# Lab #6: The neurogenic crustacean Heart

**Introduction and Background by Ian Cooke**

This experiment examines the physiology of a neurogenic heart with respect to its rhythmic control and its responses to stretch, responses to neurotransmitters, changes of ionic concentrations, and to temperature. You will need to understand the differences between this crustacean example and vertebrates in the anatomy of their circulatory systems, the heart and its muscles and their modes of control.

## Background

**Pacemaking and initiation of the heartbeat.** Unlike the **myogenic** vertebrate heart, the hearts of crustaceans are **neurogenic**. The lobster heart is a bag of striated muscle suspended by elastic ligaments within the **pericardial cavity** just under the dorsal carapace of the thorax. On the inside dorsal surface of this bag is the **cardiac ganglion**. It is a Y-shaped structure, about 1 cm long, with 9 nerve cells (7 strung along the stem, one in each arm of the Y). This ganglion is a mini-nervous system, functioning on its own, with stretch receptors responsive to filling of the heart that, together with 4 small neurons, help initiate rhythmically repeated bursts of nerve impulses in the 5 **motorneurons** that send axons to synapse on all the striated muscle fibers of the heart. Every contraction of the crustacean heart is initiated and its strength controlled by the magnitude of the muscle depolarization produced by the synaptic input. The extent of depolarization is dependent on the frequency, number and amplitude of the synaptic potentials produced on the striated muscle fibers at the felt-work of synaptic terminals from the motorneurons of the cardiac ganglion.

The lobster heart is an autonomous system in that it can be isolated from the animal and will continue to beat rhythmically provided it is filled with saline under mild pressure. The cardiac ganglion, in turn, is autonomous and will continue to produce rhythmic bursts of impulses after removal from the heart. The effect of muscle stretch via the **dendrites** has likely been replaced by injury currents in the dendrites resulting from the dissection. **The ganglion is a model for reliable pacemaking with a number of redundant features.** Each of the 9 neurons is capable of producing a rhythmic burst of impulses on its own. Normally 4 small neurons in the posterior portion of the ganglion drive the activity with excitatory synapses on each of the more anterior cells (their axons do not exit the ganglion). The **depolarizing** influence that sets off the bursts is synchronized by **electrotonic** connections among all the neurons. The large cells also have **excitatory synaptic contacts** with other neurons in the ganglion.

**Crustacean circulatory system anatomy.** The system is described as **semi-open** because there are non-muscular "arteries" that direct blood flow (more properly **hemolymph**) to major parts of the animal: the **brain**, the **ventral ganglia**, the **gills**, and specific areas of **muscle**. The distribution of the heart output is influenced by valves at the arteries that are under control of nerves from the **central nervous system** (**thoracic ganglia**) and by **neurohormones** released at various sites. The best characterized of these release sites are those present in the **pericardial cavity** which also release several neurohormones which act on the cardiac ganglion (see further details below).

The return of **hemolymph** to the heart is through tissue spaces, moved mostly by muscle activity and possibly by negative pressure in the pericardial cavity created by contraction of the heart.

Filling of the heart occurs through 4 **ostia** – openings through the dorsal surface of the heart – when the **elastic suspensory ligaments** pull the heart open. The ostia have valves - tissue flaps closed by the pressure in the heart as it contracts.

**Central nervous system control of circulation.** As in vertebrates, there is **inhibitory** and **excitatory regulation** of heart functioning by the CNS. There is an **inhibitory** and there are **2 excitatory axons** that originate in the thoracic ganglia and enter the cardiac ganglion from each side (left and right). When activated these alter the **burst rate** and **frequency** and **number of impulses** in the bursts reaching the cardiac muscles. In the intact animal, the heart stops for some 10's of seconds in response to stimuli that startle the animal or are threatening, such as a **tap on the carapace**. The effects of stimulating the **excitor axons** is more difficult to observe, but includes increases in heart rate and contraction strength. The excitors are reported to be active when **oxygen levels decline** and **during activity**.

**Neurotransmitters and neurohormones**. The excitatory synaptic transmitter among the cardiac ganglion cells and at the motorneuron synapses on muscle fibers is **glutamic acid**, as at peripheral neuromuscular junctions in crustaceans. The excitatory transmitter of the anterior of the two excitatory axons from the CNS on neurons of the cardiac ganglion is probably **dopamine**. The transmitter of the 2nd excitatory axon is still uncertain; it may be **acetylcholine**, but the axon's effect is sufficiently transitory and difficult to elicit reliably that its transmitter is not definitively established.

