

## Experiment AM-6: Frog Electrocardiogram

### Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

iWire-B3G ECG cable

C-ISO-F3 lead wires with flexible silver wire electrodes

Dissection tray, instruments and pins

Suture thread

Amphibian Ringer's Solution (See Appendix)

Reagent Solutions (See Appendix)

**Note: Connect the iWire-B3G to the IXTA before turning it on.**

### ECG Recording Cables and Stimulus Electrode Setup

1. Locate the following items in the iWorx kit: iWire-B3G ECG cable and the C-ISO-F3 lead wires with flexible silver electrodes.
2. Plug the iWire-B3G cable into the iWire 1 input on the front of the IXTA. Attach the color coded C-ISO-F3 lead wires into the appropriate sockets.



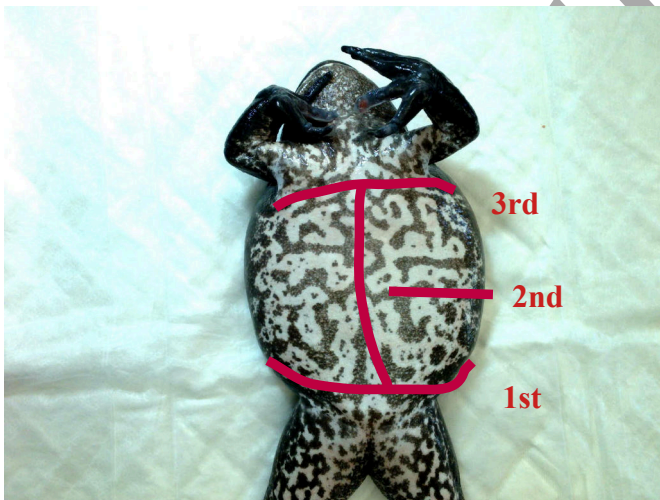
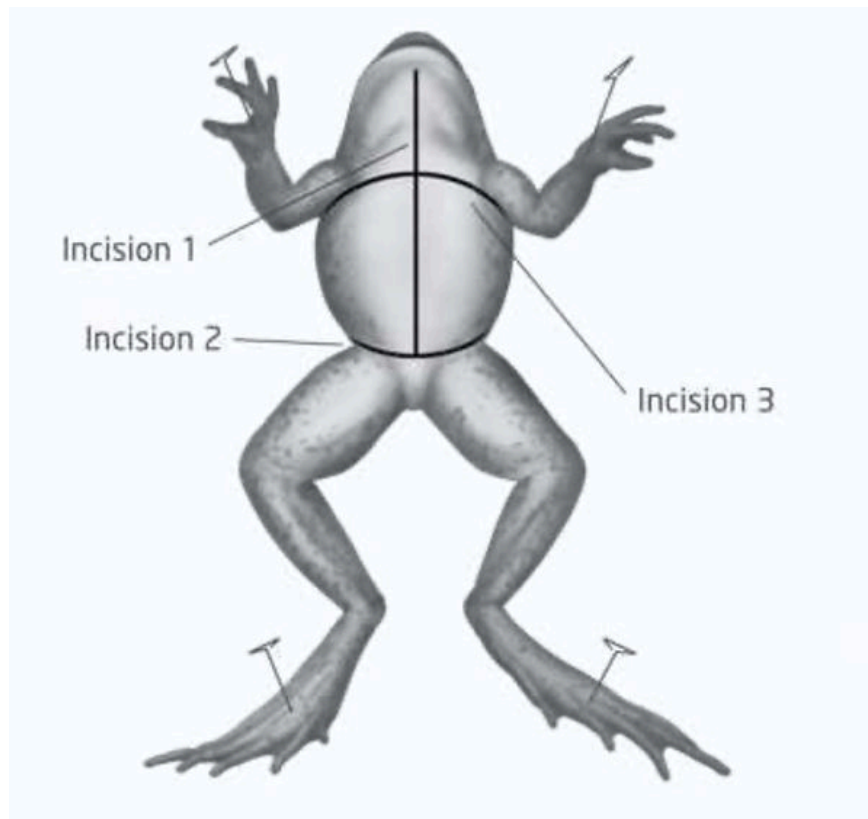
*Figure AM-6-S1: The iWire-B3G ECG cable.*



Figure AM-6-S2: The iWire-B3G and flexible needle electrodes attached to the IXTA.

## The Dissection

1. Place a frog in ice water for 15 minutes. Double pith the frog as soon as it is removed from the ice water
2. Place the frog ventral surface up, in the dissection tray. Use forceps to grasp the skin over the center of the pectoral girdle and use sharp scissors to make a cut to the skin. Use the scissors and forceps to remove the skin over the left (the frog's left) half of the pectoral girdle.
3. Use the scissors to cut through the pectoral girdle: first, in the mid-line; second, under the left arm pit. Cut with the tips of the scissors up.
4. Carefully cut the girdle away from the belly area. Lift the flap of the girdle to expose the (beating) heart. **Flush the area with room temperature Ringer's solution.**
5. While lifting the flap of pectoral girdle, cut it away from the throat region and remove the girdle from the opening. Again, moisten the heart with room temperature Ringer's solution.
6. Examine the heart. Notice that it may still be covered by a white pericardial sac. Use forceps to grasp the pericardial sac, not the heart. Cut the pericardial membrane.
7. Grasp a cut edge of the pericardial membrane with forceps and pull it to one side. Dissect away the pericardial membrane from the heart.



