Mechanisms underlying variable responses to the neuropeptide C-type allatostatin (AST-C) among individuals in the American lobster, *Homarus americanus*

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Central pattern generators are neural networks that produce steady, patterned outputs without the need for sensory input. Unlike the myogenic human heart, the cardiac neuromuscular system of the American lobster (*Homarus americanus*) is neurogenic and driven by a central pattern generator called the cardiac ganglion. The cardiac ganglion (CG) is composed of just nine neurons, making it a model system of study (Cooke, 2002).

The CG generates rhythmic cardiac output, which is not fixed, but rather functionally flexible as a result of the ability of peptides to modulate the system. One neuropeptide family that modulates the cardiac system is C-type allatostatin (AST-C). Three isoforms of this neuropeptide (AST-C I-III) have been identified. Previous research has shown great individual variation in responses to AST-C I and III (Dickinson et al., 2018). These individual responses to perfusion of AST-C I and III are characterized by either increases or decreases in contraction amplitude. It has been hypothesized that the mechanism behind this variation is differences in expression levels of four putative AST-C receptors in the cardiac ganglion.

To investigate this hypothesis, we recorded both physiological responses and molecular RNA receptor expression. First, we recorded physiological responses of the cardiac system by perfusing AST-C I and III through an isolated heart and recording the movement of the beating heart. Based on this recording, we can calculate the change in contraction amplitude to perfusion of AST-C I and III. To determine receptor expression levels, we extracted RNA from various tissues, including the cardiac ganglion, of each lobster. We focused on the cardiac ganglion samples because our hypothesis is centered around expression of the receptors in the cardiac ganglion. These cardiac ganglion RNA samples underwent Illumina RNA-Sequencing. My project this summer focused on performing bioinformatic analysis of these sequencing data to determine if there is differential expression of receptors, or other downstream factors, based on physiological response to AST-C perfusion.

I have performed bioinformatic analysis by first checking the quality of the sequencing data. Since there were errors made by the sequencing company, I cleaned the data by eliminating about 25% of the lower quality reads using a program called Trimmomatic. The data were then aligned and pseudoaligned to pre-existing transcriptomes of various lobster tissues. Through this process, I obtained counts of how many reads from the sample data mapped to specific transcripts in these transcriptomes. Results of exploratory analysis indicate that there does not appear to be differential expression of the four AST-C receptors; however, based on a subset of the data, a number of transcripts may be differentially expressed. One of these transcripts is a protein called Heat shock 90, which has been associated with molting, and therefore may support one of our secondary hypotheses, which is that increases or decreases in heartbeat amplitude in response to AST-C are dictated by the individual lobster's current molt stage (Spees et al, 2003).

Going forward, I am continuing this project during the school year to perform further analysis of these sequencing data. I plan to merge the existing lobster transcriptomes into one transcriptome in order to obtain better coverage of the possible transcripts and then align my sample sequencing data to this master transcriptome. In this way, I am seeking to answer both how and why individual responses to C-type allatostatin vary so greatly.

Faculty Mentor: Patsy Dickinson
Funded by The Arnold and Mabel Beckman Foundation (Beckman Scholars Program)

References

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