The transmitter for the inhibitory axon from the CNS in the ganglion is **gamma-amino butyric acid (GABA)**, as it is at the **peripheral neuromuscular synapses** in crustaceans.

The number of neurohormones found to be released from the neurohemal sites in the pericardial cavity includes two monoamines: 5-hydroxytryptamine (=**serotonin**), and **octopamine** in lobsters (dopamine in crabs). Peptides include proctolin, crustacean cardioactive peptide (**CCAP**), and at least two **FMRF-amide-related peptides**. These generally increase cardiac output by action on the cardiac ganglion and possibly also by effects at neuromuscular junctions. Some have differential effects on the arterial valves and thus influence distribution of the heart output. A generalization is that each orchestrates a subtly different coordinated response of the circulatory, respiratory and probably other systems to homeostatic demands, as for example to anoxia, osmotic stress, trauma and the like. The effects of some of these change when the heart is studied *in vivo* or dissected and set up in different ways.

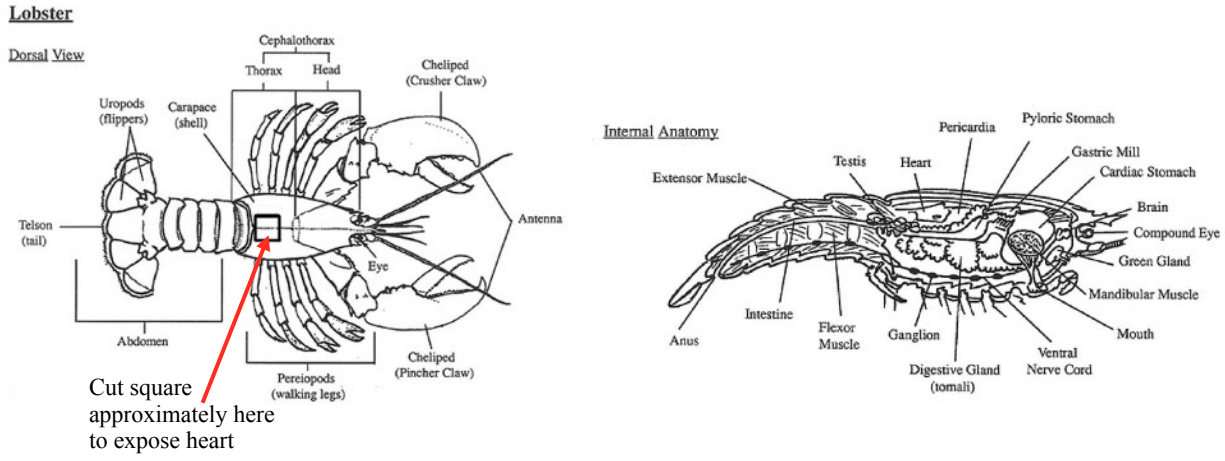


Figure 1. Anatomy of Lobster. – Note the heart is located dorsally at the caudal end of the thoracic segment. Its location is very superficial - just under and attached to the carapace by ligaments.

# Equipment and Materials

PowerLab
Bridge Pod + Force Transducer Stimulator Bar
Mounting stand with micropositioner
Thermocouple Pod and thermocouple
Thread
Duct Tape
Barb-less hook
Dissection tools
We will have Lobster Ringer's (saline solutions) of the following flavors:

Cold (-10°C; freezer) Warm (20°C room temp) Baseline (0°C; ice bath) - Sodium
+Epinephrine + Serotonin +Glutamate +Dopamine + GABA

# Procedures

## A. Setup and calibration of equipment

1. The "lobster heart" settings file has been set up to take the force transducer on Input 1 and the thermocouple on Input 2.
2. If your force transducer has a pod output, connect it directly. Otherwise, you will need a Bridge pod.
3. The thermocouple requires a T (temperature) -pod.
4. Zero the force transducer. (Force Channel Function pop-up menu, select Bridge Pod, then adjust to zero).
5. Set up your mounting stand with the Force Transducer mounted on the micropositioner (as in the toad heart experiment).

## B. Lobster dissection

***Work quickly!! Lobsters do not last as long as toads - as little as 30min or as long as 1hr.***

**1.** Use duct tape to secure the lobster to the dissection tray so that it does not move during the lab session. You will need a lot to wrap around the bottom of the tray. It does not stick if wet. Make sure to securely tape down the powerful tail and the claws.

