Control of Contraction of the Frog Heart

Rob MacLeod

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1 Purpose and Background

1.1 Purpose:

This lab builds on the previous one using the same basic preparation of the exposed frog heart to which we attach a force transducer to measure contraction. In this lab, we also measure the ECG to provide a measure of the heart's electrical activity and then apply a series of drugs to the heart. The goal is to investigate the response of the heart to a series of substances, many of which arise in the normal function of the frog.

1.2 Background

Background for the preparation is the same as for the previous lab so please see that for pointers.

1.3 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.
- Two bioamplifiers
- Force transducer
- 2 magnetic clamp stands
- Bipolar electrode
- Oscilloscope
- Computer with acquisition program (`C:\\bioen\CB8ChScope`)
- 20 ml vials for drug samples
- Plastic eye-droppers
- suture needle with thread attached
- Batteries for the force transducer

- medium sized vial containing Ringer's solution, composed of:
  - NaCl: 200 ml (stock 4M),
  - KCl: 20 ml (stock 1M),
  - $MgCl_2$: 20 ml (stock 1M),
  - $CaCl_2$: 4 ml (stock 1 M),
  - NaOH: 25.8 ml (stock 1 M)
  - D-Glucose: 1.8 g,
  - Hepes: 11.44 ml (stock 1 M),
  - pH: 7.4,
  - De-ionized water: to make 2 L,
  - Total Volume: 2 L.
- Set of drugs to evaluate (and their dosages):
  1. AcetylCholine: 1, 5 and 10 mM
  2. Atropine: 1 mg/ml
  3. Cadmium Chloride: 0.5 mM
  4. Caffeine: 30 mM
  5. Cold Ringer's solution
  6. Epinephrine: 50 uM
  7. KCl: 1 M

1.4 Effect of drugs

Each of the drugs you will apply in the lab will have some effect on the function of the heart, one that usually relates to the response of individual cardiac cells to the drug. See the list of drugs below and your class notes and support text for explanations of the expect response to each drug.

2 Procedure

2.1 Preparing the force transducer and circuit

Please carry out the following steps—these are the same steps as in the previous lab. Figure 1 shows the circuit diagram. (Note **Do not start the frog dissection until you have completed all the setup steps!**):

1. Setting up the measurement circuit:
   (a) Connect the battery to the pressure transducer and hook up the wires from it to the input of one of the bioamplifiers.
   (b) Place a T-connector on the output of the bioamplifier and then connect one end to the input of the oscilloscope and the other to the inputs for the computer A/D converter. Use channel one for both the oscilloscope and A/D converter.
   (c) Adjust the settings on the bioamplifier to get a clean signal on the oscilloscope in which you can see **gentle** bending of the force transducer. Start with the following settings on the bioamplifier:

