

Frog Heart Experiment

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1 Purpose and Background

1.1 Purpose:

To examine the effects of pre-tension, temperature, and various drugs on the frog heart muscle, specifically contraction strength and heart rate.

Background

There are a number of external influences that can affect cardiac output. Some of these are mediated by the autonomic nervous system and others are a response to changes in temperature and ionic concentrations. For background, look in your textbook and class notes for information on the effects of neurotransmitters on cardiac rate and contraction. We will use drugs in the lab that either are neurotransmitters or act through other mechanisms to alter the physiology of the whole heart.

Frog anatomy/physiology

There are a number of excellent web sites you can peruse to find background information relevant to this lab. We will cover some basics in class, but please at least go through the online dissection site at frog.edschool.virginia.edu/Frog2.

The list of possible web sites to visit include:

- frog.edschool.virginia.edu/Frog2: a great dissection site with interactive videos and animations from the University of Virginia.
- <http://www.altoona.psu.edu/academics/www/mns/bioal/Frog/default.htm>. Frog Dissection for Biology 110 Penn State Altoona Biology.
- www.biologyjunction.com/frog_dissection.htm. Massengale's Biology Junction.
- www.biologycorner.com/worksheets/frog_alternative.html A virtual dissection from the Biologycorner.com.

2 Procedure

2.1 Materials

The equipment required consists of:

- Digital camera to take photos of the frog during dissection
- Dissection pan with 4 needles
- Dissection kit you used in the anatomy experiment.
- iWorx ETH-256 Dual Bioamplifier
- iWorx FT-100 Force Transducer
- 2 magnetic clamp stands
- iWorx A-BST-100 Bipolar electrode (nominally for stimulating but also for recording)
- Oscilloscope
- Computer with acquisition program (CB8ChScope)
- 20 ml vials for drug samples
- Plastic transfer pipettes
- Suture needle with thread attached
- medium sized vial containing Ringer's solution, composed of:
 - NaCl: 126 mM
 - KCl: 4.4 mM
 - MgCl_2 : 1.0 mM
 - CaCl_2 : 1.1 mM
 - NaOH: 25.8 ml
 - Dextrose: 11 mM
 - Hepes: 24.0 mM
 - pH: 7.4
 - De-ionized water: to make 2 L,
 - Total Volume: 2 L.
- T-connectors
- Alligator clip
- 8-pin DIN-to-alligator clip
- BNC cables
- Lab notebook

