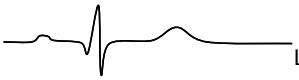
Lab Report Comments

Lab II: Frog Heart Experiment

BIOEN 6000
Spring 2010

Rob MacLeod, Brian Birchler, Cris Lapierre



Technical Aspects

Report: Introduction

- Introduction:
  - Brief background relating to report material; state broad (and specific) objectives and means of achieving them.
  - Specific example:

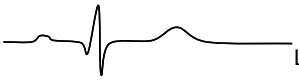
The purpose of this lab was to examine the effects of various drugs and temperature on the pulse rate and contraction of the heart. Both endogenous and exogenous substances can affect all aspects of cardiac behavior, altering heart rate, action potential shape, and strength and sequence of contraction. The animal preparation was the exposed, *in situ* heart of a pithed bullfrog. The experiment used a wide variety of drugs including those naturally occurring as neurotransmitters in the body and some others that are part of clinical treatments. To measure the action of these agents, we used a force transducer attached to the apex of the heart and bipolar electrodes placed in contact with the epicardial surface. Results in general showed a good correlation with expectations based on known mechanisms with some exceptions described in detail.

Statement of purpose. Background. Preparation Interventions and measurements Summary of results and conclusions.

Technical Aspects

Report: Methods

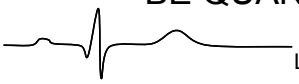
- For the purpose of these reports, an extremely concise summary. Place more emphasis on methods essential to achieving objectives, rather than technical details
- Examples specific to frog lab:
  - "The force transducer was calibrated via the hanging weight method."
  - "We obtained freshly decapitated, pithed frogs and dissected them to expose the heart."
  - "Contraction and electrogram signals were measured with a force transducer attached via suture to the apex of the heart and a bipolar electrode respectively. "
- Provide slightly more detailed statements when examining Frank-Starling and chemical interventions



Technical Aspects

Report: Results

- Concise, factual summary of what happened (no interpretation or explanation)
- Should be the easiest section to write… simply state what the data are.
- Include plots of all relevant data and think about how best to present
  - overlap plots
  - summary plots
- Draw reader's attention to specific features in the results that are relevant to the discussion to follow
- Do not let methods slip into results
- Avoid letting discussion slip into results
- BE QUANTITATIVE



Technical Aspects

Report: Results

(an excellent example from a classmate)

The calibration curve for the force transducer is shown in *Figure 2*. ~~With each successive 0.23 grams added to the force transducer, the voltage of the signal increased~~. The conversion from voltage to weight was found to be 0.103 volts per gram.

Successive stretching of the heart to observe preload caused the resting tension and the force of contraction to increase. *Figure 3* shows how the stretching affected the heart. No reduction in contraction strength was found with increased tension.

The results of the application of chemicals on force of contraction is shown in *Figure 4*. ~~The time as well as the force are the same on all graphs for comparison between chemicals~~. The effects on the heart rate and magnitude of contraction are shown in *Table 1*. The ECG data in *Figure 5* shows how the pacing of the heart changed in response to the experimental conditions.

Both the cold Ringer's solution and the caffeine decreased the heart rate while increasing the magnitude of contraction. The cadmium chloride, acetylcholine, atropine, and potassium chloride all decreased heart rate and the the force of the contraction. The acetylcholine and the KCl both stopped the heart. Epinephrine both increased the heart rate and the magnitude of the contraction. All changes can be seen quantified in *Table 1*. ~~Similarly, *Figures 4 and 5* show the contraction and ECG as measured by the force transduce and the dual electr~~ode. The ECG rate by ~~in~~ large followed the same frequency as contraction.

and

Technical Aspects

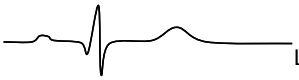
Report: Discussion

- Chance to
  - explain/validate your data
  - explore implications of the results on general mechanisms
- In the case of the frog lab:
  - Report (provide references) established mechanisms of action for various interventions
  - Explain why or why not your data produced the expected behavior (a good place for discussing potential limitations of the lab)
  - Reflect on how the various interventions might arise in the frog's natural environment

Technical Aspects

Report: Conclusions

- Concise summary of laboratory objective, accomplishments, and relevance.
- Mostly a formality (not all journals require this section), but writing a brief recap is good practice (e.g., for writing abstracts for conferences).
- Can be included at end of Discussion or its own section.