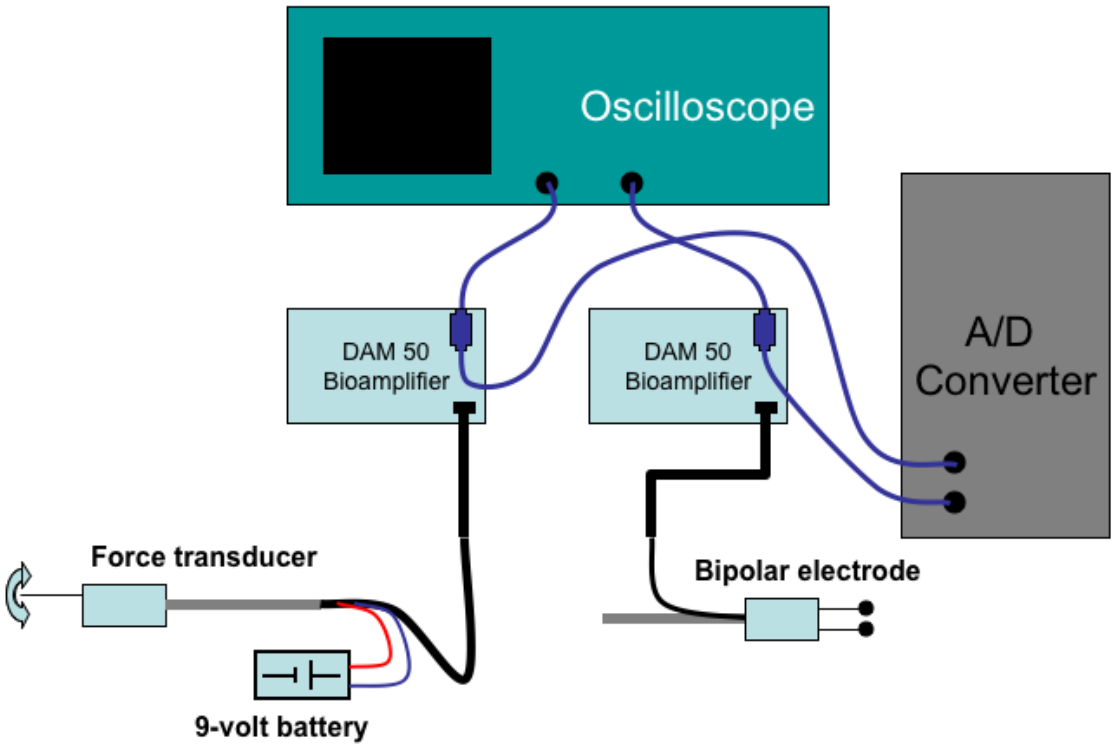

Figure 1: Circuit diagram for the recording of contraction and electrograms from the frog heart.

- DC coupling
- Low filter at lowest frequency setting
- High filter at low to moderate frequency
- Gain at or near maximum

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

- DC coupling
- 1 Volt/div
- $\approx$ 0.5 s/div

(d) Launch the acquisition program (`C:\bioen\CB8ChanScop`) 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 signal.

2. Calibration of the force transducer:

(a) Instead of the full calibration procedure, carry out a single sensitivity measurement with about 2 g of paper clips and note the resulting voltage change. Note this value in your lab report.

2.2 Frog Preparation

Once you have everything set up and the force transducer calibrated, you can move on to the frog preparation as follows (again, just as in the previous lab):

