This PRO Lesson explains how to prepare the frog heart and describes the hardware and software setup necessary to record cardiac rate and contractile responses of the frog heart.

**Introduction**

The vertebrate heart is *myogenic*; that is, the beat originates within the heart without the need for an external stimulus to be delivered by the nervous system or the endocrine system. Although the heart generates its own beat, external agents can alter the rate of the heartbeat and in some cases the strength of the heartbeat. Agents that either increase or decrease the heart rate are called *chronotropic agents*. Chemicals that alter contractility of cardiac muscle are called *inotropic agents*.

In the laboratory the surgically exposed frog heart can be used to study the effects of *autonomic control of heart rate*. The effects of sympathetic stimulation can be simulated by applying epinephrine directly on the heart. Indirectly, sympathetic effects on heart rate can be observed by blocking parasympathetic input with atropine sulphate, a chemical that prevents acetylcholine action.

The effects of *parasympathetic stimulation* can be observed by applying acetylcholine directly on the heart, or by dropping pilocarpine directly on the heart. Pilocarpine facilitates the release of acetylcholine from the vagus thereby simulating increased parasympathetic stimulation.

*Temperature change* exerts its effect on heart rate through influence on the nervous system as well as directly affecting cardiac metabolism. The effects of temperature change on the surgically exposed frog heart can be observed by dropping warm or cold frog Ringer’s solution directly onto the heart.
Objectives

1. To observe and record atrial and ventricular systole and diastole in the frog heart.
2. To observe and record the cardiac phenomena of refraction, ventricular extrasystole, and the compensatory pause in the frog heart.
3. To observe and record the effect of increasing and decreasing the temperature of cardiac muscle on cardiac rate and contractility.
4. To observe and record the effects of acetylcholine, atropine, pilocarpine, and epinephrine on the frequency and amplitude of cardiac muscle contraction in the frog.
5. To observe and record the positive inotropic effect of digitalis on the frog heart.
6. *optional:* To observe the property of myogenicity in the excised frog heart.
7. *optional:* To observe and record the effects of atrioventricular block induced by Stannius ligature in the frog heart.

Equipment

- Force Transducer Assembly (*SS12LA* includes S-hooks)
- Weights for calibration (must attach to S-hook)
- BSL PRO template file: *FrogHeart.gtl*
- Drug preparations:
  - Acetylcholine 2.5%
  - Atropine 5%
  - Digitalis 2%
  - Epinephrine 1%
  - Pilocarpine 2.5%
- Live frog
- Amphibian Ringer’s solution
- Goggles
- Droppers
- Examination/surgical gloves
- Dissection Kit: Scalpel, Forceps, Scissors, Dissection Pins, Tape
- Glass probes (2+)
- Dissection Pan
- Acrylic Board
- Thread (non-stretch nylon or equivalent)

Setup

If you are setting up the hardware and software from scratch, then you may want to perform these steps prior to prepping the frog since they may take some time to complete.

**Hardware:**

1. Connect the SS12LA to CH 1 on the MP30, as shown here:
2. Turn the MP30 ON.
3. Launch the BSL PRO software on the host computer.
   - The program should create a new "Untitled1" window.
4. Bring up the Frog Heart template by choosing File menu > Open > choose Files of type: GraphTemplate (*GTL) > File Name: FrogHeart.gtl.
   - You may need to re-position the windows such that the stimulator and data windows are in full view with the stimulator window lying to the right of the data window.
5. Put the tension adjuster (BIOPAC HDW100A or equivalent) on the ring stand, and attach the BIOPAC SS12LA Force transducer such that the hook holes are pointing down. The SS12LA should be roughly set so that it is level both horizontally and vertically.

6. Set the tension adjuster such that it is approximately ¼ the distance from the lowest setting. This will allow the majority of the range to be used for adding tension (raising the adjuster).
   - Do not firmly tighten any of the thumb-screws at this stage.
7. Select the 0 to 50 grams force range for this experiment.
   ● You want the lowest range to optimize output resolution and 0-50 grams should be sufficient.
8. Select and attach the small S-hook.
   
   The SS12LA comes with two "S-hooks" for attachment of line or weight. Use the smaller S-hook for 0 to 50 grams. Place the smaller S-hook in the proper hook hole on the SS12LA.