Technical Aspects

Figures: Do's and Don't's

- When referring to the figure in the text, capitalize the word Figure.
- Do not include titles above the figure/graph
  - this information goes in caption
  - the only exception is when you have multiple plots/graphs in a single figure
- Excel is barely a report quality plotting tool (unless you really put some time in)
- Scale axes consistently when comparing across multiple figures
- Label axes appropriately (a qualitative description plus correct units)
- Use legends
- Size appropriately to see necessary details (but don't explode it)
  - font size in labels should approximately match that of text
- Incorporate figures smoothly in the text

Technical Aspects

Figures - Captions

- The figure and its caption should be all inclusive. Someone can look at the figure, read the caption, and glean all pertinent information independent from the body of the text.
- Figure captions start with a short descriptive title, then continue with the full sentences, e.g.,
  - *Figure 1: Control values of contraction and electrograms from the frog heart. The upper panel contains a sample of contraction recorded before any intervention under mild pretension. The lower panel contains the simultaneously recorded bipolar electrograms from the exposed ventral surface of the (single) ventricle.*
- Pertinent to frog lab:
  - Each figure caption should have specified the concentration of drugs administered. This is crucial information and the reader should not have to look to the text to locate it.

Technical Aspects

Figures: Examples

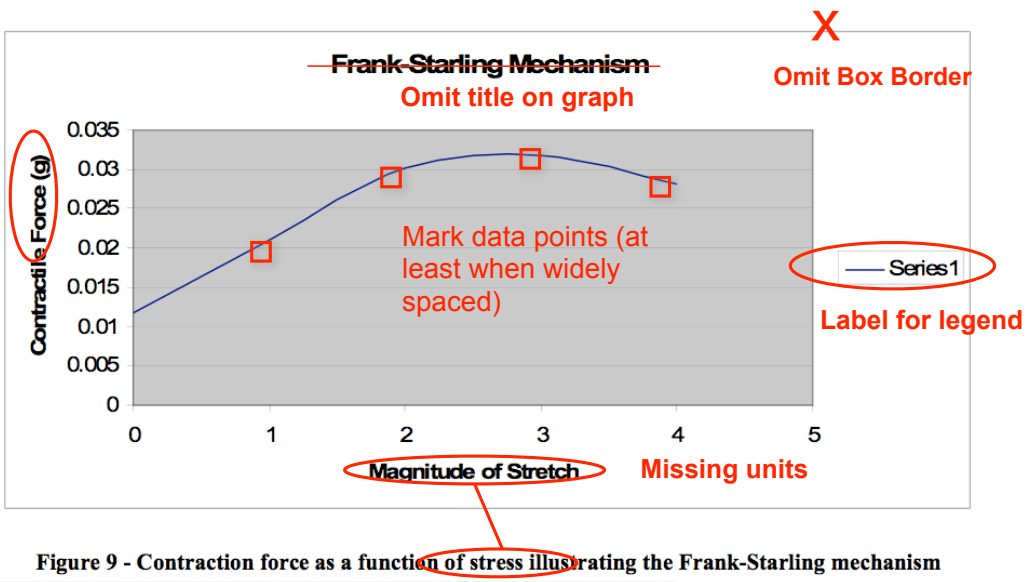


Figure 9 - Contraction force as a function of stress illustrating the Frank-Starling mechanism