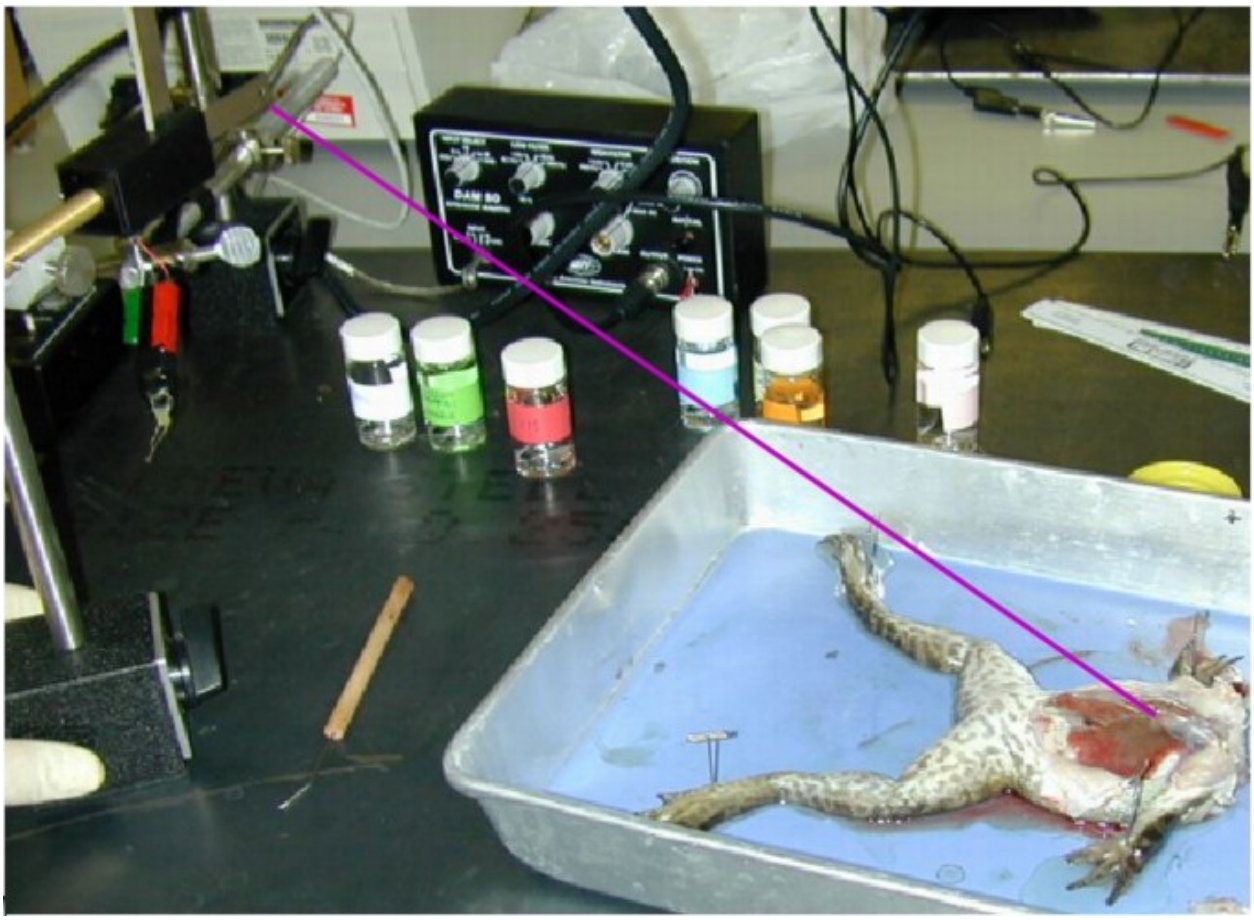

Figure 2: Photo of the complete frog preparation.

1. Obtain a pithed frog from the lab TA/Instructor and fix the frog on its back using the big needles in the pan.
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.
3. Once the heart is open, regular apply a few drops of Ringer's solution to keep is moist.
4. Attaching transducer to the frog (See Figure 2):
   (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 5 mm from the apex of the heart, and tie a loop with the suture thread. Run the other end of the suture through the hole in the transducer blade and tie a knot there as well.
   (c) Place the transducer at the end of the pan, elevated about about 20 cm above the table surface with the blade oriented perpendicular to the thread that connects it to the heart.

The thread from the frog heart to the transducer should be relatively flat (horizontal) (see Figure 2 for reference).

(d) Apply a ground wire between the metal dissection tray and the large metal plate on which you are working. This should reduce the noise levels substantially.

(e) Now apply enough tension to the thread that you start to see a signal on the oscilloscope that reflects the contraction of the heart. Sensitivity of the 'scope should be in the range of 1–5 V/div.Adjust locations 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, but not that it yanks the heart from the animal. 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 accidently touch the post or the thread so as not to change the orientation of lose the reference signals, which will be important later in the experiment.

(f) Obtain a record of the normal heart contractions in normal Ringer's solution. Save it on the computer and as a reference on the scope display so that you will be able to observe the changes in heart rate and contraction strength. **Repeat this reference recording before each application of a drug!**

2.3 Electrogram recording

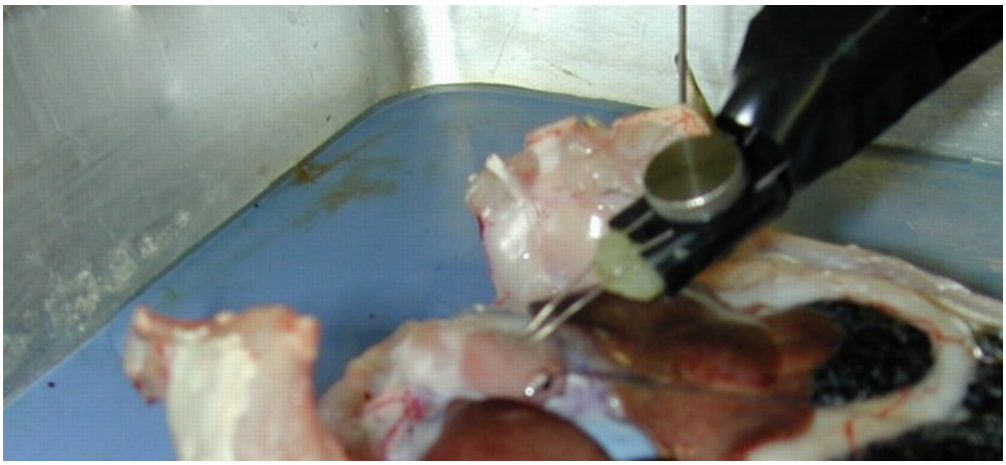

Figure 3: Exposed heart with applied bipolar electrodes. The electrodes should touch the exposed heart lightly.

Now for the electrogram, the signal one can record directly from the heart surface, as follows (see Figure 3):

1. Arrange a second bioamplifier with the output going to the second channel of the oscilloscope and the channel 2 of the A/D converter.

2. Try the following settings on the bioamplifier:

   - AC coupling
   - A-B mode
   - Low filter at lowest frequency setting

- High filter at low to moderate frequency
- Gain at or near maximum

3. Take a bipolar electrode holder, attach it to a magnetic stand that can lift up and down, and place the electrodes in contact with the heart surface.

