Lab #5: Physiology of the *in situ* Amphibian Heart

This experiment explores the basic principles of cardiac muscle physiology, including contraction force, ECG, the effect of temperature, and the effect of neurotransmitters on cardiac regulation in a myogenic heart (Withers pp. 715-719 or HWA 651-654).

Background

Studies of isolated organs were pioneered in the late 19th century when scientists such as Sidney Ringer (1835–1910) developed a perfusion solution (Ringer's solution) that could sustain an isolated organ from a pithed animal. A classic example of this phenomenon is the frog heart, which will continue to beat *in situ* for several hours allowing for the study of basic cardiac functions.

The heart is made up of specialized tissue called cardiac muscle. Cardiac muscle differs from skeletal muscle both morphologically and functionally. Probably the most striking and fascinating feature of its contractility is that it is able to initiate its own rhythmic contractions **intrinsically** without requiring **extrinsic** stimulation from outside the heart. This occurs because cardiac muscle cells have "leaky" cell membranes that allow calcium and sodium ions to slowly leak into the cells. This leaking causes a slow **depolarization** to threshold, thus causing the firing of an action potential, and initiating muscle contraction. The cells that are most "leaky" to ions and that depolarize fastest control the rate of contraction of all other cardiac cells; thus, they act as **pacemakers** for the rest of the heart. The depolarization of pacemaker cells spreads to the entire heart via electrical connections between cardiac muscle cells called gap junctions.

Although no external stimulation is required to maintain the heartbeat, cardiac rate and output is highly modulated by extrinsic signals from the ***sympathetic*** (synapses in brain or spinal cord) and ***parasympathetic*** (synapses in other tissues) **autonomic nervous systems** (see Withers pp. 352-355 or HWA 406-409). These nerve fibers release a variety of **neurotransmitters** (see Withers pp. 239-243 or HWA pp. 342-343), which can increase or decrease the rate and contractile properties of the beating heart. These molecules are able to influence heart rate by changing the rate of spontaneous depolarization of the heart's "pacemaker" cells, located in the sinoatrial (SA) and atrioventricular (AV) nodes of the mammalian heart. In the frog, the **sinus venosus** is analogous to the SA node. Molecules that either increase or decrease the heart rate are called **chronotropic (chrono- for time) agents**, and molecules that alter contractility of cardiac muscle are called **inotropic agents**. Signals from the sympathetic nervous system accelerate cardiac activity while signals from the parasympathetic nervous system decelerate cardiac activity.

The heart also responds directly to physical factors such as **temperature**. For ectothermic organisms, body temperature can and does change, sometimes rapidly during day-night transitions or other typical environmental temperature variations. Temperature change exerts its effect on heart rate through both intrinsic and extrinsic means. Intrinsic effects (internal to the animal) are those that impact temperature-sensitive aspects of cardiac metabolism, cellular, and subcellular processes. Extrinsic effects (external) are modulated through the temperature sensory mechanisms of the nervous system.

In addition, the contractile properties of the heart are dependent upon the physical characteristics of the cardiac muscle fibers. In the early 1900s, Ernest Starling's investigations demonstrated that the **energy of cardiac contraction** is proportional to **the initial length of the cardiac muscle fibers**. This statement became known as **Starling's law of the heart**, a major concept in cardiovascular physiology.

Methods

In this laboratory we will perform experiments on the exposed heart from the cane toad, *Rhinella marinus* (formerly *Bufo marinus*) to demonstrate the effects of intrinsic, extrinsic, and physical factors on cardiac function. The effects of **temperature** change on the surgically exposed toad heart will be observed by bathing the heart with warm or cold Ringer's solution (i.e., dropping directly onto the heart). **Starling's Law** will be observed by **stretching** the ventricle. You will also demonstrate the **effects of direct stimulus** on heart function as well as the **block of intrinsic signaling** from the atria to ventricle. The effects of extrinsic factors of the **sympathetic** and **parasympathetic** nervous systems will be demonstrated by application of Ringer's solution containing physiological amounts of **neurotransmitter** directly on the heart.