**Note:** To calibrate the transducer for the full range, you will need to have a weight representing the upper limit of the range (50 grams) and it must be attachable to the small S-hook.

### Hints for minimizing measurement error:

A. The SS12LA Force transducer must be level on the horizontal and vertical planes.
B. Set up the Tension Adjuster and Force Transducer in positions that will minimize movement when tension is applied. In other words, try to keep the point of S-hook attachment as close as possible to the Ring Stand support (see assembly picture).
C. Position the Tension Adjuster such that the Adjustment knob is easily accessible so you will not bump cables or the tray during adjustment.
D. Position the Force transducer cables such that they cannot be easily pulled or bumped. If necessary, use tape to adhere cables to the tray or ring stand to relieve strain or pull.
E. Set up the dissection tray such that it is very stable and will not wobble or rock.
F. Make sure the end of the frog muscle is firmly pinned to the dissection tray, such that it will not rise up when tension is applied.
G. Position the entire setup on a solid workbench such that it will not wobble if the table vibrates.
H. Use an attachment line that will not stretch under tension (such as non-stretch nylon).
I. Use a knot that will not allow the line to lengthen or come undone under tension.
J. Always keep the line attached to the SS12LA straight up and down (perpendicular to transducer).
K. Do not have excessive pre-tension on the muscle.

### Calibration

No calibration is required when you use the template.

*You are now ready to prep the frog and adjust the SS12LA Force Transducer position.*

### Frog Prep

Double-pith the frog (if necessary, see Application Note BSL-A01 Frog Prep for details). You should apply amphibian Ringer’s solution to the frog in five-minute intervals.

1. **Expose the frog heart**

   Cut the skin from the groin to the throat of the frog.
Cut through the pectoral girdle to expose the heart in the pericardial sac.

2. **Attach a small hook tied with thread through the frog heart**  
   
   *TIP*: Non-stretch nylon thread recommended rather than cotton thread.

3. **Loop the thread from the frog heart to the force transducer S-hook**

   Pull the thread so there is a little tension on the thread.  
   Position the frog so the thread from the heart is vertical, so the heart's not pulling at an angle (you want a truer reflection of the heart's contractile force).  
   Don't worry about the stand height at this point.  
   Use a square knot and tie it twice to make sure it won’t come undone when the heart contracts.  
   Don't worry about the thread tension at this point.  
   Sever the additional thread.
4. **Moisten the frog with Ringers Solution.**

5. **Slowly adjust the transducer stand height** so the connection between the heart and the transducer is relatively taut — be careful not to tear the heart out! Use the fine adjuster knob (at the top of the stand) to remove any remaining slack such that the slack is gone but the heart is not torn out.

6. **Position the force transducer**

   Confirm that the frog preparation is firmly attached to the dissecting pad and/or pan, is positioned below the ring stand and that a line has been tied from the frog heart.
Position the Tension Adjuster and/or Force Transducer such that:

- The top of the Force transducer lies on a level horizontal plane.
- When the line from the heart tendon is tied to the S-hook on the SS12LA, and made taut, it will be straight up and down.
- When the line is made taut, there will be approximately 4 inches of line from the heart tendon to the "S-hook."

7. **Attach the heart line to the transducer**

Slide the tension adjuster/force transducer assembly down the ring stand to a point where you can loosely hang the loop off the S-hook.

Slide the tension adjuster/force transducer assembly up the ring stand to a point where the line slack is removed but the muscle is not stretched.

Adjust the assembly such that the thread line falls vertically.

For a true reflection of the muscle's contractile force, the muscle must not be pulled at an angle.

Tighten all thumb-screws to secure the positioning of the assembly.

8. **Adjust transducer pretension**

Use the tension adjuster knob to make the line taught — very little tension is required.

**DO NOT OVER-TIGHTEN!**

Let the setup sit for a minute, then re-check the tension to make sure nothing has slipped or stretched.

When completed, your setup should look similar to this:
Running the Experiments:

*Note*
This recording is set up for the Append mode, so when the acquisition is stopped then re-started, data will be added onto the previous data. A marker will automatically be inserted with a timestamp to indicate the new segment start time.