2. **Pack the lobster with crushed ice to keep it cool! This step is crucial because Maine lobsters come from cold (near 0C) water; allowing them to warm for long periods will hasten their demise. Keep all solutions cold. Add more ice as it melts.**

3. Study where you will cut the exoskeleton on the top center portion of the cephalothorax to expose the

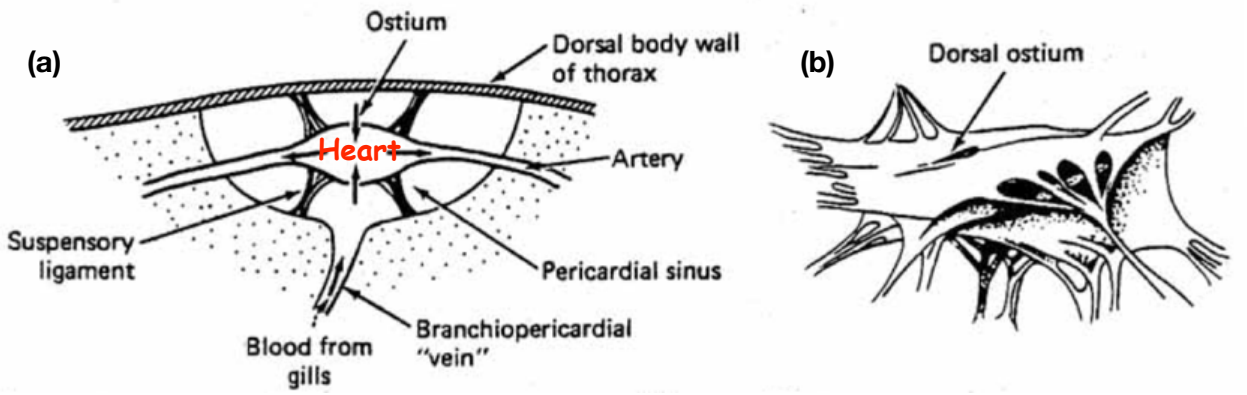


Figure 2. Lateral view of the heart and surrounding tissues. (a) Cross section. The heart is just under and connected to the carapace via suspensory ligaments. (b) The ligaments surrounding the heart.

heart (Fig.1). In the next step, you will **carefully** cut a 1" square piece of the exoskeleton from the top center portion of the cephalothorax to expose the heart. The heart will be whitish and pulsating and the hemolymph is translucent. Be **careful not to rip the heart out of the animal** nor to drive the point of the scissors down into the body of the animal – keep the scissors as shallow as possible. **Lobster tissue is VERY SOFT! Please take care.**

**4.** Starting from the posterior margin of the cephalothorax, *start gently and slowly lifting the square of the exoskeleton*, and with a sharp pair of heavy scissors, *begin cutting, little by little, two sides of a 1" square piece of exoskeleton to expose the beating heart*. As you go, *you will have to gently cut the suspensory ligament underlying the exoskeleton* in order to lift and remove the square of exoskeleton (Figure 2b). Cut the top of the square to free the exoskeleton. The heart should continue to beat. Work quickly to hook the heart and replace the tension from the cut ligaments.

5. **Keep the heart irrigated with cold (ice bath) saline solution at all times.**

6. Cut a length of string roughly 18in long. Tie one end to the small barb-less hook with a knot. Attach the small hook through the apex of the heart (as in toad lab). The lobster heart is not under pressure so it's OK to puncture the heart.

**7.** Tie the other end of the thread to the force transducer, using a square knot. Remove the slack in the thread by adjusting the micropositioner on your mounting stand. **Note: Do not over-tighten the thread! (The heart may tear).**

8. **Adjust the string to apply tension to the heart is. Tension is necessary to substitute for what the ligaments (which you cut away) would be doing!**

9. Make sure that the heart and the force transducer are aligned such that the thread is directly vertical and that the force transducer is on a horizontal plane.

10. Place the exposed tip of the thermocouple wire as close as possible to the exposed heart.

## C. Final Setup

==> **Baseline is iced Lobster Ringer's (**Ringer's on **ice) because it is a Maine lobster.** All solutions should be kept on ice (except for warm Ringer's).

- Place the thermocouple close enough to the heart so that it will measure the temperature of the solution bathing the heart.
- Record baseline heart rate for 30 seconds. You should be able to achieve about 6-7C for baseline. **Record your baseline temperature.**
- You should see a heartbeat waveform in the Force channel. Adjust the tension on the heart with the micropositioner if you get a weak signal in the Force channel, just enough to get a good signal. Do not overtighten.