2.2 The instruments

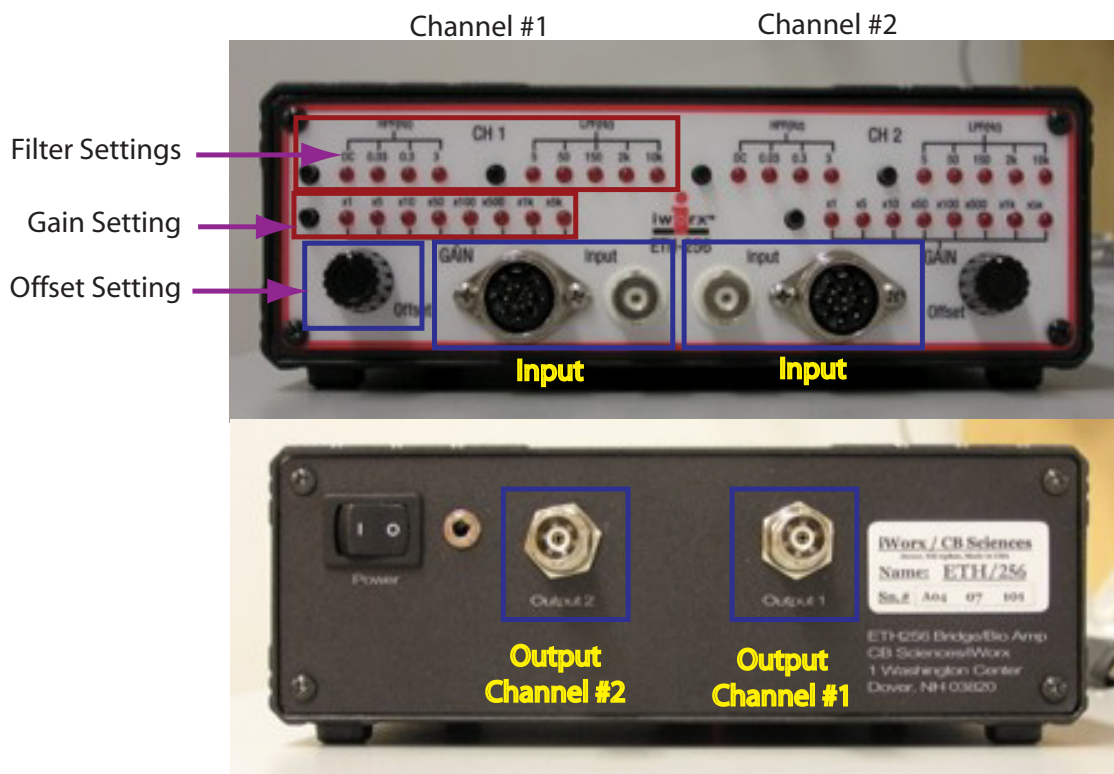


Figure 1: Photograph of the front and back panels of the ETH-256, dual channel amplifier.

The photos in Figures 1 show the dual channel amplifier that we will use in this lab (and others to follow). Dual channel means it can individually amplify two independent input signals. Note the paired input connectors and the fact that either channel can take either a force transducer or just measured biopotentials. When used with the force transducer, the amplifier provides the power to drive a bridge circuit for accurate readings of deflection of the transducer blade.

Note also that the outputs from the amplifier are on the back of the unit, as are the power switch and power cable connector.

Figure 2 shows the force transducer and bipolar electrodes that are the main sensors we will use. Resistors connected to the force transducer blade change resistance proportional to the amount of deflection. These resistors, in turn, are part of a bridge circuit, which produces a change in output voltage proportional to blade deflection. The bipolar electrode consists of two wires that we will place in contact with the heart (or other electrically active tissues) and detect the potential difference between the wires (a *lead*).

Note that both instruments are fragile so treat them with care.

2.3 Preparing the force transducer and circuit

Please carry out the following steps (Note **Do not start the frog dissection until you have completed all the setup steps!**):

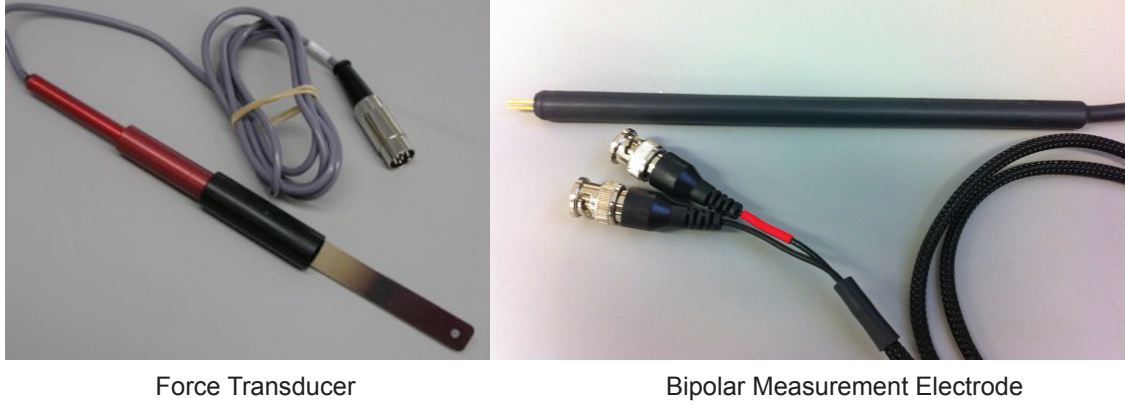


Figure 2: Photograph of the force transducer and bipolar measurement electrode.

