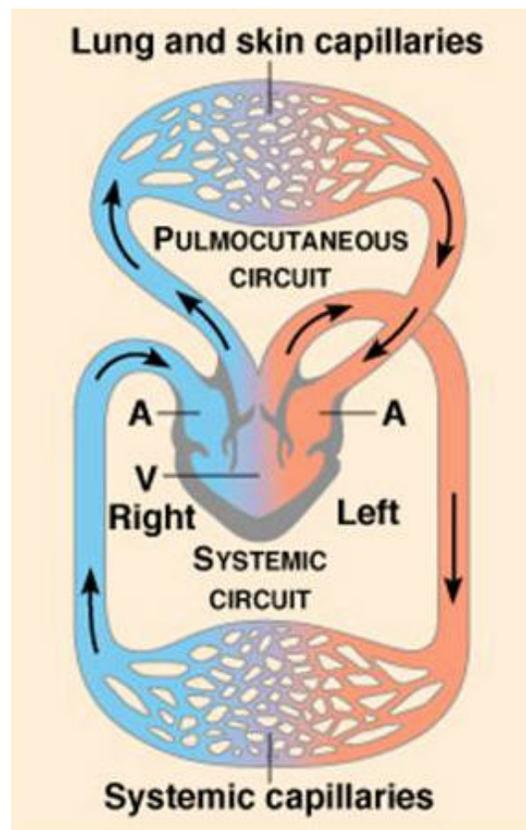


Frog Heart Physiology

iWorx/Jarzem/ Ziser, 2004/Wayne,2005

I. Introduction.

The heart of the frog has three chambers, one ventricle and two atria. Blood leaves the heart from the ventricle through a single truncus arteriosus which is short and soon branches into two aortic arches which loop left and right and dorsal to the heart to rejoin as a single aorta in the mid dorsal region of the body cavity. Each aortic arch has a branch leading to the lungs and skin where oxygenation occurs. Carotid arteries also branch off the aortic arches and supply the head region. Veins bring blood to the left and right atria. Both atria then empty into the single ventricle. Blood from the ventricle thus enters either the pulmonary or body circulation.



Because there is only a single ventricle there is some mixing of oxygenated and deoxygenated blood. Ventricular folds prevent a complete mixing of the arterial and venous blood.

Excitation of the frog heart heart is **myogenic**, that is, contraction of the heart originates within the muscle itself. In Amphibians, such as the frog, the pacemaker is the **sinus venosus**, an enlarged region between the vena cava and the right atrium. This the cells of the **pacemaker** are termed “**leaky**”, meaning that calcium ions leak into the cells. Leaking of positive ions causes a slow depolarization to threshold, thus initiating an action potential that quickly spreads throughout the muscle. The atria are very conductive, and the action potential spreads readily across these two chambers. The major route for the transmission of action potentials from the SA node to the ventricle(s) is by way of a set of modified conductive muscle cells that compose the **bundle of His** embedded in the septum separating the two atria. In animals with a four-chambered heart, it continues through the septum separating the two ventricles.

The cells that are most leaky to calcium initiate action potentials more frequently and set the rate of contraction for entire heart structure. Each region of the heart has its own **intrinsic** rate of beating.

Nervous control of the heart is primarily regulated by medulla of the brain, and the heart is innervated by both sympathetic and parasympathetic nerve fibers that terminate at the SA node. The neurotransmitters released by these nerves affect both heart rate (chronotropic effects; chronos = time) and the strength of contraction (inotropic effects), by influencing the timing and magnitude of ion currents across the cell membrane.

The vagus nerves contain parasympathetic cholinergic neurons that release acetylcholine at their terminals. This neurotransmitter binds with muscarinic receptors and initiates many effects, including a cascade that increases the number of K⁺ channels in the open position; thus keeping the membrane near the equilibrium potential for K⁺ and making depolarization more difficult.

The sympathetic cardiac nerves contain adrenergic neurons that release epinephrine or norepinephrine, depending on the species. (Amphibians release both of these neurotransmitters.) These neurotransmitters bind β₁ adrenergic receptors at both the SA node and in the myocardium. This binding can cause a variety of effects, including an increased inward flux of Ca⁺⁺ into the cell.

The function of the muscarinic and adrenergic receptors in the heart can be revealed by examining how the heart responds when these receptors are activated or inactivated. Compounds that activate a receptor are called agonists, while compounds that inactivate a receptors by binding to it are called antagonists. Most medications used in the treatment of heart disorders are antagonists for either muscarinic or β-adrenergic receptors.