Required Equipment

PowerLab
Force Transducer
Stimulator Bar
BioAmp + microhook lead wires
Mounting stand with micropositioner
Thermocouple Pod and thermocouple
Suture thread
Straight pins
Barb-less hook
Dissection tools
Eyedropper
Ringer's solution, room temp
Cold Ringer's (5°C)
Warm Ringer's (40°C)
Ringer's with:
- Acetylcholine (0.1 mg/mL)
- Epinephrine (1% solution)
- Pilocarpine (2.5% solution)
- Atropine sulfate (5% solution)
- Caffeine
- Cadmium Chloride
- KCl

Calcium-free Ringer's
Potassium-free Ringer's

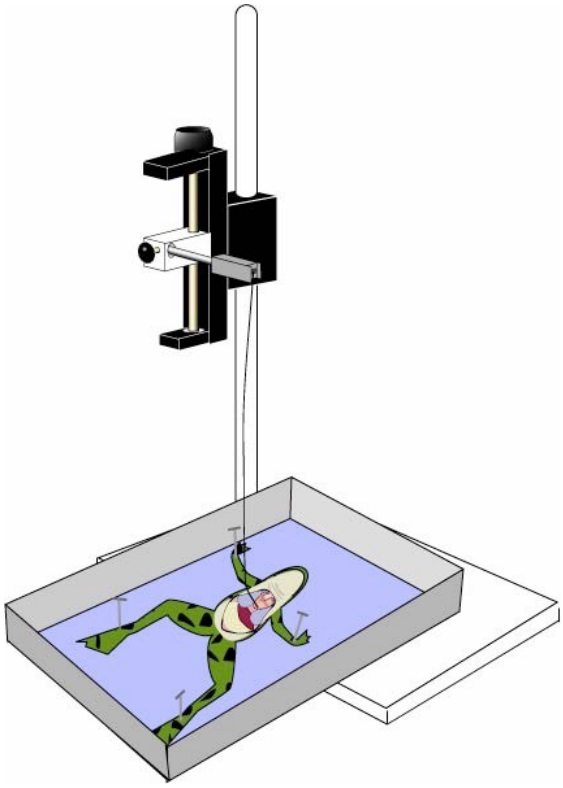


Figure 1. Setup of dissected toad and Force Transducer.

Procedures

A. Setup and calibration of equipment

1. Set up your mounting stand with the Force Transducer mounted on the micropositioner (Figure 1).
2. Connect:

   Force transducer -> PowerLab (pod Input 1).

   Thermocouple -> t-Pod -> Input 2.

   Hook electrode (micrograbber) leads into the BioAmp cable, BioAmp -> Input 3.
3. Software setup:

   "Toadheart" Settings file.

   Select Bridge Pod from the Force Channel Function pop-up menu.

   Zero the bridge pod reading by using the zeroing knob on the front of the bridge pod.

B. Toad dissection procedure

Refer to the toad dissection guide for diagrams of this procedure.

1. Obtain a double-pithed toad from your instructor. Secure the animal ventral side up to a dissecting board using straight pins.
2. Lift the **abdominal skin** with forceps, make a longitudinal incision from the thorax to the abdomen using a scalpel or scissors.
3. Peel back the skin to expose the sternum and ribs.
4. Using sharp scissors, cut through the **sternum** to expose the **thoracic cavity**. You should see the **heart** in its **pericardial sac**.

5. Grasp the **pericardium** with forceps and carefully cut it away, exposing the heart. Apply Ringer's solution to the heart every two or three minutes to prevent desiccation.
6. Attach a small hook tied with thread through the toad's heart. Push the hook through the apex of the heart. **Note: Be very careful NOT to pierce the ventricular cavity!** The hook is used to tie the heart to the force transducer to measure the force of heart contractions as they are transmitted through the string.
7. Adjust the micropositioner so that the force transducer is closer to the bottom and you have room to adjust upward. Gently lift the heart away from the animal's body cavity and tie the other end of the thread to the force transducer using a square knot, taking up slack so that the string is straight. Trim the string.
8. Raise the micropositioner to remove any additional slack and bring the heart to a vertical position. **Note: Do not over-tighten the thread! Doing so can damage the heart.** The thread should be vertical and the force transducer horizontal.
9. Place the thermocouple near the exposed heart to record its temperature, but do not touch it.
10. Locate the ECG leads: **Positive**: right wrist, **Negative**: left wrist, **Ground**: right ankle (same as Human ECG). However, you will cut a slit in the skin overlying a large muscle and attach the micrograbber leads in the muscle belly to make good electrical contact. Keep moistened with Toad Ringer's solution.

C. Recording baseline heart rate

Record for 30 seconds. You should see a heartbeat waveform in the Force channel and an ECG in the ECG channel. Adjust the tension on the heart with the micropositioner if you get a weak signal in the Force channel, but be careful not to over-tighten the thread. If the ECG signal is weak or noisy, try covering your toad with an aluminum foil "tent" to block electrical noise from the room lights or your computer. Ask your TA to check.