To save recorded data, choose

File menu > Save As… > file type: BSL Pro files (*.ACQ) File name: (Enter Name) > Save button

To erase all recorded data (make sure you have saved it first), and begin from Time 0, choose:

MP30 menu > Setup Acquisition > Click on "Reset" button

- Data may be distorted if the transducer line is not pulling directly vertical from the frog heart to the S-hook. Align the frog as detailed in setup and observe to make sure that the heart is not twisting the thread. If it is twisting, you will need to CAREFULLY remove the hook and repeat the setup.
- RATE data is calculated and always trails the actual rate by one cycle.
- RATE data may display artifact from table movement, heart movement (from, for instance, breathing on the heart), and chemicals touching to the heart. To get the best data, keep the experimental area clean, clear and stable.
- Apply temperature variants to the heart before applying drugs to the heart since temperature has no chemical impact on the heart. You do not have to return to normal heart beats between cold and hot; confirm cold effect recorded, apply hot, record hot.
- Record 5-10 cycles per segment. You do not need a continuous record; you do not to record the onset of the effects.

1. Click on Start and record 5 cycles of the normal rate.
2. Click on Stop.
3. Apply solution.
   - Suggested recording sequence is for qualitative data (not quantitative, so you do not need common baseline):

<table>
<thead>
<tr>
<th>Application</th>
<th>Qualitative Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot</td>
<td>increases rate; cold or hot first doesn't really matter</td>
</tr>
<tr>
<td>Cold</td>
<td>slows rate; not necessary to stabilize with room temp Ringers after hot since qualitative study</td>
</tr>
<tr>
<td>Substance</td>
<td>Effect Description</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>rate falls, vigor/amplitude not greatly impacted</td>
</tr>
<tr>
<td>Atropine</td>
<td>blocks acetylcholine = parasympathetic blocker = &quot;parasympatholidic&quot;</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>acts like acetylcholine = &quot;parasympathomimetic&quot;</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>increases amplitude and frequency</td>
</tr>
<tr>
<td>Digitalis</td>
<td>strength of contraction changes, effects amplitude but not so much rate</td>
</tr>
</tbody>
</table>

4. When effect is observed, click on the Start button.
5. Record effect for five (5) cycles.
6. Click on Stop.
7. Stabilize with room temp ringers (not necessary between hot and cold saline/Ringers)
8. Apply next solution and repeat recording loop (jump to next for digitalis).
9. **Flush with ringers solution before applying digitalis.**
10. Check/adjust thread tension — Can’t have slack in transducer line because the increased contractile response induced with digitalis will be used up to tighten the slack and you won’t see that on the record.
11. Check that the heart has returned to normal frequency and amplitude.
12. Apply digitalis.
13. Observe increase in vigor of contraction (increased amplitude).
14. Record for five (5) cycles.
15. Click on Stop.
16. Use File > Save as to save your data file.

**Additional study**
You can tie a Stannius ligature and/or excise the frog heart. Note that the excised heart continues to generate a beat. Note further that if you split the heart chambers, each will generate its own beat, at a different rates.

**Analysis**
The BSL *PRO* offers a wide array of measurement and analysis tools to help you isolate the data segments of interest for your particular experiment.

RATE data may display artifact from table movement, heart movement (from, for instance, breathing on the heart), and chemicals applied to the heart. To get the best data, keep the experimental area clean, clear and stable.
**Normal heart:**

The waveform amplitude and rate may differ based on frog size and vitality, but the nature of the waveform should not vary significantly from the one shown here.

If your data does not resemble the waveform shown, check to make sure that the line from the hook to the transducer is pulling directly vertical and not twisting.

If your line is twisting. You will need to CAREFULLY remove the hook and repeat setup for a direct line.

**Real-time RATE**

The real-time RATE function is calculated from the selected areas and will always appear in the next rate sequence. That is to say, the calculated rate always trails the actual rate by one cycle.

**Drug Effect**

Heart rate and amplitude are effected by temperature and chemical changes, as shown in this graph. The qualitative effect of each is briefly noted below.
Appendix

GRAPH TEMPLATE SETTINGS

Click here to open a PDF of the graph template file settings.