

Investigating Neuropeptides and Receptors in Transcriptomes of *Homarus americanus*

Madi Thies, Class of 2021

Central pattern generators (CPGs) are neuronal networks that produce a consistent rhythmic motor output to generate behaviors such as eating, walking, and breathing. One pattern generator is the cardiac ganglion (CG), which controls the heart of the American lobster, *Homarus americanus*, and is responsible for generating patterned bursts of action potentials, each of which results in a contraction of the heart. The cardiac ganglion includes four premotor and five motor neurons, as well as stretch-sensitive dendrites which extend from the CG neurons (Cooke, 2002). In order to maintain homeostasis in response to changing sensory inputs, there is often modulation from neuropeptides which play a key role in the underlying flexibility of this neuronal network.

Due to the importance of neuropeptides in the flexibility of the CPGs, predicting which neuropeptides and corresponding receptors are present can provide more information on their role in modulation. The transcriptomes are created from mRNA extracted from brain, heart muscle, and cardiac ganglion tissues. These hold valuable genetic information that codes for proteins, including the neuropeptides and receptors. Using these transcriptomes, we were able to analyze them with transcriptomics using an *in silico* approach. Transcriptomics has been shown to be a useful tool to predict which neuropeptides and receptors are present in the RNA of the American lobster (Christie et al., 2017). Homology-based BLAST searches and bioinformatics guidelines from previous researchers allow these neuropeptides and receptors to be discovered and documented in the transcriptomes of the CG, heart muscle, and brain of the American lobster (Christie et al., 2017).

A previous study investigated the peptides present in the eyestalk ganglia of the lobster. Using transcriptome mining, researchers were able to discover a number of predicted and undiscovered neuropeptides present in the transcriptome. These findings provide sequences that can be used as query sequences to search through other transcriptomes from the lobster. These include the neuropeptides CCAP, RPCH, proctolin, myosuppressin, and tachykinin, as well as a variety of amines including dopamine, octopamine, tyramine, octopamine-tyramine, and serotonin. All of these peptides were searched in the CG, heart muscle, and brain transcriptomes of the lobster. Once neuropeptides are identified, predictions regarding the cleavage sites and post translation modifications can be made to provide more information about the neuropeptides and their role. All of the neuropeptides listed above were identified in the brain tissue. Each neuropeptide except for CCAP was identified in the cardiac ganglion tissue and none of the neuropeptides were identified in the heart muscle tissue.

The complementary receptors were also searched for and identified to see if these neuropeptides have a receptor in order to elicit a response. Well vetted sites such as Flybase or Uniprot provide known receptor sequences from other species such as the fruit fly, *Drosophila melanogaster*, that can be used as query sequences, in addition to the query sequences from *Homarus americanus*. Receptors for all of the studied neuropeptides were identified in the brain, cardiac ganglion, and heart muscle tissues of the American lobster.

Moreover, preliminary work started investigating the presence of the Piezo and Trek-1 channels and the no-mechanoreceptor and transient receptor potential-gamma proteins. Previous research has shown that these channels and proteins may be involved in the stretch feedback system in *Homarus americanus*. Stretch is part of the positive feedback pathway that is also responsible for the underlying flexibility of the neuronal network along with the neuropeptides. Piezo and Trek-1 channels are newly discovered. While there remains little known about them, it is predicted that they, along with no-mechanoreceptor and transient receptor potential-gamma proteins, are involved with mechanoelectric feedback: the conversion of mechanical forces into biological signals. This feedback may play an important role in the stretch feedback system (Geng et al., 2017). Interestingly, the Piezo and Trek-1 channels and no-mechanoreceptor and transient receptor potential-gamma proteins were predicted to be in the brain and cardiac ganglion tissues, and further research is necessary to determine if they are found in the heart muscle tissue.

Faculty Mentor: Patsy Dickinson

Funded by Henry L. and Grace Doherty Charitable Foundation Coastal Studies Research Fellowship

References:

Cooke, I. M. (2002). Reliable, responsive pacemaking and pattern generation with minimal cell numbers: The crustacean cardiac ganglion. *Biological Bulletin*, 202(2), 108-136. doi:10.2307/1543649

Christie, A. E., Roncalli, V., Cieslak, M.C., Pascual, M.G., Yu, A., Lameyer, T.J., Stanhope M.E., & Dickinson, P.S. (2017) Prediction of a neuropeptidome for the eyestalk ganglia of the lobster *Homarus americanus* using a tissue-specific de novo assembled transcriptome. *General and Comparative Endocrinology*. 243, 96-119.

Geng, J., Zhao, Q., Zhang, T., & Xiao, B. (2017). In touch with the mechanosensitive piezo channels: structure, ion permeation, and mechanotransduction. *Current Topics in Membranes*. 79, 159-195.