4. Connect the wire from the electrode to a bioamplifier. Connect the reference lead to one of the pins that hold down the feet of the frog. Adjust the electrode location so as to get a clean signal of both atrial and ventricular electrograms.

5. Record the electrogram together with the contraction signal on the computer.

2.4 Application of drugs

For each of the drugs listed below, record first a stable baseline as control and then apply 2–10 drops of the drug; start small and work up **only if there is no response within a few minutes**. After whatever changes that arise have settled, make a second recording and then wash out the drug with the Ringer's solution.

Please apply the drugs in the order below, especially the KCl! Make sure to take control recordings before each application of the drug and make sure to provide plenty of time an Ringer's solution after each drug to wash it out.

1. Cold Ringers
2. Epinephrine
3. Cadmium chloride
4. Caffeine
5. ACh
6. Atropine (ask the instructor for this drug)
7. KCl

Note the changes you see in the heart after each of the drugs; do not just trust the computer but rather look at the oscilloscope and at the heart itself and write down your observations.

Epinephrine and **ACh** as natural antagonists so if the heart rate or contraction should drop too low, apply epinephrine as needed to restore.

From `www.rxlist.com/cgi/generic3/atrop_cp.htm`:

- **Atropine** is commonly classified as an anticholinergic or antiparasympathetic (parasympatholytic) drug. More precisely, however, it is termed an antimuscarinic agent since it antagonizes the muscarine-like actions of acetylcholine and other choline esters.

- **Atropine** inhibits the muscarinic actions of acetylcholine on structures innervated by postganglionic cholinergic nerves, and on smooth muscles which respond to endogenous acetylcholine but are not so innervated. As with other antimuscarinic agents, the major action of atropine is a competitive or surmountable antagonism which can be overcome by increasing the concentration of acetylcholine at receptor sites of the effector organ (*e.g.,*, by using anticholinesterase agents which inhibit the enzymatic destruction of acetylcholine). The receptors antagonized by atropine are the peripheral structures that are stimulated or inhibited by muscarine (*i.e.,*, exocrine glands and smooth and cardiac muscle). Responses to postganglionic cholinergic nerve stimulation also may be inhibited by atropine but this occurs less readily than with responses to injected (exogenous) choline esters.

3 Lab Report

Combine the lab reports from both the frog labs into a single document that documents your findings. Include very brief background and methods sections and concentrate on showing the following:

- Plots of the measured signals, both contraction and ECG, for each of the drugs, including baseline signals record before each drug application. (Use MATLAB for these).
- Create a table of all the interventions (drugs) that you applied and then list the effect of each on 1) heart rate, 2) strength of contraction, 3) morphology (shape) of contraction signal, and 4) morphology of the electrogram.
- Attempt to explain the nature and the mechanism of the changes you observed after application of the drugs.
- Did conditions return to baseline after every intervention? Why not?

Here are some additional suggestions, most of them general in nature but illustrated using this lab report as an example. The goal is to develop your ability to describe what you did, what you saw, and especially interpret what it might mean. In practical terms, this means a lab report that contains the following:

Introduction: include a brief statement of the purpose of the labs. Do not regurgitate the background sections that I provided in the description but rather try and summarize the important points in your own words.

Methods: **briefly** describe the steps in the measurements you performed; assume you are writing this description for a fellow student with your level of knowledge in physiology and bioengineering. Assume they have access to the lab description but are probably not anxious to read it carefully.

Results: describe in some detail the results of the measurements and observations you made. Include plots and graphs where appropriate and for each one, include some text describing the contents. Again, imagine explaining to fellow students the contents of the figure/graph/table and draw their attention to the features that you consider important or meaningful. Do not hesitate to include observations that are outside the specific tasks or questions in the lab description, especially if they tie into the discussion below.

Discussion: discuss what your results tell you about the behavior under examination. Try and focus each part of the discussion around a specific question or hypothesis and present the evidence for all the possible answers. For example, we proposed that stretching the frog heart would increase contraction; do the data support this presumption? We came up with expectations of the action of all the drugs you applied to the heart; explain each expectation, briefly the physiology behind it, and then describe whether your preparation responded as predicted. If not, speculate about why not and make sure to give reasons for any speculations?

Conclusion: a short statement of what you think you learned form the lab.

The tips for homework assignments provided at
www.cvrti.utah.edu/~macleod/bioen/be6000/homeworks/homework-tips.html also apply to lab reports.