In this lab the chronotropic and inotropic effects of two agonists, epinephrine and acetylcholine along with atropine, digitalis and various ions will be studied.

CAUTION: *Some of the chemicals and drugs you will use in this experiment can be dangerous if gotten on skin or in mouth.*

II. Preparation of the Frog

Follow the directions in the frog heart movie on line and the step below:

1. Obtain a double-pithed the frog
2. Place the frog on its dorsal surface.
3. Using the sharp end of a pair of blunt/sharp scissors make a small penetration into the abdominal cavity of the frog.
3. Carefully cut abdominal wall with a pair of scissors and cut towards the sternum. Lift the scissor as you cut to prevent cutting of the heart bloods vessels or other internal organs.

4. Cut through the pectoral girdle to expose the heart in the pericardial sac.
5. Carefully remove the pericardial sac.
6. Cut any connective tissue attachments (not the vessels around the atria) so that the heart beats freely
7. Using forceps to grasp the apex of the ventricle and push the point of the 'J'-hook into the apex of the ventricle. Push the hook through the ventricle wall until the bend of the hook is inside the heart.
8. Connect the thread from the frog heart to the force transducer S-hook Use a square knot and tie it twice to make sure it won't come loosen when the heart contracts.
9. Position the frog so the thread from the heart is vertical. If it is pulling at an angle much of the contraction of the frog heart will not be observed.
10. Don't worry about the stand height or thread tension at this point. cut any additional thread so it won't get in the way of the transducer

Remember to bathe the heart in Ringer's periodically

III. iWorx Heart Physiology setup

1. The hardware has been set up for you
2. Read through the instructions on operating the software to refresh your memory
3. Start the software (settings = AK204; Muscle #4) After a short time, LabScribe will appear on the computer screen with the Muscle #4 Settings.

IV. Experiments in Frog Heart Physiology

Activity: Baseline Frog Heart Activity (Control)

1. Click <Start> and then <Autoscale> and you should see a rhythmic deflection of the trace indicating that you are recording the beats of the frog's heart. If not try clicking <autoscale>. If you still do not see a clear deflection or if the deflection is very slight, increase the tension on the thread by raising the transducer even higher on the ring stand.
2. Type "resting" in the <marks> area and press <Enter> on the keyboard and allow the program to run about 30 sec.
3. Click <Stop>

4. Use the <Marks> icon or scroll to the appropriate section of your data using the arrow beneath the data screen.
5. Make measurements of heartbeats about every 10 seconds after the application by:
 - a. Move the mouse to place the pointer on one cursor, click the mouse, holding down the button, and drag it to the peak of a wave, then release the button
 - b. Repeat with the second cursor by dragging it to the peak of the next wave and reading T2-T1 in the top left corner of the screen; this is the **time interval**
 - c. convert your time value to **rate(beats/minute) = (60 seconds/ (your time values))** and record this value on the table on your data sheet
 - d. Then drag the second cursor to the next trough and read the value in volts in the frog heart channel title area near the top right of the screen; this represents the **amount of contraction**
 - e. record this value in the table on your data sheet

Moisten the heart with frog Ringers solution frequently

Activity: Assessing Physical and Chemical Modifiers of Heart Rate

Effects of Temperature

1. You will be flooding the frog heart with frog ringers at the following temperatures: 2°, 12°, 22°, and 32°; begin with the coldest solution and proceed in order of increasing temperature
2. flood the heart with each temperature long enough to allow the heart to attain a constant rate.
3. You can measure the heart rate on the iworx station ($60 \div [T2-T1]$) as described below or use a clock to count # of beats for 15 seconds then multiply by 4

Using iWorx:

- a. Click <Start> and you should see a rhythmic deflection of the trace, if not, also press <autoscale>
- b. Type “2° Ringer’s” in the “marks” area; apply 10 drops of frog Ringer’s solution (at ~2° temperature) to the heart through a Pasteur pipette and press the <Enter> key
- c. After about 30 seconds press <stop>
- d. Type “12° Ringer’s” on the keyboard; and apply about 10 drops of the proper Ringer’s solution to the heart and press <Enter> on the keyboard
- e. After about 30 seconds press <stop>