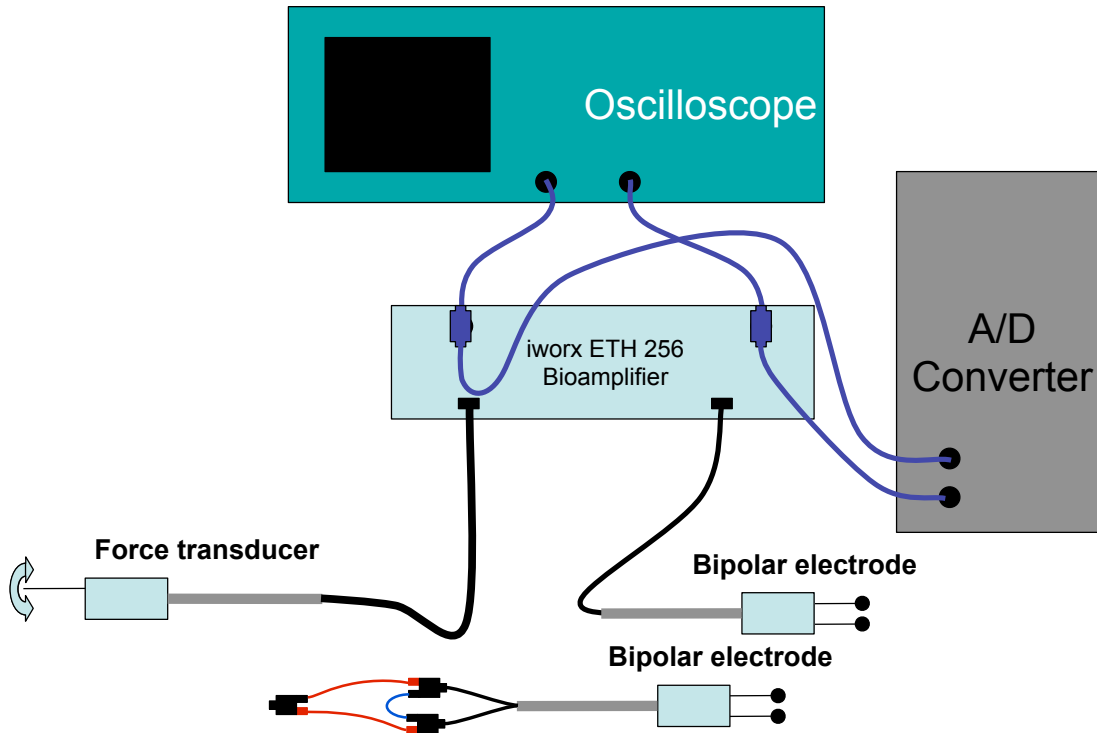


Figure 3: Circuit diagram for the recording of contraction and electrograms from the frog heart. Note detail at figure bottom of the connections for bipolar electrogram.

1. Setting up the measurement circuit according to Figure 3 (force transducer component):
 - (a) Connect the force transducer to the CH 1 8 pin DIN input of the iworx ETH-256 dual channel bioamplifier.
 - (b) Place a T-connector on the corresponding output of the bioamplifier and then connect one end to the input (CH 1) of the oscilloscope and the other to the input (0) for the

computer A/D converter using BNC cables.

- (c) Adjust the settings on the bioamplifier to get a clean signal on the oscilloscope in which you can see the response to **gentle** bending of the force transducer. Start with the following settings on the bioamplifier:

- Low pass filter (LPF) at lowest frequency setting (5 Hz)
- High pass filter (HPF) at DC for the Frank-Starling measurements and possibly at 0.03 or 0.3 Hz for the subsequent measurements.
- Gain at x5–x100, adjust this as necessary.