Caption is missing details. Use consistent expressions for the same quantities

Technical Aspects

Figures: Examples

Nice layout of time signals

Fonts a little small

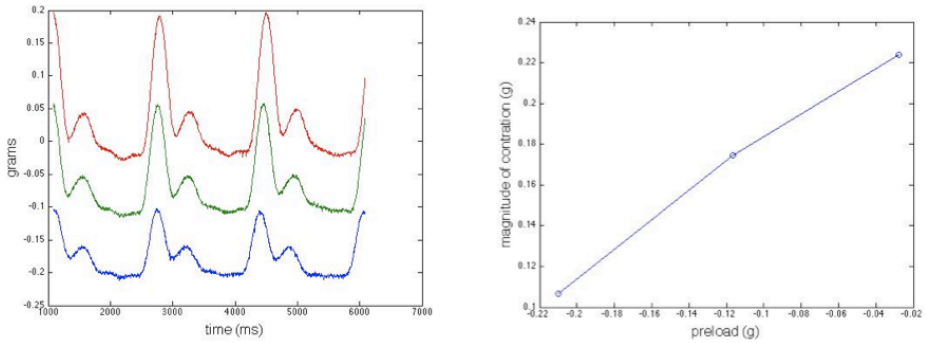


Figure 3: The left shows increased magnitude with increased pretension. On the right, the magnitude of contraction as a function of the preload is shown.

Caption missing title, e.g., “Results of Frank-Starling measurements”.

Technical Aspects

Figures: Examples

Cluttered plots

Poor signal quality

Good legend

Fonts discretized

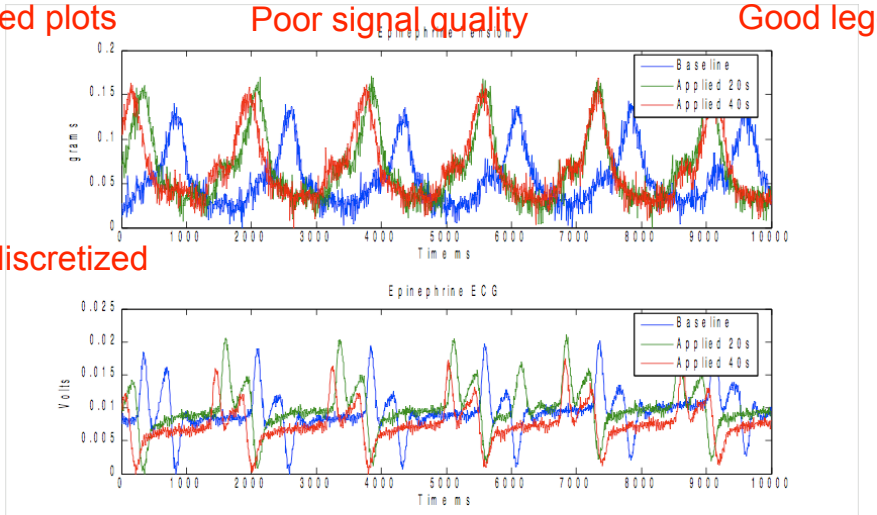
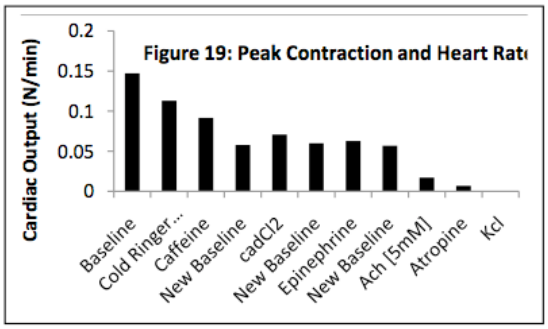


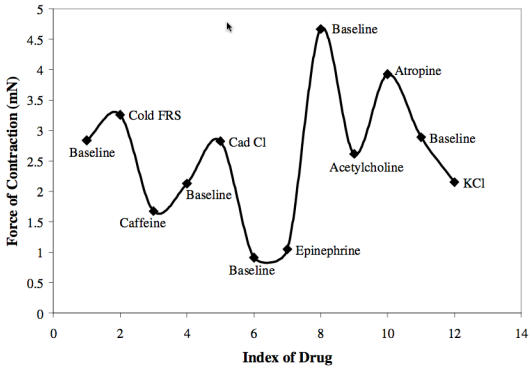
Figure 9: Baseline and Applied Epinephrine Solution. The stimulant clearly increases the tension and amplitude of the ECG.

Needs more explanation and less interpretation.