- f. Wait about 1 minute or until the heart seems to have recovered (ie. the amplitude and rate have returned to that observed at 'resting').before proceeding.
- g. Once a normal, resting heartbeat has returned, type "22° Ringer's" on the keyboard; apply 10 drops of this ringer's solution to the heart and press <Enter> on the Keyboard.
- h. after about 30 seconds press <stop>.
- i. Wait about 1 minute or until the heart seems to have recovered (ie. the amplitude and rate have returned to that observed at 'resting').before proceeding.
- j. Once a normal, resting heartbeat has returned, type "32° Ringer's" on the keyboard; apply 10 drops of this warm ringer's solution to the heart and press <Enter> on the Keyboard.
- k. after about 30 seconds press <stop>.
- l. Use the <Marks> icon or scroll to the appropriate section of your data using the arrow beneath the data screen.
- m. Make measurements of heartbeats about every 10 seconds after the application by:
 - i. Move the mouse to place the pointer on one cursor, click the mouse, holding down the button, and drag it to the peak of a wave, then release the button
 - ii. Repeat with the second cursor by dragging it to the peak of the next wave and reading T2-T1 in the top left corner of the screen; this is the **time interval**
 - iii. convert your time value to **rate(beats/minute)** = (60 seconds/ (your time values)) and record this value on the table on your data sheet
 - iv. Then drag the second cursor to the next trough and read the value in volts in the frog heart channel title area near the top right of the screen; this represents the **amount of contraction**
- n. record this value in the table on your data sheet

5. Calculate the **Temperture Coefficient** for Frog Heartbeat:

An increase in temperature, within a narrow range, speeds up the rate of chemical reactions. The temperature coefficient, Q₁₀-coefficient is an expression of how much the speed of the process increases with a temperature increment of 10 ° C.

$$Q_{10} = \frac{\text{heartrate at given temperature}}{\text{heartrate at [above temp - 10°]}}$$

- a. Use the formula above to calculate the Q_{10} at each temperature interval
- b. Record your values on the table on your data sheet

Effects of Chemicals

1. Use the same procedure above to test the effects of various chemicals on the heart beat:
2. Some or all of the following chemical will be available for your use:

Acetylcholine *Atropine Sulfate* *Epinephrine* *Digitalis*
Calcium ions *Potassium Ions* *Sodium Ions*
3. Click <Start> and you should see a rhythmic deflection of the trace, if not press <autoscale>.
4. Type the name of the chemical you are testing in the “marks” area; apply ten drops of the chemical you are testing onto the heart and press <Enter> on the Keyboard
5. after about 30 seconds Click <Stop>
6. Flush the heart with frog Ringer’s solution until it returns to a normal heart beat
7. Repeat this procedure for each of the chemicals you are testing.
8. Use the <Marks> icon or scroll to the appropriate section of your data using the arrow beneath the data screen.
9. Make measurements of heartbeats about every 10 seconds after the application by:
 - a. Move the mouse to place the pointer on one cursor, click the mouse, holding down the button, and drag it to the peak of a wave, then release the button
 - b. Repeat with the second cursor by dragging it to the peak of the next wave and reading T2-T1 in the top left corner of the screen; this is the **time interval**
 - c. convert your time value to **rate(beats/minute)** = (60 seconds/ (your time values)) and record this value on the table on your data sheet
 - d. Then drag the second cursor to the next trough and read the value in volts in the frog heart channel title area near the top right of the screen; this represents the **amount of contraction**
 - e. record this value in the table on your data sheet

Graphing your Results:

1. Plot the data for each set of experiments (temperature and chemical treatments) on a bar graph. For each of the experiments, make one bar graph showing the **heart rate** at different treatments; make another bar graph comparing the **amount of contraction** at different treatments compared to the control.

Activity: Investigating the Refractory Period of Cardiac Muscle

1. An important characteristic of *skeletal* muscle is its ability to go into tetanus with repeated stimulation. This usually occurs when the muscle receives between 40 and 60 stimuli per second (40-60 Hz).
2. Attempt to induce tetanus in *cardiac* muscle of the frog heart:
 - a. if not already attached, attach electrodes to the iWorx station
 - b. Adjust the electrodes on the ring stand so that the tips both touch the ventricle of the frog's heart
 - c. Select <Preferences> from the <Edit> menu and click <stimulator> to display the stimulator panel
 - d. Click <low voltage> and enter the following parameters if not already set:

Pulse Width	=	10 ms
Delay	=	50ms
Amplitude	=	4 Volts
Frequency	=	40 Hz
Number of Pulses	=	300

This will produce a stimulus train of 300 pulses each four volts in amplitude and 10 ms in duration at a frequency of 40 per second.
 - e. Close the stimulator screen
3. Click <Start> and you should see a rhythmic deflection of the trace, if not press <Autoscale>
4. Type "tetanus-40hz" and press <enter>
5. After about 10 seconds click <stop>
6. If tetanus was not produced, repeat the procedure, increase the frequency to 50 Hz then 60 Hz
7. Attempt to determine the refractory period by resetting the stimulator parameters:
 - a. Select <Preferences> from the <Edit> menu and click <stimulator> to display the stimulator panel
 - b. Click <low voltage> and enter the following parameters if not already set:

Pulse Width	=	10 ms
Delay	=	50ms
Amplitude	=	5 Volts
Frequency	=	1 Hz
Number of Pulses	=	30

This will produce a stimulus train of 30 pulses each four volts in amplitude and 10 ms in duration at a frequency of 1 per second.