## D. Effect of temperature on cardiac function

1. Record 30 seconds of baseline data.
2. Bathe the heart in cold Ringer's (from freezer; ~-10°C) for 15 seconds. Once the heart reaches the desired temperature (as close to 0C as possible), record for 30 seconds. Record the temperature.
3. Bathe the heart with baseline (iced) Ringer's until baseline values return.
4. Repeat steps 2 and 3 to obtain cold (from freezer), baseline (on ice), and "warm" (room temp) treatments. Record the actual temperature on the heart.
5. Click Stop. Return the heart to baseline temp before continuing to Part E

## E. Starling's law of the heart

*Starling's law addresses cardiac performance when cardiac muscle is stretched*

1. Click Start and record 10 seconds of baseline data.
2. While recording, slowly increase the tension on the heart by turning the micro positioner knob by 1mm. Add a comment "1 mm stretch". Record for 15 sec.
3. Repeat set 2, 1mm at a time. Try to obtain data for a total of 3mm stretch from the baseline condition.

4. Immediately return the micropositioner to its original position to reduce the tension on the heart.
5. Click Stop.
6. Allow the heart to recover for two minutes before proceeding to Part F.

## F. Effects of drugs on the heart

*You will be provided with a suite of variants of saline solutions that contain cardioactive neurotransmitters, other drugs that may affect cardiac activity, and solutions that have altered ion composition. For each experiment, keep the heart at a constant baseline (low) temperature.*

**Be sure to apply these drugs in the order indicated.**

**Glutamate**
**Dopamine**
**Epinephrine**
**GABA**
**Serotonin (or 5HT)**
**Sodium-Free**
**Sodium-Enriched**

***FOR EACH DRUG:***

- Record 15 seconds of baseline data.
- Apply two or three drops of the drug to the heart. Comment.
- Record 15 seconds of good data (that means 15 sec after it stabilizes).
- Rinse the heart with baseline (iced) Lobster Ringers solution and allow the heart two minutes to recover.

Also try demonstrating heart rate changes by tapping on the carapace

**CLEANUP! Save your data to your flashdrive.**

# LAB REPORT:

In this laboratory, you have not been given specific instructions on which data to record, which analyses to perform, or which questions to answer. This is intentional. The expectation is that you will be able to use the structure of the lab approaches in this and the previous week's labs to formulate your own questions about the experiments you did today and to perform your own analyses. The hallmarks of good scientific writing are brevity, clarity (unambiguousness), logical flow, and beautiful ideas. **Please be direct, to the point, and concise! so that your ideas can shine.**

**Introduction:**
What are the ideas involved in this experiment? Do not regurgitate the background sections provided in the lab manual. Instead, be thoughtful and introduce the ideas that you need to set up your hypotheses. (Avoid any off-topic background or ideas - get to the point). Present the questions that you are addressing and the hypotheses you are testing. Be sure that the rationale for your hypotheses are clear.

**Methods:**
Briefly describe the methods that you used to test your hypotheses. Cite the lab manual for setup and standard procedures. Focus on experimental design (the intellectual content of what you are doing). The amount of information in your methods section should be sufficient for someone with the lab

manual to be able to understand how you tested and analyzed your hypotheses and be able to reproduce your study. Be sure to include any deviations!

**Results:**
Tell us what you found. Describe the outcome of your experiments and observations in a manner that best addresses your hypotheses. To do this, you must choose the comparisons and present them in the manner that most clearly shows whether your hypotheses are supported. Use tables and/or figures to show these important results, but **each table or figure must make a point** (i.e., address a hypothesis), and **data should be presented in only one way**; do not present the same data in the text and as a table, or as a table and a figure. Your written results section can be brief, but you must state the findings that address each of your hypotheses (and refer to any figures/tables). Present, but do not interpret your results. Interpretation is next.

**For the Discussion:**
Interpret the results of your experiments in the context of the hypotheses stated in the introduction. Focus each part of the discussion around a specific hypothesis. What do your results mean? How could different aspects of what you explored work together? Which might be more important? Also, you may want to think about the differences between neurogenic and myogenic hearts (lobster heart vs. toad heart). Toward the end, conclude by including a short statement about what was learned in this experiment, and what new directions could be taken in follow up studies (be specific).

Citation:

Cooke, I.M., Butler, M.A. (2015). Zool430L Animal Physiology Lab Manual: #6 The Neurogenic Crustacean Heart.