The influence of Sema2a on the peripheral nervous system of developing *Gryllus bimaculatus*

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This summer, I received the incredible opportunity to participate in Neurobiology research in Professor Horch's cricket lab for 8 weeks. I aimed to investigate the effect of a protein, Semaphorin 2a (sema2a), on the peripheral nervous system of developing crickets (*Gryllus bimaculatus*) while in early embryonic stages.

Sema2a is a part of a family of proteins known as the Semaphorins – a group of highly conserved proteins that are expressed in vertebrates and invertebrates. Sema2a is thought to be involved in axonal guidance and synapse formation in early invertebrate development, but its role has yet to be fully studied within cricket development.

To do so, I planned to inject freshly laid cricket eggs with double-stranded RNA (dsRNA) designed to inhibit the expression of sema2a during embryonic development. After a few days, the embryos would grow to a stage where I could clearly see the axonal path of the tibial pioneer neuron (TiPN1) of the peripheral nervous system, located in the developing limb bud. I would visualize this neuron by dissecting the embryo out of its egg, mounting the embryo on a slide, and using immunohistochemistry to highlight the peripheral nervous system under a fluorescent microscope. I expected to see that the TiPN1 axon would fail to migrate toward the central nervous system, supporting sema2a's role as a repulsive guidance cue to the TiPN1 axon, just as demonstrated in grasshopper embryos.

Unfortunately, our lab ran into a number of setbacks that negatively impacted many of our projects. At the beginning of the summer, the crickets were laying very few eggs. While we normally expect to collect several hundred cricket eggs after an overnight deprivation of egg laying material, we were only obtaining a couple dozen each day. This meant a few of us were unable to practice injecting and dissecting to the extent that we would have liked, leading to us spending many hours troubleshooting later in the summer.

Several weeks later, we began the process of synthesizing our dsRNA using a method known as Gibson Assembly. While Gibson Assembly is commonly used in many labs, this summer was the Horch lab's first attempt at doing so, and we encountered many challenges, such as shipping delays of necessary materials and unsuccessful reactions. While I was eventually able to synthesize my desired strand of dsRNA, its concentration was far too low to use in any injection trials, and there was not enough time left at that point in the summer to retry the reaction.

I, along with my lab partner Kyla Gary, decided to use leftover dsRNA of a different protein (Toll 6-1) from a prior experiment and go through our entire procedure as planned. While the short timeframe at the end of the summer and the small sample size we had to work with prevented us from obtaining any significant results, I feel very fortunate that we were able to see how our experiment may have played out if things had gone our way earlier in the summer.

Despite my lack of concrete data, I have taken away many valuable lessons and learned a great deal from this experience. Most importantly, this lab revealed the immense patience and diligence required to successfully obtain any results. Even with the hours I spent researching the subject and perfecting my methods, I ran into many unforeseen obstacles, and had to find ways to work around them. I have a much greater appreciation for scientific research after this experience, and I am immensely grateful for the time I spent in the Horch lab. Finally, thank you to Professor Horch, the Fellowships Office, and INBRE-NIH for this outstanding opportunity; I am excited to continue my education in Neuroscience, and I look forward to seeing where it will take me.

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