General Comments I

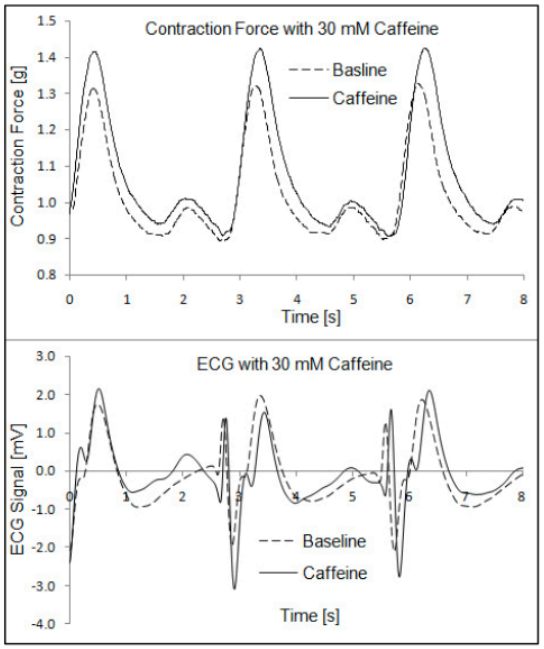
- Overall quality was very high!
- Descriptions of results generally very good to excellent
- Some nice examples of reworking the raw data to illustrate a point; try to replace tables with graphs when appropriate
- But method of extracting single value of contraction rarely documented





General Comments II

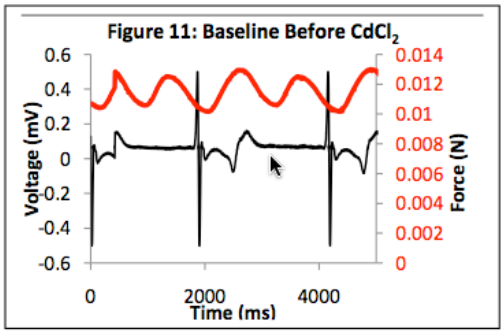
- When comparing signals, time align first beats to allow better comparison
- Use past tense to describe results
- Be realistic in reporting significant digits from a measurement; 5-10% is as good as it gets!
- Include as much data as necessary to justify points in the discussion.

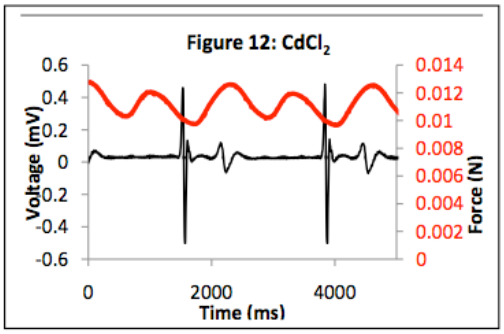


General Comments III

- Electrograms are from the heart
  - no P waves
  - bipolar recordings
- Overlay of contraction and EGs

Note identical scales





Use of Photos

- Labels essential
- Great inclusion of ruler in many reports!

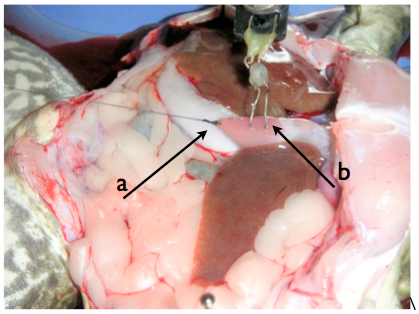


Figure 1: Experimental Set Up. a) Suture in Ventricular Apex b) Bipolar Electrode