On the oscilloscope, try the following settings (make sure all settings are in calibrated mode, *i.e.*, latched into fixed settings):

- DC coupling
- 200 mV–5 V/div
- ≈ 0.5 s/div

- (d) Launch the acquisition program (CB8ChScope) computers for acquiring the signals. Then select sampling parameters from the program (sampling rate of 100–200 is adequate) and run it to make sure it acquires a signal. **Note:** Once you have obtained a clean signal, record all settings (amplifier and acquisition program) in your lab notebook. If you ever change a setting, make a note of the new setting and when you changed it in you notebook as well.

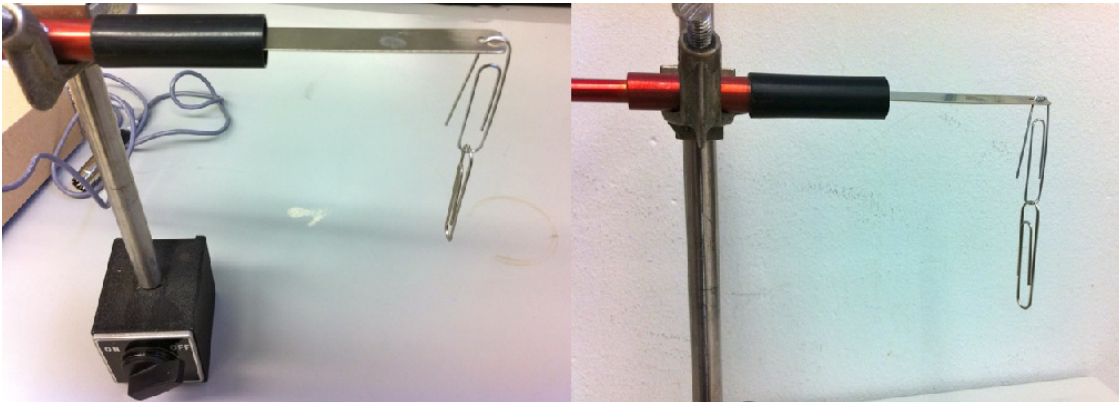


Figure 4: Calibration of the force transducer. Note the use of the magnetic based (left-hand panel) to stabilize the transducer.

2. Calibration of the force transducer (see Figure 4) Note that calibration can occur at the start or the end of the experiment. (What would the advantage be of one option over the other?):
- (a) Mount the transducer in the magnetic stand and rotate the transducer so the flat side of the blade is parallel (horizontal) to the table; deflections in the up and down direction should cause the signal on the oscilloscope to change.
 - (b) The zero-offset is controlled via the offset knob on the bioamplifier. Adjust the offset accordingly to make maximum use of your recording range. Because we do not anticipate

bi-directional movement of the transducer blade (the heart only tugs in one direction), setting the offset to zero will waste half our recording range. Therefore set the offset such that the entire voltage range displayed on CB-Scope (and the oscilloscope) is utilized.

- (c) Weigh a set of 3-4 paper clips of different sizes and then hang them alone and in combination from the transducer blade and note the total weight and the associated deflection of the signal on the oscilloscope (or computer). The resulting table of values will be the basis for calibration of the transducer.
- (d) When you are done, rotate the blade of the transducer back into the vertical orientation and, if necessary, reposition the offset. You should now be ready to perform the measurements of the contracting heart.

2.4 Frog Preparation

Once you have everything set up and the force transducer calibrated, you can move on to the frog preparation as follows (see Figure 5)

