## The role of different neuron types in controlling the response to a neuropeptide in the cardiac ganglion of the American lobster, Homarus americanus

#### Josie Tidmore, Class of 2024

Central pattern generators (CPGs) are neural circuits that produce patterned neural activity that underlies rhythmic motor behaviors such as breathing, walking, and swimming. Neuromodulation of these circuits enables each CPG to produce multiple variants of rhythmic patterns. Such flexibility can be achieved through the action of neuromodulators, which include a plethora of molecules, with neuropeptides being the most prevalent and diverse group of neuromodulators (Delcomyn 1980; Marder and Calabrese 1996).

The cardiac neuromuscular system of the American lobster, *Homarus americanus*, is a useful model for understanding the role of such neuropeptides in the modulation of patterned outputs (Cooke 2002). The nerve tissue of this system, called the cardiac ganglion, is a central pattern generator responsible for the reliable, rhythmic activation of the heart. The cardiac ganglion (CG) is composed of nine neurons—five motor and four premotor neurons—that control the contraction of the heart through patterned bursts of action potentials (Cooke 2002). Motor and premotor neurons are electrically and chemically coupled in order to deliver efficient and precise communication. These neurons exhibit spontaneous bursting activity, which drives muscle behavior (Williams et al. 2013). Previous studies have illustrated that premotor and motor neurons are capable of producing independent bursting patterns when decoupled, suggesting distinct physiological roles (Oleisky et al. 2020).

Studies have identified several neuropeptides involved in the modulation of the CG. One such neuropeptide is SGRNFLRFamide (SGRN), a peptide endogenous to the American lobster which has been shown to elicit modulatory effects on the cardiac neuromuscular system. Previous studies have characterized the role of SGRN in the isolated CG, which found that the neuropeptide elicits a decrease in burst frequency and an increase in burst duration (Dickinson et al. 2015). However, its role in the motor and premotor neurons of the CG is not as well-understood. My summer research project sought to further examine the effects of SGRN across the two cell types of the cardiac ganglion which would potentially contribute to a better understanding of the mechanisms that underlie the flexibility of pattern generators.

In order to examine the role of SGRN on neuronal activity, the heart was isolated via manual microdissection from the carapace of the lobster, and the CG was then dissected out. Petroleum jelly wells were built around the anterolateral nerves to monitor motor neuron output and around the trunk of the CG to monitor premotor neuron output through extracellular recordings. Baseline recordings of the intact CG when perfused with physiological saline and then SGRN were taken as a control. Using a protocol of decoupling described in previous studies, the premotor and motor neurons were separated by physical ligation of the trunk of the CG just anterior to the motor neuron 4 using suture silk (Fig. 1; Oleisky et al. 2020). Once bursting activity recovered after ligature, recordings were taken of the CG in saline and then in SGRN solution.

The results of these experiments suggest that, although the response to SGRN is variable, in general, this peptide may act to stabilize neural activity. By decoupling the motor and premotor neurons, it was found that the premotor neurons are more important in determining the effect of SGRN on the CG. SGRN causes a decrease in burst frequency and an increase in burst duration in the premotor neurons, while its effect on motor neurons was not as pronounced, suggesting that premotor neurons are the driver of the response to SGRN observed when the CG is intact. Future investigation to confirm these results may provide greater insight into the mechanism(s) by which SGRN acts on the motor and premotor neurons of the cardiac ganglion.



Figure 1. Schematic diagram of the cardiac ganglion (CG) of the American lobster, *Homarus americanus*. Organization of the cardiac ganglion with ligature and petroleum jelly well placement. Four small premotor neurons located in the posterior trunk of the cardiac ganglion are electrically and chemically coupled to the five large motor neurons, which are located in the anterior portion of the cardiac ganglion. Purple indicates motor neurons and green indicates premotor neurons. Purple and green ovals indicate the location of recording sites. Site of the ligature is indicated by thread loop. (adapted from Oleisky et al. 2020).

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