Analyzing the molecular underpinnings of injury response in *Gryllus bimaculatus* CNS using single-cell RNAseq

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The central nervous system (CNS) has long been known to possess the remarkable ability to restructure and rearrange itself in processes like learning and memory formation. During development, the CNS has a much greater potential to take new forms and make new connections. The presence of growth factors and axonal guidance molecules direct this process and are also thought to play a role in the CNS injury response. Nonetheless, injury to the adult CNS often has devastating effects. Following injury to the CNS, dendritic retraction and eventual apoptosis can be expected, as there is no mechanism for recovery in many organisms, like mammals. In such cases, this mechanism is thought to be inactive after development. Organisms like insects, however, seem to retain this mechanism past development and through adulthood, and have demonstrated a quite remarkable ability to recover, at least partially, from injuries to the CNS. The present study focused on the Mediterranean field cricket, *Gryllus bimaculatus*, as a model for neural plasticity in the CNS, more specifically, in the auditory system. Crickets have their main auditory organ, the tympanal membrane, located below the joint of each foreleg. Auditory input passes through this membrane into Nerve 5, which runs along the leg and connects to a bundle of neurons of the CNS, known as the prothoracic ganglion. In the prothoracic ganglion, the auditory input is then relayed to two ascending neurons, AN1 and AN2, the auditory interneurons, which send signals up to the cricket's brain. The dendrites of the ascending neurons that make connections with Nerve 5 in the prothoracic ganglion typically respect the midline of the prothoracic ganglion. This guideline is hypothesized to be a molecular and/or chemical barrier with repulsive cues that prevent the dendrites from crossing over. Research on the cricket, however, has demonstrated that in the event of an injury to the cricket's auditory system, where auditory input is lost (known as deafferentation), the dendrites of the auditory interneurons begin to cross over the midline of the prothoracic ganglion and eventually form functional connections with the contralateral Nerve 5. This phenomenon results in some restored functionality, including the ability to respond appropriately to predatory signals, such as bat calls. Because the molecular mechanism for this injury response is still not very well understood, my study aimed to glean this mechanism through the study of individual cells in the prothoracic ganglion. The primary technique used in these experiments was single-cell RNAseq, in which RNA transcripts are isolated from individual cells, in this case AN2 cells, which are the neurons primarily involved in escape behavior mediated by the auditory system. Once these transcripts are extracted from the individual cells, they can be sent for sequencing, and with the results, a micro-scale transcriptome of each individual cell can be assembled. These small transcriptomes can be mined for key transcripts such as those of known developmental guidance cues, to help determine if the presence of certain guidance molecules may play a role in the remarkable example of neuroplasticity exhibited by the cricket's auditory system. The transcripts can also be mapped onto the full transcriptome of the cricket prothoracic ganglion, which was assembled by a previous member of the Horch lab, Harrison Fisher, with the goal of comparing those transcripts expressed in the individual cell with all those expressed in the prothoracic ganglion as a whole. Targeting specific cells allows for the detection of small changes in expression over time as the cricket auditory system recovers from injury. Changes in expression are tracked at three different time points after injury: 24 hours, 3 days, and 5 days. This summer in the Horch lab, I was able to extract RNA from control crickets which received an injury to the lower segment of the leg, the "foot", thus leaving the CNS uninjured, as well as from experimental crickets, which had one ear removed. Using Qubit Fluorometric Quantitation technology, I was able to confirm the presence of within-normal-range levels of RNA in my samples, which is the first step in assessing the quality of the RNA samples prior to sequencing. These samples were obtained from 3-day deafferented crickets. I will go on to collect samples from more 3-day deafferents and controls as well as 24-hour and 5-day crickets. After the quality of the RNA samples is fully assessed, they can then be sent for sequencing, and the later phases of the project can be approached. I hope to continue this project as an independent study in the Spring of 2018 as well as during the Summer of 2018, and develop this study further as an Honors project in my senior year.