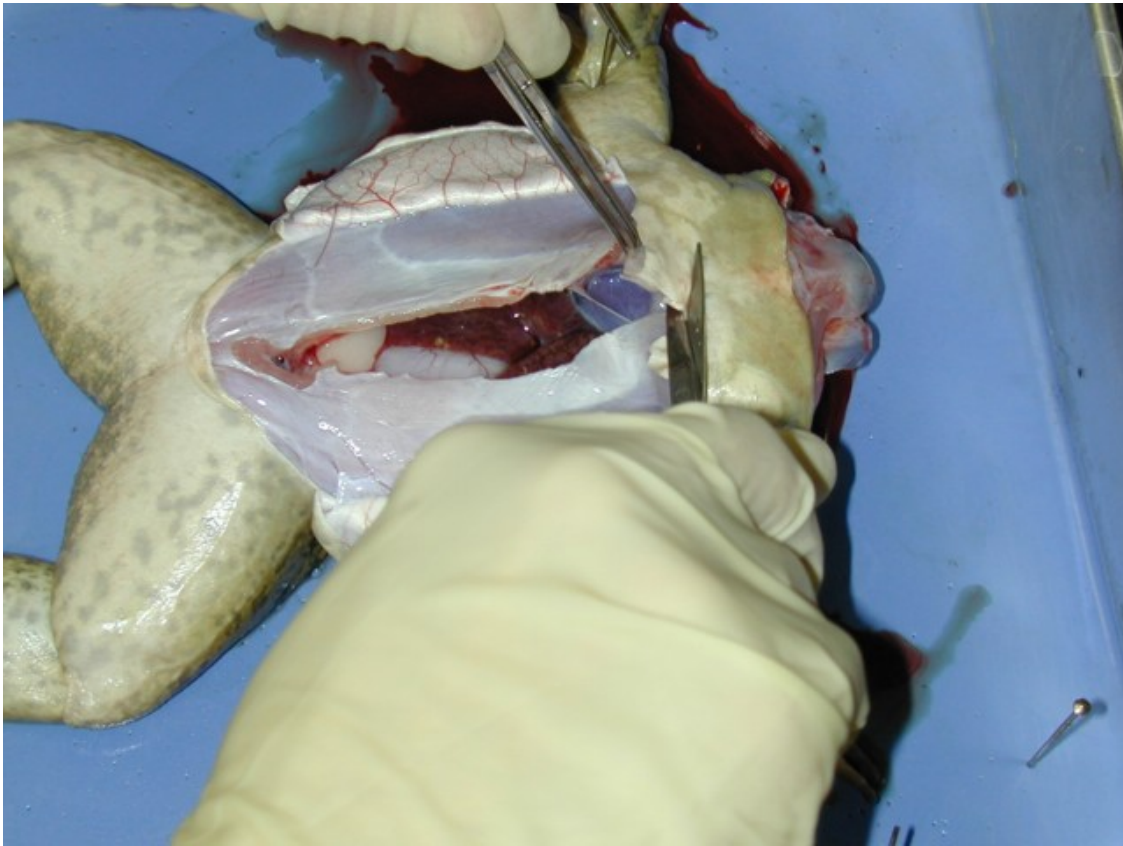


Figure 5: Dissection of the frog showing the open skin flaps, the removal of the sternum and, underneath, the exposed heart inside the pericardial sack.

1. Obtain a pithed frog from the lab TA/Instructor and fix the frog on its back using the big needles in the pan. Open the thorax of the frog with a central incision and two flaps, which

is also shown nicely through a series of images in the web site frog.edschool.virginia.edu/Frog2. Go to the point of the Layer One section and focus on the heart. The point here is not to perform a detailed dissection but to make you familiar with the general anatomy and comfortable with the preparation of the animal. **Do not cut or remove any organs other than the skin and some of the ribs covering the heart.**

2. To expose the heart, make sure to remove the lower and middle sections of the rib cage as they will interfere with the transducer you will use to measure contraction. Cut low enough so that added drugs are able to drain from the thorax. The heart of the bullfrog is quite large and red and should be slowly beating. Figure 5 shows the process of removing the ribs and sternum with the exposed heart below. If the frog is still cold, the rate may be very slow so run some Ringer's solution over the heart to help it warm up. Observe the atria and single ventricle of the heart and note the sequence of contraction of each.
3. Once the heart is open, regular apply a few drops of Ringer's solution to keep it moist.
4. If you have a camera available, take photos of the thorax and mark organs on them. If you do not have a camera, find images from a classmate and label them for your lab report.