- c. Close the stimulator screen
8. Click <Start> and you should see a rhythmic deflection of the trace, if not press <Autoscale>
9. Type “refractory” and press <Enter> on the keyboard
10. After about 10 seconds click <Stop> and examine your recording. Look for extra ventricle beats (See Figure 4-24). If you see no difference in the heart wave profile try each or both of the following:

Decrease stimulus frequency by tenths of a Hz (eg. try .9 Hz)
Increase voltage amplitude if possible (Maximum possible is 5 volts)
and repeat steps 8, 9 and 10
11. Once you have generated an extra ventricular beat select <save> in the <file> menu. (If you have not successfully generated an extra ventricular beat, note that fact on your data sheet and skip to the next activity).
12. Scroll to the appropriate section of data.
13. Locate region with an extra ventricular contraction (Fig 4-24)
14. Click the two cursor icon (Fig 4-26) so that two blue vertical lines appear over the recording window.
15. Use the mouse to drag a cursor to the peak of the last normal ventricular contraction before the extra contraction
16. Repeat with the second cursor by dragging it to the peak of the extra ventricular contraction and release the mouse button
17. Read and record T2-T1 in the top left corner of the screen; this is an estimate of the refractory period of the frog cardiac muscle and enter this time on your data sheet

Activity: Demonstrating the Intrinsic heart rate:

1. When you have completed all your experiments with the frog heart, use scissors to remove the heart from the frog and place it in a small petri dish containing frog ringers solution.
2. Use a watch or timer to count the beats per minute. This is the intrinsic heart rate. Record this value on your data sheet.

V. Completing Your Report

1. Complete the information requested on your data sheet

2. You should have recorded average heart rates for the control and each treatment in the table provided
3. You should have graphed the results for the change in heart rate and change in amount of contraction for each experiment.

Cleanup:

Place the frog in the plastic bag and discard in the trash

Do NOT discard the “J” hooks, clean, dry and return them to your tray

Clean and dry your dissecting tools and return them to your tray

Turn off the iWorx station

Drag your saved file to the recycling bin

Name: _____

Group: _____

Due Date: _____

Frog Heart Physiology

Record the baseline data and the data from the results of your experiments with temperature and chemicals on the attached table. Plot the average values for each treatment on a bar graph.

1. How did temperature, heat and cold, change the heart rate from the baseline? Explain.

Temperature (°C)	Heart rate/min	Q ₁₀
2°		
12°		
22°		
32°		
	Average Q₁₀:	

2. How did your average temperature coefficient compare with others in the class? Was the value fairly constant or did it change with increasing temperature? Explain.

- 3a. Describe the effect that you would expect each of the chemicals below to have on heart rate and amplitude of the heart beat and explain your reasoning
- 3b. Then describe the effect that each chemical you actually tested had on the frog heart rate and amplitude compared to the control (your baseline rate) and explain any discrepancies from what you expected (what was the *specific* cause of any change in heartrate):

Acetylcholine:

Atropine Sulfate:

Epinephrine:

Digitalis:

Calcium Ions:

Sodium Ions:

Potassium Ions:

3. Were you able to demonstrate tetanus in the frog heart? Why or why not?

4a. What is the intrinsic heart rate of your frog's heart?_____

4b. What specifically produces this effect?

Treatments		typical reading from 1 st 10 seconds		typical reading from 2 nd 10 seconds		typical reading from 3 rd 10 seconds		Average of three readings	
		Rate (b/min)	Contraction (volts)	Rate (b/min)	Contraction (volts)	Rate (b/min)	Contraction (volts)	Rate (b/min)	Contraction (volts)
Temperature	Baseline Control								
	Cooled Ringer's								
	Heated Ringer's								
Chemicals	Acetylcholine								
	Atropine								
	Epinephrine								
	Digitalis								
	Calcium Ions								
	Sodium Ions								