Mechanisms underlying variable responses to isoforms of the neuropeptide C-type allatostatin (AST-C) in the American lobster, *Homarus americanus*

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My honors research centers around understanding the mechanism underlying the variable physiological responses to the perfusion of AST-C isoforms through the lobster heart. My hypothesis is that amidation of AST-C isoforms may influence binding to four putative AST-C receptors, thus causing the varying physiological responses we see. I came to Arizona with the hopes of performing AST-C receptor binding studies at the USDA-ARS lab in Maricopa, AZ to see if amidation was indeed influencing receptor binding. These studies are an important addition to my honors research and cannot be conducted at Bowdoin due to the lack of resources in that particular area. However, due to the government shut down, the USDA lab was closed for the entirety of my visit to Arizona. Therefore, I was not able to conduct receptor binding experiments. Nevertheless, I was able to make the best of my time in Arizona and make progress on my honors work as well as learn vital computer based lab skills from our collaborators, Andy Christy from the Békésy Laboratory of Neurobiology at the University of Hawaii at Manoa, and Joe Hull from the USDA-ARS lab in Maricopa, Arizona.

My first task was to analyze my physiological data from lobster whole heart perfusions of AST-C amidated and non-amidated isoforms and to re-analyze previous Bowdoin students data so as to maintain a consistent data analysis method. Once all 47 data sets were compiled, I ran statistical tests in prism to determine if the distribution and means of responses to amidated vs. non-amidated isoforms differed from one another. My data do not support my original hypothesis, and suggest that amidation is not responsible for the differences in cardiac responses across peptides.

After reviewing these data with my honors advisor, Patsy Dickinson, I decided to look into other possible mechanisms underlying the variability of responses to perfusion of AST-C peptides. I am specifically interesting in the structure function relationship between these AST-C peptides and the four putative AST-C receptors. I therefore decided to look at differences within the amino acid sequences between the three AST-C isoforms and visualized these isoforms in 3-D using a PEP-FOLD server program. I then formed a new hypothesis for my honors research: the position of tyrosine and alanine in the amino acid sequence of the three AST-C isoforms is responsible for the variability in responses to perfusion of AST-C. To test this hypothesis, I designed two new AST-C isoforms: one where tyrosine replaced alanine in the endogenous form of AST-C II and one where alanine replaced tyrosine in the endogenous form of AST-C III (Figure 1). We ordered these peptides from GenScript USA and they have since arrived at Bowdoin and I have begun experiments with them.

During my time in Arizona, I also met with Joe Hull from the USDA lab and learned how to create primers with a fellow Bowdoin student, Emily Oleisky. I also helped Patsy Dickinson and Andy Christie with the various papers that they are working on by looking on databases such as PubMed for papers on particular receptors that pertain to their work.

AST-C isoforms	Endogenous	Designed
AST-CII	SYWKQ**CAFNAVSC**F	SYWKQ**CYFNAVSC**F
AST-CIII	GNGDGRLYWR**CYFNAVSC**F	GNGDGRLYWR**CAFNAVSC**F

Figure 1.
Endogenous and designed AST-C isoform sequences. Tyrosine and alanine are reversed in designed peptides.

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