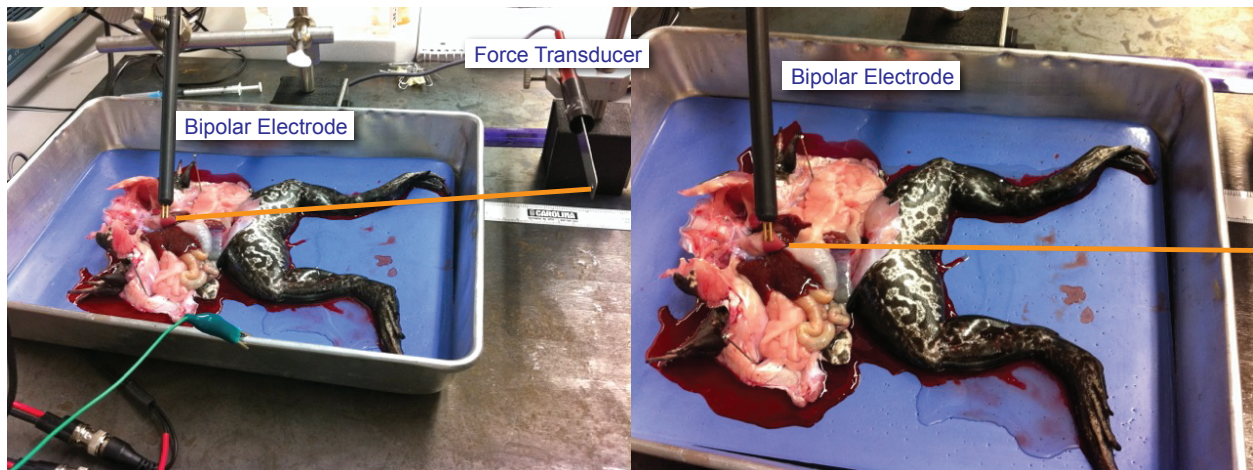


Figure 6: Photos of the complete frog preparation including the thread (enhanced in orange) connecting the heart to the transducer.

5. Attaching transducer to the frog (See Figure 6):
 - (a) Very carefully, cut open and remove the pericardium from the heart so you can see it fully exposed.
 - (b) Using the curved needle and suture provided, run the needle through the lower part of the ventricle, about 3–5 mm from the apex of the heart, and tie a loop with the suture thread. Then clip off the needle and discard it carefully in the sharps container (red plastic). Run the other end of the suture through the hole in the transducer blade and tie a knot there as well. Make sure there is at least 30 cm of suture available between the heart and the force transducer.

- (c) Place the transducer at the end of the pan, elevated 5–10 cm above the edge of the dissection pan with the blade oriented perpendicular to the thread. The thread from the frog heart to the transducer should be quit flat (horizontal) so that you apply tension to the long axis of the heart. See Figure 6 for reference.
- (d) Use alligator clips to attach a wire between the metal dissection tray and the large metal plate on which you are working. This can reduce the electrical noise levels substantially when we start to perform electrocardiographic measurements.
- (e) Now apply enough tension to the thread such that you see a signal on the oscilloscope that reflects the contraction of the heart. Sensitivity of the oscilloscope should be in the range of 500 mV-5V/div, depending on the gain setting of the amplifier. Adjust location and tension so as to generate as clean a signal as possible, ideally one that reveals the separate components of atrial and ventricular contractions. Make sure the tension of the thread is just enough to pull the thread taught and lift the heart slightly. Check also that there is no obstruction from the side of the pan or any other object. Place the pan and the stand well away from the edge of the lab bench and always be careful not to touch the post or the thread accidentally. Otherwise, any change in orientation will alter the resting tension and the reference signals, which will add error to subsequent measurements.

