effect of toll 7 knockdown on the PNS of developing *Gryllus bimaculatus*
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The *Gryllus bimaculatus* auditory system is greatly important to their survival; a loss of an ear triggers an impressive feat of neuronal plasticity. Unilateral deafferentation of the ear triggers corresponding neurons to extend their dendrites across the midline and form functional synapses with auditory neurons corresponding to the functional ear (Hoy et al., 1985). This pattern of growth across the midline is rare, and so this study aimed to identify potential molecular mechanisms in crickets that explain how developing neurons direct axons via their growth cone tips towards their targets. Through the study of the cricket, *G. bimaculatus*, we can begin to broaden our understanding of the molecular processes underlying anatomical plasticity in living organisms.

One family of proteins that influence neuronal shape in vertebrates is the neurotrophins. Recently, neurotrophic-like molecules and their receptors have been found in invertebrates. One of these signaling pairs, the Spätzles and their Toll receptors (Spz-Toll), was discovered in fruit flies, *Drosophila melanogaster* (Zhu et al., 2008). The Toll signaling pathway has been studied extensively in *Drosophila* but has yet to be explored in *Gryllus*. I specifically focused on Toll 7, due to its similarity to the Toll 7 protein found in *Drosophila* (Bando et al., 2022). This similarity between the Toll family protein types found in both species is important because *Drosophila* and *Gryllus* are significantly separated genetically. The existence of the Toll family in both species, specifically Toll 7, may indicate its importance to life (Pechmann et al., 2021). This summer I studied the impact that Toll 7 had on the developing peripheral nervous system of *G. bimaculatus* embryos.

To elucidate the role of Toll 7 during development, I performed a knockdown study to control protein expression. The method I used employed RNA interference (RNAi) which uses double stranded RNA (dsRNA) to interrupt the translation of mRNA into proteins. To treat the crickets with dsRNA, a solution was created which consisted of 0.5μl of rhodamine dye, followed by 0.5μl of injection buffer, and 4μl of dsRNA (Toll 7 or GFP control). Cricket eggs were then injected and allowed to incubate for five days upon which the embryos were extracted from their egg casings. This was one of the most difficult parts of the process. The embryos were then treated with a series of soaps and finally with an antibody called anti-horseradish peroxidase (anti-HRP): this antibody binds to a protein called Nervana, which is present in all nervous tissue. Using anti-HRP allowed for visualization of the peripheral nervous system which I used to quantify the effects of the Toll 7 knockdown. The anti-HRP treated embryos were mounted onto slides and imaged using confocal microscopy; the images allowed me to begin to determine the role of Toll 7. Both the images of the embryo as a whole as well as images of the embryo Tibial Pioneer Neuron 1 (TiP1), found on the first leg, were captured. Images of TiP1 were analyzed using software called Imaris and the relative volume of the axon extending from TiP1 as well as the number of filopodia were used to discern differences between Toll 7 and the GFP control. The entire process took a total of a week to complete, and there were numerous steps that caused the yield to decrease due to their difficulty. The extraction of the embryos from their egg casings as well as the mounting of the embryos proved extremely difficult. However, through numerous trials I was able to improve my yield and contribute more to the study.

Though some successful knockdowns were performed, the sample of Toll 7 knockdown embryos (n=6), and the sample of GFP control embryos (n=1) was not large enough to deduce any meaningful conclusions. Nonetheless, images of the Toll 7 knockdown were captured and analyzed. Additionally, the numerous trials that were completed helped increase the survival rate of the embryos throughout the course of the summer.