D. Effect of temperature on cardiac function

1. Record 30 seconds of baseline data at room temperature.
2. Irrigate the heart with the icy cold (~5°C) Ringer's solution for 15 seconds (use dropper). Measure heart temperature by placing the thermocouple in the solution close to the heart but **without touching the heart**. Once the heart reaches the desired temperature, record for 15 seconds.
3. Bathe the heart in room temperature Ringer's until baseline values return.
4. Record heart rate at a range of temperatures. Repeat steps 2 and 3 using Ringer's that is ~10°C warmer each time, up to 40°C (**don't cook the heart!**). Try to get readings at the coldest temperature, room temperature, and warm (the highest temperature the heart stabilizes at with 40°C Ringer's).
5. Bathe the heart in room temperature Frog Ringer's before continuing to Part E.

E. Starling's law of the heart

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

1. Record 10 seconds of baseline data. Note the position of the micropositioner (in mm). Comment "Starlings".
2. While recording, increase the tension on the heart by moving the micropositioner 1-2mm at a time (by turning the knob). If 1mm produces a response, stick with 1, if its hard to see, try 2mm. Add a comment to your data file called "stretch Xmm". Record for at least 5 heartbeats.
3. Repeat the stretch 1-2mm at a time to get 4 stretch recordings.
4. Immediately return the micropositioner to its original position to reduce the tension on the heart. Stop recording. Allow the heart to recover for one minute before proceeding to Part F.

F. Effects of drugs on the heart

You will be provided with Ringer's solutions that contain drugs that affect cardiac activity, cardioactive neurotransmitters, and vary in ion composition.

==>> Be sure to apply these drugs IN THE ORDER indicated.

1. **Acetylcholine** - released by the parasympathetic nervous system.
2. **Epinephrine** - released by post-ganglionic sympathetic nerves.
3. **Pilocarpine** - stimulates release of acetylcholine from parasympathetic fibers.
4. **Atropine + Acetylcholine** - Atropine is a plant alkaloid that blocks acetylcholine receptors in the heart. First apply atropine, and then without rinsing the heart, immediately follow the atropine application by an application of acetylcholine.
5. **Caffeine** – increases potassium sensitivity
6. **Potassium-free Ringer's**
7. **Cadmium Chloride** – blocks calcium channels
8. **Calcium-free Ringer's**
9. **High Potassium Ringers**

FOR EACH DRUG:

1. Ensure that there is at least 15 seconds of baseline data before applying treatment.
2. Apply two or three drops of the drug to the heart. Watch for an effect, if none, you can add more. Comment at each drug application. Record 15 seconds of good data.
3. Rinse the heart with Ringer's and allow the heart to return to a stable baseline (recovery).

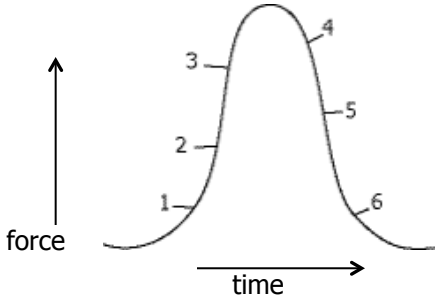
G. *Refractory period of cardiac muscle.*

(Understanding cardiac function through direct electrical stimulation)

The refractory period is the minimum time required for the heart to regenerate the membrane potential required for contraction.

Setup

1. Attach bipolar stimulator to the positive and negative analog **output** terminals on the PowerLab (2 BNCs).
2. Click **Start** to begin recording. In the chart menu, select **Macro: refractory period**
3. Gently place the tips of the stimulating electrode on the ventricle.
4. Click the **Stimulate** button in the Stimulator Panel to stimulate the heart.
5. Stimulate the heart at each of the following times in the cardiac cycle (see diagram below):



Study Questions

- At what point (1–6) were you able to get a second heartbeat?
- What happens to the heartbeat interval when you observe a second beat?

H. Heart Block

1. Using a heavy thread, place a single-loop ligature around the heart at the junction of the atria and ventricle.
2. Take two short pieces of thread and run each one through the ligature on opposite sides of the loop as releasing threads. You will use these to open the ligature at the end of the experiment.

3. Tighten the ligature slowly and observe the beating of the atria and ventricle for changes in rhythm. **Do not tighten the ligature so much that you cut the heart (this is hard to do).**

Can you see any of the following types of heart block? Take notes in your lab notebook.

- **First-degree block.** The interval between atrial and ventricular contraction is prolonged.
- **Second-degree block.** Some impulses fail to reach the ventricle so that the ratio of atrial to ventricular beats is altered. You may see 2:1, 3:1, 5:1, 8:1 types of heart block.
- **Third-degree block.** Impulses fail to pass through the AV node and bundle of His and the ventricle may start its own independent rhythm of beating, or you may see only the atria contracting.