2.5 Acquiring electrograms

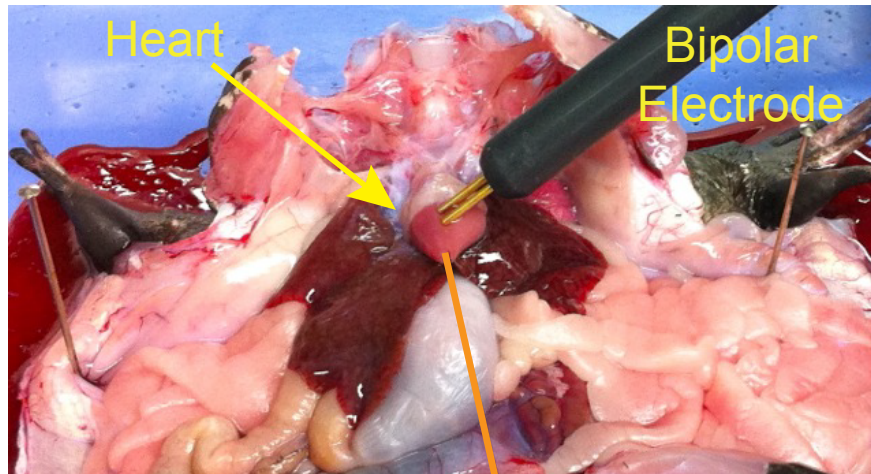


Figure 7: Exposed heart with applied bipolar electrodes. The electrodes should touch the exposed heart lightly on the surface of the ventricles.

Now, to visualize and acquire the “electrogram”, the bioelectric potential from the surface of the frog heart, carry out the following steps referring to Figures 3 and 7:

1. Obtain three BNC to banana converters/splitters. Connect the BNC outputs from the electrode to two of the BNC/banana adapters. Connect the two black banana ends together. Connect the two red banana ends to the remaining BNC/banana adapter and connect it

directly to the input of the bioamplifier. To further improve signal quality, use alligator clips to connect the common negative poles to the ground on the frog (pan and plate) and to the oscilloscope ground (use a banana cable).

2. Attach the bipolar electrode to the second input channel of the iworx ETH-256 bioamplifier.
3. Attach a T-connector to the second output of the bioamplifier and split the output between the second channel of the oscilloscope and channel 1 of the A/D converter.
4. Try the following settings on the bioamplifier:
 - High pass filter (HPF) at 0.03 or 0.3 Hz. The purpose of this filter is to remove the low frequency drift.
 - Low pass filter (LPF) at 50 or 150 Hz. The purpose of this filter is to limit high frequency noise; too low a setting also removes information at cardiac frequencies, especially important for the electrical signal.
 - Gain typically in the range of x10 to x100 but adjust as needed to get good quality signal.
5. Attach the bipolar electrode to a magnetic stand that can lift up and down, and place the electrodes in contact with the heart surface. It should just lightly touch the ventricles but experiment with electrode placement to achieve strong signals. For additional improvement in signal and to reduce sensitivity to heart motion, try placing the electrode underneath the heart, *i.e.*, so that the heart sits on top of the electrode.
6. Record the electrogram together with the contraction signal on the computer and adjust gains, offsets, and filter settings to generate high quality signals.

Note: Once you have obtained a clean signal, record all settings (amplifier and acquisition program settings) in your lab notebook. If you ever change a setting, make a note in your notebook of the new setting and when you changed it.

3 Experimental protocol

There are a set of interventions that you should carry out to see how the heart responds to external stimuli, both mechanical and chemical.

3.1 Response to stretch (Frank-Starling mechanism)

Now try and replicate the effect of the Frank Starling mechanism with the preparation by measuring contraction and progressively stretching the heart to simulate the effect of increased venous return.

You can perform this test without the bipolar electrode connected.

1. Arrange the pan and transducer so that there is just enough tension for the thread to clear the edge of the pan (adjust the height of the transducer if necessary) and for you to get a contraction signal. Take this as the baseline value and make a 5–10-second recording of the

force signal on the computer. **Note:** the signal processing after the experiment will consist of extracting both the tension (force) before contraction and the peak force of the contraction so make sure the signal quality is adequate for these measurements.