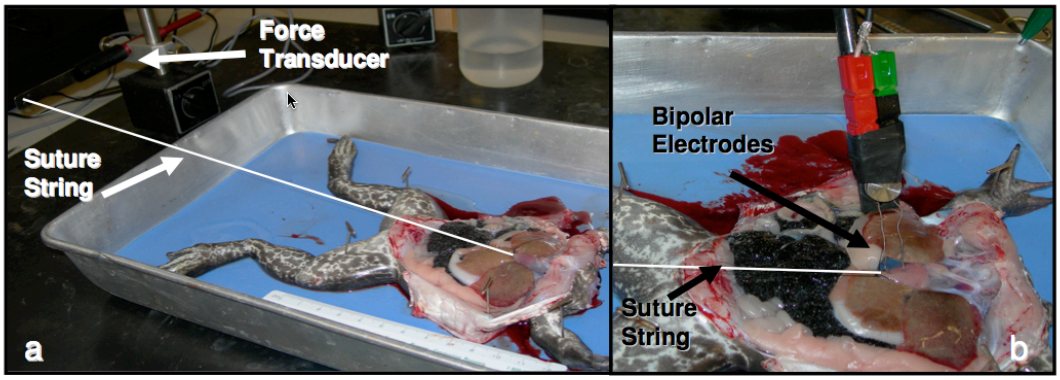
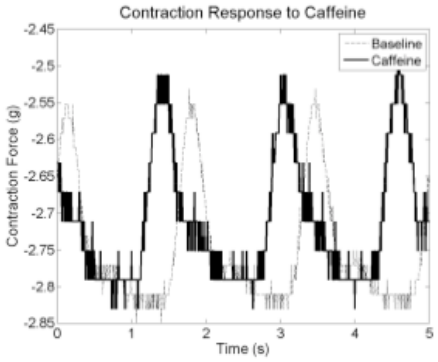
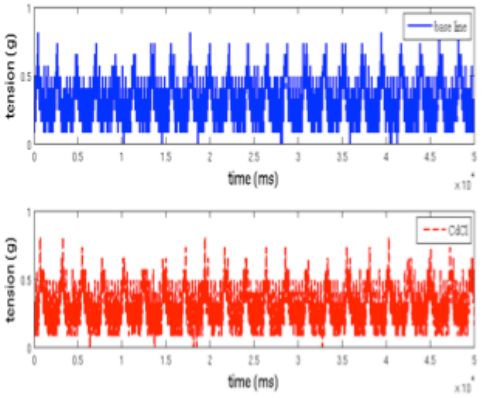


Figure 2. Overview of the test system. (a) The experimental setup for the force measurements. (b) The bipolar electrodes placed on the surface of the frog heart.

Signal Quality

- In a few cases, poor signal quality because of discretization errors (gain too low)
- When data unusable, borrow (with credit) from others





Signal Quality II

- Gain has to match A/D converter

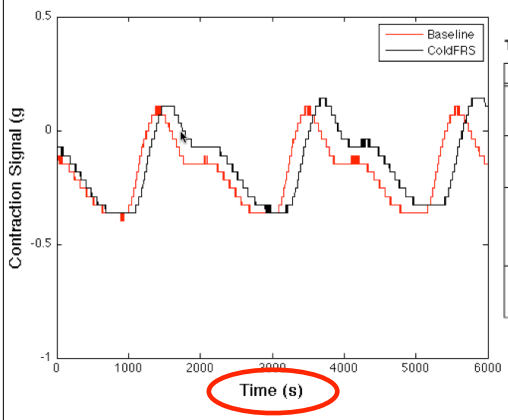


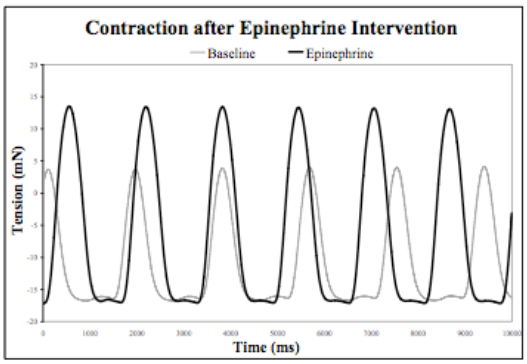
Table 1. Common ADC Converter Resolutions

