Analyzing the molecular components of injury response in Gryllus bimaculatus using single-cell RNAseq
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INTRODUCTION

Unlike peripheral nervous system, which exhibits robust regrowth in response to injury, the central nervous system (CNS) in mammals must overcome numerous obstacles in order to regenerate. On the other hand, many invertebrates show a high degree of regeneration, even in the CNS. Studying these animal models will help elucidate the mechanisms behind neuronal regeneration in order to improve our capacity to treat CNS injuries. In the cricket, *Gryllys bimaculatus*, auditory neuron 2 (AN-2) displays compensatory growth in response to injury resulting in functional recovery 4 to 6 days post injury (Pfister et. al., 2013). Removing the tympanic membrane on one side of the cricket removes the source of input (deafferentation) for neurons such as AN-2, which fires in detection of 15kHz bat calls. The dendrites of AN-2 grow across the midline to make synapses with the auditory input coming from the contralateral side to regain function. This project seeks to explore the molecular mechanism behind this compensatory growth response by sequencing the messenger RNA transcripts (RNAseq) in the prothoracic ganglion—where AN-2 is located—, and in single AN-2 cells.

METHODS

Prothoracic ganglia were extracted 24hrs, 3days, or 7days post-deafferentation or control amputation. Ganglia were collected from crickets backfilled 24hours post control amputation to control for the stress of backfilling for single cell extractions. For single cell extractions, fluorescent dye was backfilled into the neck connective of the prothoracic ganglion, through which the axon of AN-2 projects. This dye fluorescently labels the cell body of AN-2, visually facilitating the single cell extraction. Messenger RNA was purified the extracted tissues and will be sent to Alpha Hudson (GA) for Illumina Next Generation Sequencing.

RESULTS

Single cells were successfully extracted from the prothoracic ganglion (confirmation by confocal microscopy (figure 1 B, C). Once the RNA samples are sequenced, a *de novo* transcriptome will be constructed and used to determine differential expression between deafferented and age-matched control ganglia. Furthermore, AN-2 cells will be sequenced. Future work should perform RNAseq experiments on deafferented single cells and age-matched controls at multiple time points post-deafferentation. In addition, analyzing the RNAseq data for AN-2 could elucidate a tissue specific promoter, which could be utilized to create transgenic crickets with specific neurons genetically encoding a fluorescent labeled, eliminating the need to perform challenging and time-consuming backfills, greatly facilitating other experiments.

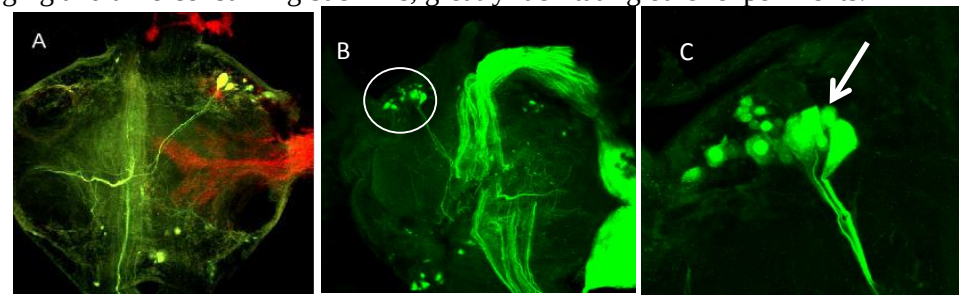

Figure 1. A) Confocal image of backfilled prothoracic ganglion. Cell bodies of AN-1 and AN-2 visible in the ventral anterior quadrant (contralateral to the backfilled connective). Image courtesy of Ana Garcia-Moreno and Monique Lillis. B) Confocal image of backfilled prothoracic ganglion following single cell extractions. C) Arrow indicates the residual fluorescence where the cell body was removed.

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References:

Pfister A, Johnson A, Ellers O, Horch HW (2012) Quantification of dendritic and axonal growth after injury to the auditory system of the adult cricket Gryllus bimaculatus. Frontiers in Physiology 3:367.