After producing a complete heart block (third degree), release the ligature by pulling on the release threads and see if a normal AV beat is restored.

BEFORE YOU LEAVE - CLEANUP!!!

Place your toad into a ziplock bag. Rinse all of your dissection gear and set it to dry so that it will not rust.

Suggestions for a full lab (all sections) or IWS **(focus on results and discussion, see IWS for hypothesis)**

Always follow the lab report content guidelines.

Introduction

The introduction introduces the ideas that will go into the study (i.e., the hypotheses). So here you may want to describe cardiac muscle and how the rhythmic activity of the heart is created (why would it be called a "myogenic heart"), and of course include the pacemaker. Describe how signals are transmitted throughout the heart (and you may want to point out some of the ways in which cardiac muscle that differs from skeletal muscle). You will want to hit upon the general mechanisms that can modify the heart rate (that you explore in these experiments). End with hypothesis or hypotheses constructed around the aspects of heart physiology you investigated. Keep it brief, ~3-4 paragraphs for a 5 page report.

Methods

Describe the subject and preparation. Briefly describe the physiological parameters that you measured in order to test your hypotheses (easier to understand if you organize it around the questions). Also describe the techniques and equipment used to measure heart rate and contractile force. Describe the experimental design - the control and experimental treatments (keeping in mind your hypothesis). Describe how you will analyze your data to address the hypothesis.

Results

Recording of the heartbeat and ECG

You may want to include a sample of your data to demonstrate data quality - a screen shot showing a clear trace of the Force and ECG in a cardiac cycle at rest (we should be able to clearly see the wave form or it is not a good figure for checking data quality). Indicate atrial contraction and ventricular contraction.

Collect data (in your notebook) such as indicated by the ECG table below from the resting frog heart. Don't forget to collect data on your other important sources of information (heart rate and force output). As you go through each of the tested variable, the baseline may change, therefore adjust as necessary as you analyze your data. You will of course need to collect matched data for your baseline vs. experimental treatments.

	P-wave and atrial contraction	QRS complex and ventricular contraction	Atrial contractions (beat to beat)	Ventricular contractions (beat to beat)
Time difference (sec) between:				

Effect of temperature

Include a graph (such as a bar plot) showing the effects of temperature on your Toad's heart.

The Q_{10} effect

What is the value of Q_{10} for the toad heart rate?

$$Q_{10} = \left(HR_2 / HR_1\right)^{10/(t_2 - t_1)}$$

Where: t=temperature, HR_1 is heart rate @ t_1; HR_2= heart rate @ t_2

Starling's Law of the Heart

Demonstrate Starling's Law using a scatter plot of force amplitude vs. the relative stretch (% of resting length).

Effect of drugs on heart rate

Demonstrate the effects of each of the drugs on the cardiac cycle. Include how heart rate and force output changed from baseline after applying each drug. Create a bar graph showing the relative heart rate (% of resting heart rate -- how do you do that?) and relative force output for each drug. For all drugs that apply, demonstrate what changes occurred in the ECG analysis.

Refractory Period

On a figure, show where you applied the electric stimulus and the response. Describe where in the cardiac cycle you stimulated and report what resulted from the stimulation.

Blocking

Comment on the timing of atria to ventricle contraction and/or the ratio of the contractions as it applies. What did you observe? What degree of heart block could be inferred through the results?

Discussion

Here are points to consider for enriching your discussion. See grading rubric for overview.

1) Describe the basis for the delay between the atrial and ventricular contractions.

2) How did temperature affect heart rate? What do you suppose is a consequence of being a poikilotherm?

3) What is Starling's Law of the Heart? Does your data support this law?

4) Describe the mechanisms by which the following drugs affect heart rate:
    a) Acetylcholine
    b) Epinephrine
    c) Atropine followed by acetylcholine

5) How long was the delay between the QRS complex and the observed ventricular contraction? Explain the mechanism for this delay.

6) Based on your results, what happens to a frog's metabolism and heart rate in cold weather? Does ambient temperature affect human heart in the same way? What is the significance of this?

7) The effect of acetylcholine on cardiac muscle is the opposite of its effect on skeletal muscle. Can you explain the mechanistic basis for the difference?

8) Epinephrine mimics the effects of which branch of the autonomic nervous system?

9) During exercise, venous return of blood to the heart increases. Based on your knowledge of Starling's Law, what happens to stroke volume during exercise? How would this help?

10) Discuss what a refractory period is in respect to the cardiac cycle, and describe the evidence for the refractory period in the data you collected.

11) What is happening when you are cinching the heart junction between the atria and the ventricle (around the bundle of his)? Based on your observation, what degree of heart block was observed and how can you support your claim with evidence from your data.