**Predicting and Searching for Specific Neuropeptides and Protein Receptors in the Brain and STG of *Libinia emarginata, Pugietta producta, Chionoecetes opilio***

**Grace Lee, Class of 2022**

Central pattern generators (CPGs) are biological networks of neurons that generate rhythmic outputs central to motor behaviors: like walking, respiration, and heartbeat. To adapt to shifts in internal and external environments, many animals have CPGs. An example of a simple CPG is the stomatogastric ganglion (STG) in the decapod crustacean stomatogastric nervous system (STNS). The STG is comprised of 30 neurons that neuromodulators can act on to conduct the digestive contractions of foregut muscles (Selverston 2017). As seen in the STG, CPGs are predisposed to modulation by neuromodulators. Neuromodulators are generally peptides or amines that regulate action potential firing patterns and either enhance or diminish electrical reactions after binding to matching receptors. As a result, neuromodulators play a role in plasticizing and diversifying the behavior of neural circuits to sensory feedback (Dickinson et al. 2019).

Although the importance of neuromodulation is widely known, scientists have yet to uncover how conserved and abundant certain modulators are in different crustacean species and neural circuits. Hence, this summer, the lab decided to adopt a bioinformatics approach: we searched for common crustacean neuropeptide precursors/preprohormones and their complementary G-protein coupled receptors (GPCRs) in the brain and STG of spider, kelp, and snow crabs (*L. emarginata, P. producta, C. opilio,* respectively).

We analyzed many neuropeptide ligands and GPCR neuropeptide receptors; some examples include proctolin, myosuppressin, red pigment-concentrating hormone, and tachykinin. Known neuropeptide and GPCR sequences were used as query sequences to search for closest matches in the RNA transcriptome databases. For *Libinia* and *Pugietta* brain and STG, searches were completed on the public CLC server from the University of Hawaii. Mining in the *Chionoecetes* transcriptome was a little more complicated: searches were done locally, which involved the use of the Bowdoin High Performance Computing Grid (HPC). Deduced protein hits were validated with reciprocal blasts in NCBI and FlyBase databases and structural features and domains of GPCRs were found using Pfam, Topcons, and Wolf Psort. Only viable neuropeptide preprohormone hits were post-translationally modified. Using Veenstra 2000 paper and NeuroPred as guidance, dibasic cleavage sites in the preprohormone were predicted, signal peptides were cleaved off, disulfide bonds were marked, and tyrosine residues were sulfinated.

Common trends observed in the preprohormone processing were the presence of mature bioactive peptides and variation in the linker peptides among multiple protein isoform hits for the same target query sequence. In the future, more work can be done in investigating the function of such differences in the linker peptides. I also encountered some alignment inconsistencies and poor blast hits in the *Chionoecetes* Brain and Eyestalk transcriptome, alluding to a possible assembly problem. This issue was potentially ruled out after finding high quality blast hits in the database for generic proteins present in all neurons. Therefore, future measures need to be taken to solve the problems in the *Chionoecetes* database.

This broad-range research is a start to my critical examination of decapod crustacean neural circuits and their evolved flexibility to deliver an output optimal for any situation. Now with an organized and comprehensive blast database, I can build off past students’ theses concerning behavioral diversity’s effect on the extent of neuromodulation. More specifically, I can further compare these crabs from the same superfamily with different feeding patterns (opportunist-feeding *Libinia* and *Chionoecetes* vs. specialized-feeding *Pugietta*) and see if and how neuromodulator and receptor sequences vary in the STNS. I can also use collaborator’s mass spectrometric data to verify or refute the presence of possible protein hits.

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