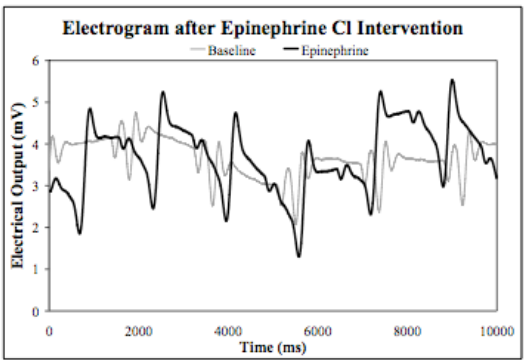
	8-bit	12-bit	16-bit	18-bit	24-bit
Distinct Levels	256	4,096	65,536	262,144	16,777,216
Resolution, ±10 V scale	78.4 mV	4.88 mV	305 µV	76.4 µV	1.192 µV
Resolution in °C, K type TC, ±0.5 Volt Full Scale input range (~25 °C)	97.7	6.10	0.38	0.10	0.00149
Dynamic Range in dB	48.2	72.2	96.3	108.4	144.5

Units have to match numbers

Electrical vs. Mechanical

- ECG amplitude does not predict contraction!



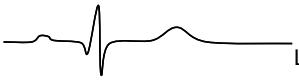


Explanations and Text

- Include adequate text; even without figures, there should be enough text to tell the story.
- Keep striving for conciseness and specific word choices.
- Excellent explanation of mechanisms but some reports needed (more) references.
- Commas are free so use them!

Suggestions?

What would you do differently next time?



Chemical Interventions

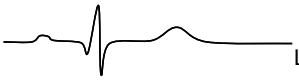
Caffeine

- Limited knowledge of caffeine's influence on amphibian cardiac physiology
- Caffeine does not cause unloading of the SR as in mammalian physiology
- Caffeine does cause an increase in the sensitivity of myocardial contractile elements (actin thin filaments) to calcium, although the mechanism is unknown
- Theoretically, this should cause an increase in contractile properties of the heart

Chemical Interventions

Cadmium Chloride ($CdCl_2$)

- Divalent cadmium ion (Cd^{2+}) competes with Ca^{2+} for entry into the myocardial cell via the L-type calcium channels.
- Cd^{2+} lacks the specificity to interact with the Ryanodine receptor of the SR or the actin thin filaments within the sarcomere.
- Overall effect is a reduction in contractility



Chemical Interventions

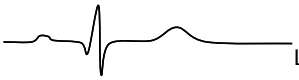
Epinephrine

- Secreted by the sympathetic division of the autonomic nervous system; acts primarily on β adrenergic receptors within the heart
- A positive inotropic and chronotropic agent
  - Increases Ca^{2+} conductance in pacemaker cells of SA node, accelerating the heartbeat (more rapid depolarization via I_f)
  - Increases Ca^{2+} conductance in myocardial cells and enhances Ca^{2+} uptake by SR
    - Enhances speed and strength of contraction
    - Shorter systole means longer diastole… increased ventricular filling increases cardiac output
- Epinephrine facilitates excitation in all elements of the heart (SA node, atria, AV node, Purkinje system, ventricles)

Chemical Interventions

Acetylcholine (ACh)

- Secreted by the parasympathetic division of the autonomic nervous system, specifically the branch of the vagal nerve that innervates the heart; acts on muscarinic receptors
- Primarily a negative chronotropic agent, although has some effect on ventricular myocardium as a negative inotropic agent
  - Increases hyperpolarization in cells of SA node by enhancing I_k and reduces depolarization by decreasing I_{Ca} and I_{na}; results in an overall reduction of heart rate
  - Same mechanisms can result in an AV node block
  - Reduced calcium current in ventricular myocytes responsible for slight reduction in contractility



Chemical Interventions

Atropine

- Reduces parasympathetic (vagal) activity on heart by competing for binding sites of muscarinic acetylcholine receptors.
- In a normal individual this acts to increase heart rate (vagal tone usually dominates).
- In the denervated frogs (decapitated so no more autonomic control) it simply acts to reverse the effect of the applied ACh.

Chemical Interventions

Potassium Chloride (KCl)

- Excessive increase of extracellular potassium causes the following:
  - permanent depolarization (resting potential normally maintained by a high intracellular potassium concentration)
  - loss of excitability
  - eventual cardiac arrest during diastole.