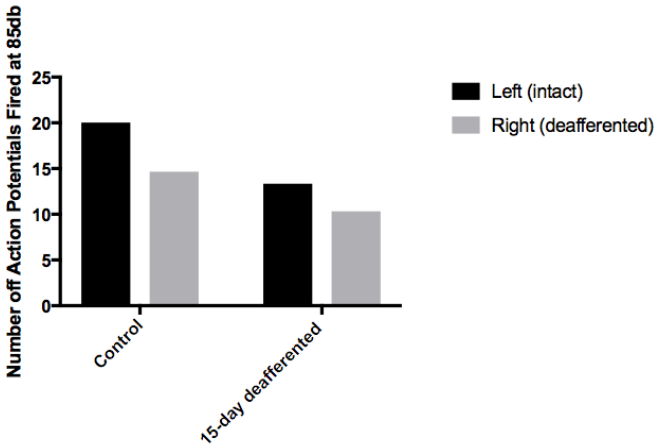

Figure 1. Number of action potentials fired by AN-2 in response to an 85db and 15kHz stimulus for a control (n=1) and a 15- day post deafferention (n=1) female cricket. The AN-2 response was averaged across three sounds pulses.

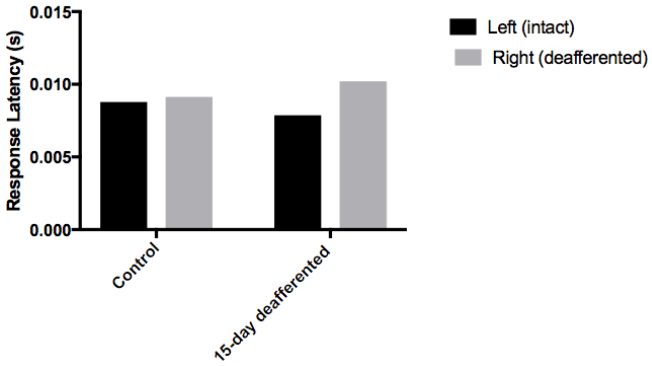

Figure 2. Latency of AN-2 response to an 85db and 15kHz stimulus for a control (n=1) and a 15- day post deafferention (n=1) female cricket. The AN-2 response was averaged across three 85 db sounds pulses.

Acknowledgements and References

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