The role of feedback in determining the stability of the lobster heart in response to temperature perturbations

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The Dickinson lab studies central pattern generators, which are neuronal circuits that control rhythmic behaviors, such as walking, breathing and heartbeat, without the need for sensory input. The lobster cardiac ganglion is a simple central pattern generator responsible for the lobster's heartbeat. As a result, the lobster cardiac ganglion is a good subject of study in order to understand central pattern generators at the simplest level. These findings can then be applied to more complex central pattern generators, such as those in humans.

The *Homarus americanus* lobster inhabits the north east shore of North America and can survive in temperatures ranging from 0-30˚C. As a result, my project used the changing condition of temperature to identify the feedback systems at play in helping the lobster heart maintain stability. This is to say, how does the cardiac ganglion, a neuronal circuit that requires no sensory input, continue firing at 0˚C as well as 30˚C?

There are two known feedback loops present in the lobster cardiac system: the positive stretch feedback system and the nitric oxide negative feedback loop. My project focused on the nitric oxide negative feedback loop because it is a feedback pathway that has been shown to be conserved across invertebrates and vertebrates, namely humans. When the heart muscles contract they release nitric oxide, and this has been shown to decrease the action potential burst frequency of the cardiac ganglion. It has been widely proposed that nitric oxide pathways stabilize systems, therefore, we proposed that when the nitric oxide pathway is present in the lobster cardiac system, it would help stabilize the heart in response to changing temperatures.

We initially tested this hypothesis using two preparations: a whole heart and an isolated cardiac ganglion preparation. In the whole heart preparation, we used a force transducer to measure contraction amplitude and frequency of an intact heart that has been removed from the body of the lobster. In the isolated cardiac ganglion preparation, we removed the cardiac ganglion from the whole heart preparation, thereby separating it from the surrounding muscle tissue, and recorded burst frequency using a suction electrode. The difference between the two preparations is that in the whole heart preparation, the nitric oxide feedback pathway is present, whereas it is not present in the isolated cardiac ganglion preparation because the muscle tissue has been removed. Each preparation was then subjected to a temperature ramp that increased the temperature of physiological saline being perfused through or across the preparation by 0.75˚C/min. Then once the heart/cardiac ganglion crashed, meaning it failed to beat/burst more than twice in thirty seconds, the preparation was cooled and this "crash temperature" was recorded. The crash temperatures were compared to see which preparation was more stable, i.e. had a higher crash temperature. As shown in Figure 1, the isolated cardiac ganglion (ICG) crashed at a significantly higher temperature than the whole heart. This was the opposite of our prediction because these data suggest the nitric oxide pathway is destabilizing the whole heart, causing it to crash at a lower temperature.

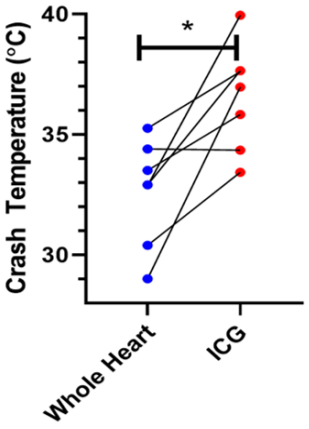

Figure 1. The isolated cardiac ganglion (ICG) crashed at significantly higher temperatures compared to the whole heart preparation (n=7, p=0.016, paired t test).

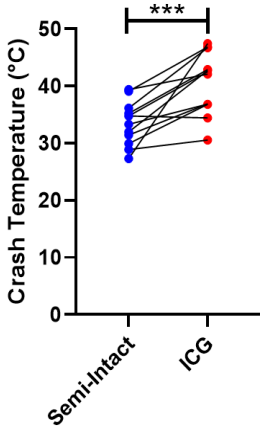

Figure 2. The isolated cardiac ganglion (ICG) crashed at significantly higher temperatures compared to the semi-intact preparation (n=12, p=0.0008, paired t test).

In order to eliminate the posibility that the muscle tissue was crashing and failing to contract even though the cardiac ganglion inside the whole heart was continuing to fire, a subsequent experiment was run which compared a semi-intact prepartion to an isolated cardiac ganlgion preparation. The semi-intact is identical to the whole heart preparation, except a suction electrode is inserted through an incision in the heart wall to be able to record the bursting of the cardiac ganlgion as well as muscle contraction. These preparations were run through identical temperature ramps and the results are shown in Figure 2. These data confirm our previous finding that the cardiac ganlgion is less stable in the presence of the nitric oxide pathway and therefore crashes at a lower temperature.

The future direction of this project, which will pursue during an honors project next year, is to apply nitric oxide in the form of a nitric oxide donor to the isolated cardiac ganlgion during identical temperature manipulations. We predict that the isolated cardiac ganlgia with applied nitric oxide will crash at a lower temperature compared to the isolated cardiac ganglia in the absence of nitric oxide. These results would support our previous findings that suggest nitric oxide is a destabilizing feedback loop in the lobster cardiac system.

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