Role of peptide modifications in differentially modulating multiple components of a rhythmic neuromuscular system in the American lobster,

may undergo further post-translational modification (PTMs) before becoming fully bioactive. These modifications regulate how peptides interact with the cellular environment, ensuring its stability and proper bioactivity. When bound to their receptor, peptides can alter neuronal function and elicit unique behaviors.

The goal of this project was to further elucidate the molecular underpinnings of peptidergic modulation through physiological experiments combined with transcriptomics and molecular biology. The CPG that drives the rhythmic contractions of the lobster heart, the cardiac ganglion (CG), serves as an ex] TJ Ee5nv Ee5nveET Q q 0.24 0 0 0.2 relatively simple network composed of only nine neurons that produces quantifiable output that responds to peptide modulators. One such modulator is myosuppressin (pQDLDHVFLRFamide). Previous research in the Dickinson lab (Stevens, et al., 2009) has shown that, when applied to whole hearts, myosuppressin causes an immediate dec contraction frequency and contraction amplitude, followed by a large increase in contraction amplitude. By applying myosuppressin to isolated CGs and externally stimulated muscle cells, it was found that myosuppressin acts both centrally, on the neurons themselves, and peripherally, at the neuromuscular junction of muscles. It was found that myosuppressin alters a number of the burst characteristics of the CG, where CG is one of the predicted , three of which exist in the CG.

In addition, it has been shown that myosuppressin exists endogenously in the lobster with two PTMs: a cyclization of the (N)-terminal glutamine and amidation at the (C)-terminus. It may also exist in forms that lack certain modifications, which may be differentially bioactive. My previous research tested three isoforms of myosuppressin on whole hearts of lobsters; one isoform with both modifications, one isoform lacking the cyclization, and one isoform lacking amidation. I found that both the fully modified isoform, and the non-cyclized isoform, were able to elicit a decrease in frequency and a decrease in amplitude followed by a large increase. In contrast, the non-amidated isoform was able to elicit a smaller decrease in frequency and amplitude, but was not able to elicit an increase in amplitude. This summer I investigated whether the differentially modified isoforms of myosuppressin were able to elicit a response in the periphery of the system, in the absence of neuronal input. To do this, I dissected out the cells of the CG and provide manual stimulation at a nerve ending to evoke contractions of the heart. In this experiment, the fully modified form of myosuppressin and the non-cyclized form caused an increase in the amplitude of nerve-evoked contractions, whereas the non-amidated form caused no change in the amplitude of contractions, suggesting that the non-amidated isoform is unable to bind either at the neuromuscular junction or muscle.

To further investigate the binding capabilities of myosuppressin, I began to characterize the expression of the predicted receptor sequences in the neural tissues of the lobster. Using real time PCR, four predicted myosuppressin sequences were identified in the CG, two of which were also identified in the muscle. One receptor was identified in muscle that was not identified in any CG samples. Future research will confirm the sequences of the predicted receptors and express these sequences in an insect cell line to test their ab