Gryllus bimaculatus

The cercal system in crickets is a mechanosensory system comprised of antenna-like appendages called cerci that detect air currents. This receptor organ demonstrates incredible anatomical plasticity in response to injury. In the cricket *Gryllus bimaculatus*, unilateral removal of a cercus causes changes in synaptic strength, in addition to some dendritic sprouting. We hypothesize that, similar to the prothoracic ganglion, well-conserved developmental cues potentially recapitulate their function to not only help in this sprouting. As a first step, we are generating a *de novo* assembly of a terminal ganglion transcriptome for orthologues of guidance molecules of four conserved developmental signaling families found in the prothoracic ganglion: Ephrin, Netrin, Semaphorin, and Slit. Due to the specificity of the terminal ganglia in term of response to injury, specifically the observed changes in synaptic strength, we posit that various synaptic proteins may also play a role in the plasticity observed post-injury.

Brown-morph mediterranean field crickets, G. bimaculatus (N= 30), from an inbred colony originally from the Hoy lab at Cornell University, were used to extract terminal ganglion RNA for construction of the transcriptome. Crickets were kept in a twelve-hour light and dark cycle at 40-60% humidity and 28 °C. Batches of juveniles, between the 7th and 8th instar stage, were removed from the original colony bins and placed in plastic cages, received egg flats for shelter, and were fed cat chow and water *ad libitum*. After reaching the 9th instar, completing their final molt, adult male brown-morph crickets were isolated and placed in a plastic cage. Once again they received egg flats for shelter, and were fed with cat chow and water *ad libitum*.

Three days after the final molt, while the cricket was anesthetized under low-temperature anesthesia, the left-side cercus of a cricket was removed from the stump/base with a razor blade ("deafferented" experimental condition) or about 0.25 mm was removed from the tip of the cercus (the control condition) for control crickets, leaving intact the majority of the appendage intact. Using a microscope, special attention was taken to ensure that no mechanosensitive filiform hairs were remaining post ablation. RNA was purified from the 30 individual terminal ganglion samples using the QIAGEN RNeasy Lipid Tissue Mini Kit (QIAGEN). RNA was eluted from the RNeasy column with 30 l of RNAse free water, and the same 30 l were put through the column three times to maximize yield. RNA was additionally purified using the TURBO DNA-free treatment (Ambion by Life Technologies). Sample qualities were further assessed using an Agilent 2100 Bioanalyzer NANO Chip (Applied Biosystems, Carlsbad, CA). RNA samples were stored at -80 °C until they were shipped on dry ice for sequencing.

The goal of this project is to generate a *de novo* assembly of a transcriptome for the cricket *Gryllus bimaculatus* terminal ganglion. Experimentally, this translates to 1) acquiring tissues from control and experimental adult crickets 24 hours, 3 days, and 7 days post-deafferentation, 2) sending the tissues for sequencing, and 3) assembling the transcriptome once the data is received. With a total of 30 crickets acquired across the three conditions, phase 1 of this project is officially complete. It is still unclear what the data obtained from these sequences will show, but, I posit that a combination of well-conserved developmental guidance molecules, in addition to various synaptic proteins, are working together to help the cricket nervous system recover post-cercal ablation. Phases