2. Move the pan a few millimeters further away from the transducer so that it increases tension on the heart slightly. Again, record a 5–10-second sample of the force signal on the computer.
3. Repeat this process in 5–10 small steps until the heart looks dangerously stretched, at each step recording the force signal on the computer. Check with the TA or instructor if in doubt about how far to stretch the heart.
4. For the report, construct a plot of peak contraction force versus pre-tension force and explain which mechanism(s) explains the results. The pre-tension is the background tension, before (and after) the time-varying signal from the heartbeat. The peak contraction is the difference between the peak tension during the contraction and the pre-tension before the contraction. The resulting plot of peak contraction versus pre-tension should look something like the Frank-Starling curves from the text (or class).
5. **Note: make sure to apply the calibration curve to all the data you acquire from the lab so that units are in grams. While force is measured in Newtons not grams, the latter (a unit of mass not force) is typically used in practice.**

3.2 Thermal and chemical stimulants

1. First obtain **and save** a record of the normal heart contractions in normal Ringer's solution. Save it as a reference on the oscilloscope display so that you will be able to observe the changes in heart rate and contraction strength directly from there. **Repeat this reference recording before each application of a drug!**
2. Apply a few drops of the following solutions directly on the heart and wait long enough to observe their effects. **This may take several minutes so be patient!!**
3. Record and save the tension and the electrogram signals on the computer. Wash with normal Ringer's solution after each solution and record again. For each intervention, save 10–20 seconds of data. If necessary, record several times—you can delete extra files later, however, you cannot come back to re-record signals. The chemicals at your disposal will be some subset of the following:
 - (a) 2°C Ringer's solution.
 - (b) 30 mM of Caffeine.
 - (c) 0.5 mM of Cadmium chloride (CdCl_2).
 - (d) 50 μM of Epinephrine.
 - (e) 1, 5, or 10 mM ACh (start small and only move to a higher dosage **only** if there is no effect).
 - (f) 1 mg/ml atropine (apply this immediately if the heart stops beating after the ACh)
 - (g) 1 M of Potassium chloride (KCl).

4 Lab Report

Make sure to be aware of what is expected in the lab report before you complete the lab; it is too late to obtain data after the lab period is over.

- Observe and record the effects of each intervention on the heart rate and contraction strength.
- Use photos to document your preparation, if only to provide a reference for later recall of what you did. Include a photo in the lab report if you think it is useful.
- Record and compare the ECG and contraction signals between the normal and intervention states. Record signals before each intervention (controls) and then at several times following the intervention. You can always select later which ones you wish to use.
- After the lab, process the data from the experiment in MATLAB (or equivalent), *e.g.*, select a window of data including at least 4 beats, filter the signals if they are noisy, and time align signals that you wish to capture in an overlay plot. From these processed signals generate time signal plots to include in your report. Include in your report plots for contraction and for electrical activity for each intervention, including the baseline recording before each intervention. How you combine them depends on your message and how they look; try to consider overlay plots (with time alignment) but avoid clutter, especially if the signals are noisy.
- Make sure your comparisons are also *quantitative*, especially in terms of changes in heart rate and contraction strength. You should present some kind of processed data for both major parts of the lab (the Frank-Starling curve is processed data from the first part of the lab). Make sure you present this data in as clear a format as possible.
- In the Discussion section, describe the possible mechanisms of the individual effects based either on class room material or a (modest) literature search. Compare your measurements to the expected responses and speculate (carefully and thoughtfully) about the reasons for any disparities.

The **lab report** this time should include the traditional components: Introduction, Methods, Results, and Discussion. Do not spend too much time repeating the methods but do provide an overview of what you did along with any notable variations from the instructions. Include appropriate statements about the rationale for the methods to indicate that you understand why you did things in the lab. Then focus on the results and discussion. Keep in mind the overall goal of characterizing the response of the heart to external interventions so that for each intervention, you report what you observed and what the mechanism(s) might have been. As an integrative step, try to tie these responses to the natural state of the heart and the frog's interactions with its environment.

Please always include the names of your lab partners in the lab report.