The Stomatogastric Ganglion's (STG) Response to Synaptically Released Modulators and Hemolymph in the *C.borealis*

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This past summer, I worked with Professor Dan Powell on a project to investigate the modulation of a neural circuit in the Jonah Crab (*C.borealis*). We sought to examine how circulating hormones and synaptically released modulators co-modulate a neural circuit. Neural circuits are constantly under the influence of neuromodulators, which include peptides, amino acids, and amines. While this concept is well understood, how exactly circuits are modulated within animals remains a mystery. Thus, we sought to unpack this question by studying a neural circuit in the stomach of the Jonah crab, the stomatogastric nervous system (STNS). The connectivity of the STNS is well known, meaning we know what neurons control this circuit and how they are connected. The STNS also produces fictive output in vitro, which means that it produces activity that we can record when we dissect the stomach of the animal. These elements of the STNS make it very useful to investigate modulation because we can apply modulators to the STNS outside of the animal and examine the effects.

Specifically, our research sought to study an interesting disconnect based on Cooke & Nusbaum's study in 2021. Previous work has shown that the pyloric circuit, one of the two neural circuits in the STNS that controls the pyloric muscle, produces a continuous triphasic pattern in vivo (within the crab) (Kushinsky et al., 2019). Furthermore, this triphasic rhythm is active in the presence of the crab's circulatory fluid, called the hemolymph. However, when we apply hemolymph to the dissected STNS *outside* of the crab, the pyloric rhythm is suppressed. Why is this? We hypothesized that there must be synaptically released neurotransmitters in the crab that excite the pyloric rhythm. In other words, there must be this excitatory input that counteracts the suppression from the hemolymph.

To test this hypothesis, we co-applied several excitatory neuromodulators to the STNS with hemolymph and measured the output of the STNS via extracellular recordings. This involved dissecting the crab's stomach, extracting just the STNS, and pinning the nervous system out. (Much of my time was spent learning and practicing this lengthy dissection.) Once the nervous system was pinned, I drew several Vaseline wells around the nerves we wanted to record so that we could isolate the electrical activity of each nerve from the bath. I placed electrodes next to each nerve and recorded the nerve activity via Spike2 software. Cool saline was continuously perfused. For each prep, I collected control data on the activity of the nerves, not in the presence of any modulator. I then applied an excitatory modulator to the STNS and recorded the output, followed by a wash with saline. After the wash, I applied hemolymph alone directly to the ganglion for about 10 minutes and then co-applied it with an excitatory modulator for an additional 10 minutes. I repeated this process for several excitatory modulators for each preparation. These excitatory modulators included serotonin, dopamine, proctolin, and oxotremorine.

Our results from the summer yielded a few key takeaways. 1. We confirmed that hemolymph suppresses the pyloric rhythm in vitro. 2. All these modulators had an excitatory effect on the pyloric circuit, and 3. Proctolin and oxotremorine are effective at restoring the triphasic rhythm in the presence of hemolymph. More experiments are required to determine the effects of these modulators when co-applied with hemolymph, as our current results are under-sampled. However, I am excited to continue this research throughout the year in my honors thesis. Some of the next steps besides repetition of these experiments include applying combinations of synaptically released modulators, stimulating nerves, and doing intracellular recordings to help provide us with a more detailed picture.

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References:

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