Investigating Functional Circadian Rhythms in the Cardiac Ganglion of the American Lobster, *Homarus americanus*

Casey Breslow, Class of 2019

Organisms interact and react to environmental change, synchronizing with environmental patterns. This is called a biological clock – a time-keeping system that helps to coordinate physiology and behavior. The circadian clock rhythm operates on a day-night timescale over a 24-hour period; it has been recorded in patterns of locomotion (Arguzzi and Sardà, 2008), visual sensitivity (Fanjul-Moles et al., 1987), and the levels of activity in enzymes (Durán-Lizarraga et al., 2001). In decapods, circadian rhythms were also recorded in the cardiac system, specifically heart rate frequency (Arguzzi and Sardà, 2007; McGaw and McMahon, 1998). This would suggest that the cardiac ganglion (CG) may be driven by a circadian timekeeping system. Proteins important in the circadian clock system (clock, cryptochrome2, cycle, period, and timeless) have been identified as present in the CG (Christie et al. 2018); however, consistent and substantial physiological evidence has not demonstrated whether a functional circadian rhythm is also present. Because the CG possesses the molecular components needed for a circadian timekeeping system, it is important to understand if this system is also functional.

The focus of my work in the Dickinson lab has been to isolate the whole heart or CG of the American lobster, and record the heart rate for at least two full circadian cycles (48 hours). To do this, a dorsal dissection was performed to extract the heart from the whole organism. For isolated whole heart recordings, the anterior arteries were tied off and connected to a force transducer to measure the force and frequency of the heart-beat. For CG recordings, the isolated whole heart was detached from the carapace and a fine dissection was performed to remove the CG. Electrodes were used to measure the bursts generated by the CG. Both the isolated whole heart and CG setups were perfused with lobster specific saline. To extend the longevity of these isolated tissues, glucose was added to the saline and the saline was autoclaved to hinder bacterial growth.

From these preliminary recordings of the isolated whole heart (Figure 1) and CG (Figure 2), some patterns of changing frequency over 24-hour periods were identified. In the whole heart recording, a decrease in frequency was seen at around hour 10-12 across three 24 hour days (Figure 1). With some data points missing, these data are not complete, but suggest the possibility of a circadian pattern being present. In the CG recording, an increase in frequency was seen at about hour 11 on Day 1, and at about hour 13 on Day 2 (Figure 2). Again, these data are not complete and clear across the recording period; however, the preliminary data supports the possibility of a functional circadian timekeeping system driving heart rate frequency of the CG.

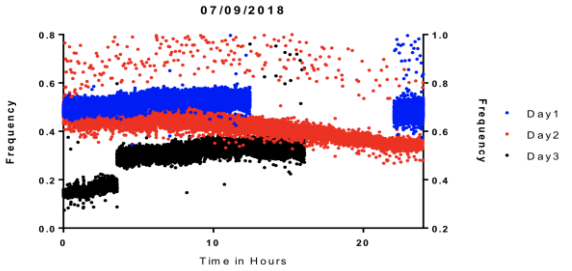

Figure 1. This graph maps the frequency of heart beats over 64-hour time period in an isolated whole heart. Some data is missing due to computer malfunction. Decreases in frequency are seen at hour 10.

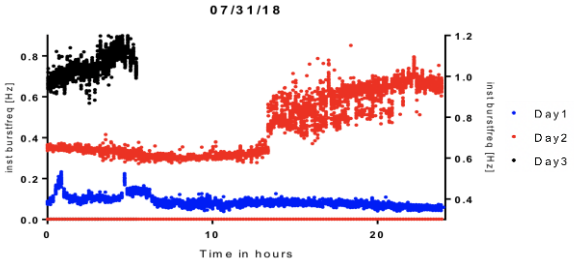

Figure 2. This graph maps the frequency of bursts over 54-hour time period in an isolated cardiac ganglion. Increases in frequency are seen at hour 12.

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