The Embryonic Development of the Cricket (*Gryllus Bimaculatus)* Nervous System

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The Horch lab is interested in the cricket model system, due to the interesting behavior crickets produce with a relatively simple nervous system. Additionally, scientists have gained an interest in crickets because phylogenetically, they are more closely related to the ancestral insect as opposed to the extensively studied drosophila and grasshoppers. By learning more about the poorly investigated development of the cricket central nervous system (CNS) development, we can conduct comparative studies looking at the development of holometabolous insects to understand the different developmental processes they undergo.

The goal of my project was to investigate the embryonic development of the cricket, *Gryllus Bimaculatus*. Specifically, I focused on the development of embryonic stages 6.0-9.0 (Donoughe et al, 2016). In order to do this, I first collected eggs laid in cricket bins and set up two test conditions: poked (experimental) and unpoked (control) embryos. In order to calculate survival rate, the environmental conditions for poked and unpoked embryos remained the same. That is, both were put in well plates with HEPES Buffered Saline (HBS) Solution with 1 % Penicillin-Streptomycin (P/S), with the experimental plate being the embryos poked with an injection needle. Once enough controls were collected, sand plates of unpoked embryos were set up purely for visualization through an Immunohistochemistry(IHC) procedure.

At first, achieving a survival rate, particularly for the poked embryos, that was on par with previous studies was tricky. Publications such as Barry et al, indicated that generally poking/injecting cricket eggs with any solution decreases survival rate. Initially, our control survival rates on days 3 &4 post-egg lay where, 615/730 (84.25 %) unpoked and 178/327 (54.53%) for the poked. To reach the minimum survival rate of 80% as recommended by Barry et al, changing the HBS & 1% P/S solution more frequently, vigilantly removing dead eggs, and altering the poking ergonomics, allowed a survival rate of 128/162 (79.01 %) for the poked embryos.

Once we assessed survival rates, we began embryonic dissections on days 3-6 after the eggs were laid which corresponded to the egg stages we were interested in. The dissected embryos were fixed and underwent an IHC staining in order to visualize the developing neurons.

Our results confirmed a developing CNS where we could visualize the brain, the three thoracic ganglia, and all the abdominal ganglia. Moving forward, we hope to complete the embryonic timeline for the unpoked and poked conditions on days 4-6 (AM & PM). Furthermore, by understanding the normal development, we can begin to manipulate previously characterized guidance molecules known as semaphorins. Semaphorins play a role in CNS fasciculation and prevent abnormal midline crossing. So, by knocking down these molecules using double-stranded RNA, we can analyze the consequent impacts it has on the cricket's CNS & PNS development and connect it to the overall question the lab is concerned with, surrounding the unusual plasticity this organism has.

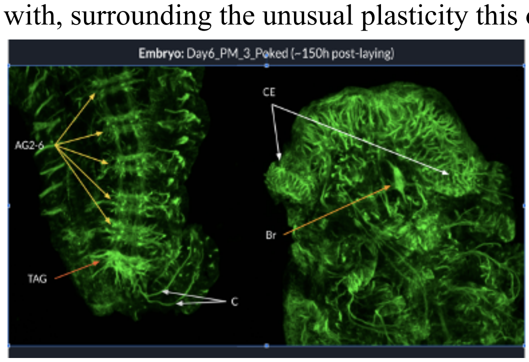

Figure 1.
An IHC stain of a day 6 pm embryo, depicts the developing ventral nerve cord ladder, abdominal ganglia 2-6, the terminal abdominal ganglion (regulates wind detection) and the cerci implicated in sensory stimuli .

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References:

Donoughe S, Extravour CG. 2016. Embryonic development of the cricket *Gryllus bimaculatus*. Developmental Biology. 411(1):140-156.