Hadley Horch
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Figures

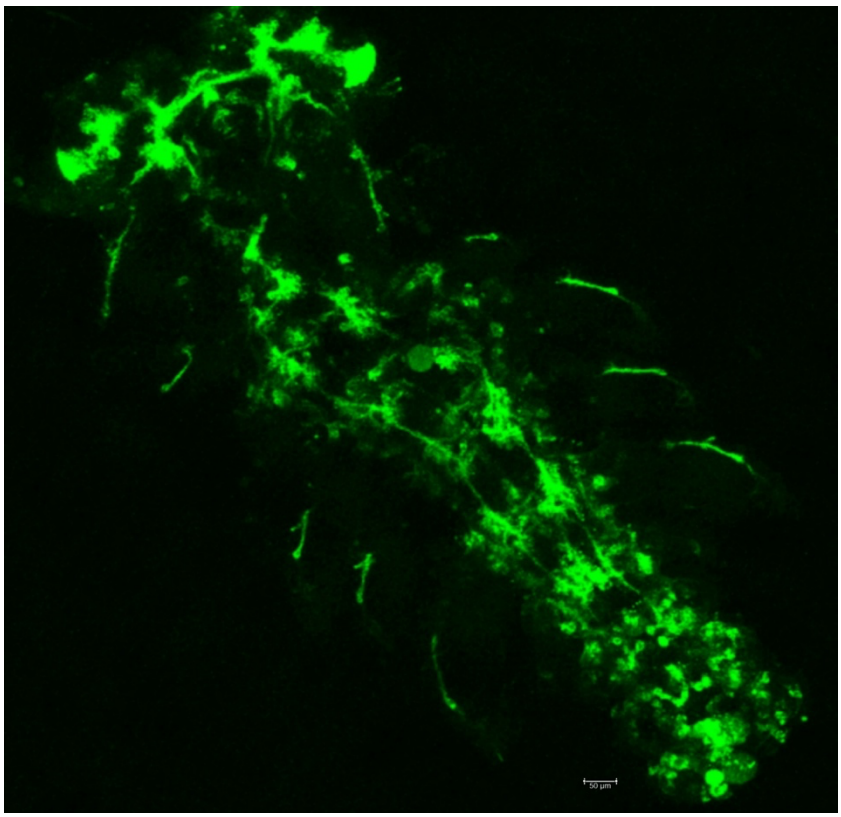

Figure 1. Image captured of the embryo central and peripheral nervous system using confocal microscopy at 100x magnification.

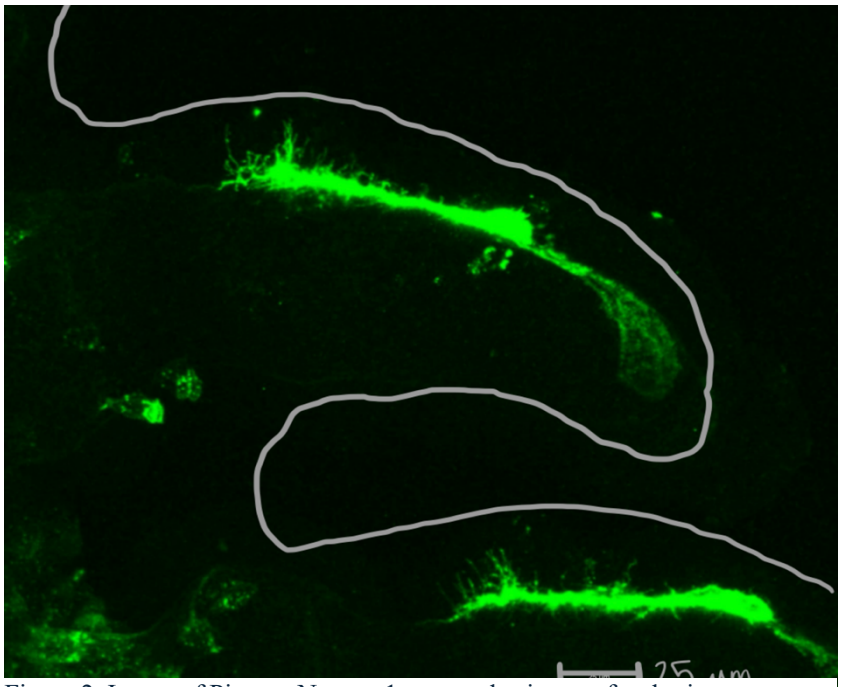

Figure 2. Image of Pioneer Neuron 1 captured using confocal microscopy at 400x magnification.

References

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