Predictions of Proteins within Brain and Stomatogastric Nervous System Transcriptomes in Northern Kelp Crabs (*Pugettia producta*)

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Having a diverse array of receptors within an organism provides a mechanism for flexibility of outputs in a simple pattern generating neural network (Dickinson 2019). Kelp crabs (*Pugettia producta*) are unique in that their diet is mainly herbivorous, while the Spider crab (*Libinia emarginata*) consumes both plants and small animals regularly. Knowing this, we can hypothesize that different receptors may be present between these species as they metabolize their diets differently. To determine this, we must first predict the sequences of these receptors. We can then predict the presence of these peptides by analyzing transcriptomes of the crabs that have been previously created to identify where they are expressed in the organism's body.

To conduct predictive searches for these neuropeptides and receptors, we first searched the *Pugettia* stomatogastric ganglion (STG) and brain transcriptomes on the University of Hawaii BLAST database. From this database we received hits of similar protein sequences within the *Pugettia* transcriptomes that matched the query sequence for the neuropeptide or receptor of interest. We then utilized Expasy Translation tool to translate the genome sequences for the hits to a FASTA protein sequence. These protein sequences predicted the possible proteins within the *Pugettia* transcriptomes. To confirm that each FASTA sequence was a unique protein, we aligned the top hits and identified which had any differences of amino acids. Then, the unique proteins were aligned to the query sequence. To validate the identity of these FASTA sequences, we entered the sequences into databases that compare them to protein sequences in other species. These databases were FlyBase (which compared to *Drosophila melanogaster*), and NCBI Blast (which allowed us to compare the sequences to other arthropods).

Receptor	Present?	Confidence
CCAP - STG	Yes	High
CCAP - Brain	Yes	High
CabTRP - STG	Yes	High
CabTRP - Brain	Yes	High
Myosuppressin - STG	Yes	Moderate
Myosuppressin - Brain	Yes	Moderate
Proctolin - STG	Yes	Moderate
Proctolin - Brain	Yes	Moderate
RPCH - STG	No	Low
RPCH - Brain	No	Low

We then utilized several tools to predict the processing of the neuropeptide sequences. To determine processing features, we had to detect the presence of a signal peptide, and the cleavage site if a signal peptide was present (using SignalP), sulfination (using Expasy Sulfinator tool), and disulfide bonding (using DIANNA). We utilized the processing rules developed by Andrew Christie, PhD. (Christie 2019). After predicting the processing of the neuropeptide sequence, we could determine the bioactive peptide sequence, and compare it to that of the query sequence. If the bioactive peptide could not be determined, we concluded that the neuropeptide did not exist within that transcriptome. Receptor sequences were entered into programs to determine their topography and structure. We utilized the programs TOPCONS, Pfam (and later hmmrscann, as the Pfam program was changed during the summer), and WoLF PSORT to predict the structural features of the peptide predicted in the FASTA sequences.

Neuropeptide	Present?	Bioactive Peptide
CCAP - STG	Yes	PFCNAFTGCG
CCAP - Brain	No	N/A
CabTRP - STG	Yes	APSGFLGMRamide,TPSGFLGMRamide
CabTRP - Brain	Yes	APSGFLGamide
Myosuppressin - STG	No	N/A
Myosuppressin - Brain	Yes	QDLDHVFLRFG
Proctolin -STG	Yes	RYLPT
Proctolin - Brain	Yes	RYLPT
RPCH -STG	No	N/A
RPCH - Brain	Yes	pQLNFSPGWamide

From these searches, we predicted the presence of certain receptors and neuropeptides within the *Pugettia* crab STG and brain. If a neuropeptide was likely present, we were able to determine the bioactive peptide from processing the predicted sequence. As receptors do not have the processing step to help predict the presence of the protein, we indicated our confidence in our findings based on reciprocal blast searches and alignment to the query sequence.

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