Determining the sites at which neuromodulators exert peripheral effects in the American lobster (*Homarus americanus*)

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Central pattern generators (CPGs) are networks of neurons that generate rhythmic patterns of output to drive behaviors such as eating, walking, and breathing. These CPGs are relatively fixed networks of neurons that produce consistent, stereotypical patterns in the absence of other inputs. However, neuromodulators like peptides acting on the network enable flexibility in the motor outputs. Rhythmic behaviors need to be flexible to allow organisms to adjust to changes in the environment and sensory input.

Neuromodulators have been shown not only to exert effects on the CPGs themselves, but also to alter the muscle movements by acting on the peripheral sites, such as the neuromuscular junction or the muscle itself.

This study was performed on the cardiac neuromuscular system of the American lobster (*Homarus americanus*). The simplicity of crustacean CPGs made the American lobster an optimal experimental model. The lobster's heart is neurogenic, so it requires neural impulses to contract. These neural impulses come from a cluster of neurons on the heart wall known as the cardiac ganglion (Cooke, 2002). Neuromodulators exerting their effects on the cardiac neuromuscular system are known to modulate the contraction of the system at multiple sites. The periphery is one of these sites. The periphery consists of multiple components, the neuromuscular junction and muscle, and in most cases, it is not known whether these sites are affected by the neuromodulators.

There had only been one study researching this question, using the peptide proctolin. In this study, it was found that proctolin exerted its effects on both the neuromuscular junction and the muscle (Wilkens, 1996). Many other neuromodulators have been shown to affect the periphery, including the highly conserved peptide, myosuppressin. The goal of this study was to determine where specific neuromodulators exert their effects: the neuromuscular junction, the muscle itself, or both. The peptide that we analyzed was myosuppressin, which has known effects on the periphery. In previous research done in the Dickinson lab, myosuppressin was found to increase the contraction amplitude largely from effects on the periphery (Stevens et al., 2009). They also predicted that myosuppressin would alter feedback from the periphery to the heart.

Neuromodulators acting on the periphery are likely to alter either presynaptic release or calcium dynamics in the muscle. In a presynaptic neuron, there is an influx of calcium entering the cell, which triggers neurotransmitters to be released at the neuromuscular junction. The neurotransmitters diffuse across the synapse to a postsynaptic cell where receptors trigger channels to open and depolarize the cell. This depolarization stimulates a release of calcium which elicits a muscle contraction. Contractions are controlled by levels of calcium within the muscle fibers, and changes in calcium or interactions of calcium with the contractile proteins would alter the extent of contraction. Thus, neuromodulators acting on the system could influence any step of this muscle contraction, some of the most obvious being the neurotransmitter release and calcium release in the muscle.

This summer I started experimentation on the more well studied of the periphery sites, the neuromuscular junction. Through stimulating the motor nerve, I was able to elicit an action potential in the motor nerve causing transmitter release. This caused a postsynaptic response depolarization of the muscle membrane potential known as excitatory junction potential (EJP) which was recorded with an intracellular electrode inserted into one of the muscle fibers. Myosuppressin was superfused over the muscle and the EJP was recorded. It was predicted that if myosuppressin is acting at the neuromuscular junction, there would be an increase in EJP amplitude.

It was found that myosuppressin did not alter the EJP amplitude. This suggests that myosuppressin is exerting its peripheral effects on the muscle and not at the neuromuscular junction. In the future, the Dickinson lab plans to determine if myosuppressin exerts its effects directly on the muscle by directly stimulating the muscle and using bioinformatics.

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