Modulatory effects of six GSEFLamide isoforms on the *H. americanus* stomatogastric nervous system Benjamin Wong, Class of 2020

This summer I worked with the Dickinson lab examining the GSEFLamide family of peptide hormones in the American lobster stomatogastric nervous system (STNS). The STNS is a network of neurons commonly found in arthropods such as insects and crustaceans. The stomatogastric ganglion (STG) is the central cluster of cells in this system, and is comprised of 30 primary neurons that form two central pattern generators (CPGs), which are neural networks capable of producing rhythmic muscular sequences without cues from outside the central nervous system.¹ CPGs can dictate rhythmic motions in invertebrates and vertebrates alike, and can be flexibly modulated by neuropeptide hormones -- short amino acid chains that act as neurotransmitters. Neuromodulation is a form of nervous system communication distinct from typical synaptic transmission, in that chemicals such as peptide hormones flood a system to act on multiple neurons at once, rather than communicating at just one synapse.

Neuropeptides are common neuromodulators that can flexibly affect the STNS, and are therefore often studied in this system. Each of the two CPGs I'm studying controls a distinct part of the lobster's stomach: the gastric mill pattern controls three teeth used to break down food, and the pyloric pattern controls an active filter that leads to the lobster's midgut.² The peptides that modulate these CPGs originate either from descending projection neurons that secrete peptides onto the STG's receptors, or from neuroendocrine systems releasing peptides into the bloodstream.^{2,3} Previous experiments by the Dickinson lab have shown that different peptide family isoforms, which vary by just a few amino acids, can have differing functional effects on the lobster cardiac system, yet similar effects on the STNS. My research this summer has considered the potential similar or distinct STNS effects of the novel GSEFLamide peptide family, which was identified last year in the Dickinson lab.⁴

To examine the effects of this family of peptides (containing six isoforms) on the STNS, each system was dissected from the lobster via a two-part procedure; the lobster was cold-anesthetized, before the foregut was removed and then manually dissected to remove each neuron. This dissection isolated the STG as well as three other STNS ganglia, and all of their corresponding motor nerves, which control the motor patterns described previously. The STG was then de-sheathed, to allow peptides to act on the system. The dissected STNS activity was then recorded using a constant cold saline perfusion system, with petroleum jelly wells around each nerve to be recorded from. Once the recording system was fully set up and baseline recording taken, one isoform from the GSEFLamide family was diluted and applied through the perfusion system. Spike2, a data recording and analysis program, was used to record the system's response to each peptide, and then analyze those changes by considering action potential burst frequency and density.

Although I am in the early stages of data analysis for this project, and am continuing to collect data, we believe that the GSEFLamides are showing similar effects in the STNS to those they had in the lobster cardiac system – one isoform, AVGSEFLamide, appears to have very little effect on either the gastric or pyloric patterns, while the other five isoforms have seemingly significant, yet similar gastric effects, and some limited pyloric effects. I look forward to continuing to research this peptide family throughout the next school year, and further analyzing my recorded data.

Faculty Mentor: Patsy Dickinson

Funded by: INBRE, IDeA Networks of Biomedical Research Excellence and The National Institute of Health

References:

- 1. Bucher D. 2009. Central Pattern Generators. Encyclopedia of Neuroscience. 691-700.
- Marder E, Bucher D. 2007. Understanding Circuit Dynamics Using the Stomatogastric Nervous System of Lobsters and Crabs. *Annual Review of Physiology*. 69:291–316.
- 3. Coleman MJ, Nusbaum MP, Cournil I, Claiborne BJ. 1992. Distribution of modulatory inputs to the stomatogastric ganglion of the crab, *Cancer borealis. Journal of Comparative Neurology*. 325:581–94.
- 4. Christie AE, Roncalli V, Cieslak MC, Pascual MG, Yu A, Lameyer TJ, Stanhope ME, Dickinson PS. 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.
- 5. Christie A.E., Skiebe P.E., and Marder E. 1995. Matrix of neuromodulators in neurosecretory structures of the crab Cancer borealis. *Journal of Experimental Biology*, 198(12), pp.2431-2439.
- 6. Li L, Kelley WP, Billimoria CP, Christie AE, Pulver SR, et al. 2003. Mass spectrometric investigation of the neuropeptide complement and release in the pericardial organs of the crab, Cancer borealis. *Journal of Neurochemistry*. 87:642–56.
- Dickinson P.S., Kurland S.C., Qu X., Parker B.O., Sreekrishnan A., Kwiatkowski M.A., Williams A.H., Ysasi, A.B. and Christie A.E. 2015. Distinct or shared actions of peptide family isoforms: II. Multiple pyrokinins exert similar effects in the lobster stomatogastric nervous system. *The Journal of Experimental Biology*, 218(18):2905.