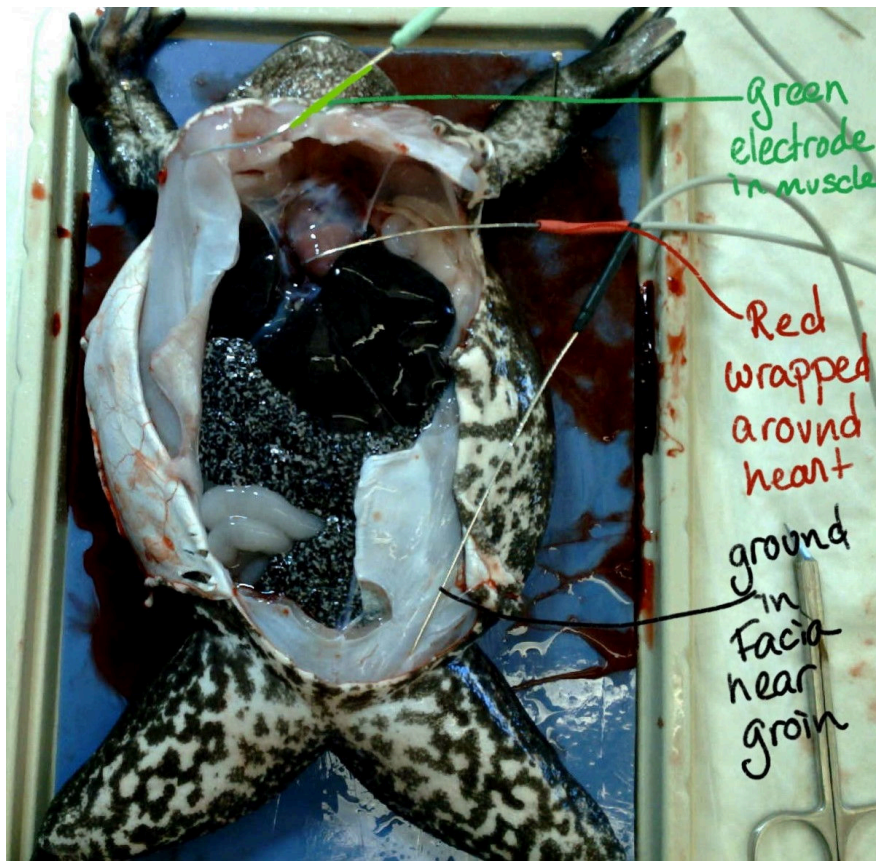


### The Preparation

1. Attach the ground “green” electrode to the upper limb just underneath the skin. Carefully insert the needle electrode into the muscles of the upper forelimb. **Do not go through the muscles into the tray below.**
2. Carefully adjust the position of the positive “red” flexible silver recording electrode so that it is resting on top of the ventricle.
3. Adjust the position of the negative “black” flexible recording electrode so that it rests just below the A-V groove slightly to the frog’s left.

**Warning:** The heart preparation used in this experiment is functional for a limited period of time. If the muscle is bathed periodically in Ringer’s solution, it will work for about four hours. To conserve time, complete all the exercises in the experiment before analyzing the data.





## Experiment AM-6: Frog Electrocardiogram

### Exercise 1: The Frog ECG and Heart Rate

Aim: To record the electrical trace (ECG) produced by the contraction of a resting heart, and to determine the resting heart rate.

Approximate Time: 15 minutes

#### Procedure

1. Type **Resting** in the Mark box.
2. Click the Record button and click the Mark button. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the resting ECG and heart rate for one minute.
4. Click Stop to halt the recording.
5. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.
6. Moisten the chest cavity with room temperature Ringer's solution.
7. Frog ECG - <https://youtu.be/umtKznPe6IQ>

### Exercise 2: Effects of Cold Temperature on the ECG and Heart Rate

Aim: To record changes in the ECG and heart rate after the heart is bathed in cold Ringer's solution.

Approximate Time: 20 minutes

#### Procedure

1. Type **Room Temp Ringer's** in the Mark box.
2. Click the Record button. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the ECG and heart rate for thirty seconds.
4. Apply ten drops of Ringer's solution (at room temperature) to the heart. Click the Mark button when the Ringer's solution is dropped on the heart.
5. Place the beaker with chilled Ringer's solution near the preparation.
6. Type **Cold Ringer's** in the Mark box.
7. Twenty seconds after the addition of room temperature Ringer's to the heart, apply five drops of cold Ringer's solution to the heart. Click the Mark button when the cold Ringer's solution is dropped on the heart.
8. Record until the heart has recovered from the effects of cold Ringer's solution.

**Note:** Recovery is when the ECG amplitudes and rate of the heart contraction have returned to the resting values.

9. Click Stop to halt the recording. Select Save in the File menu.
10. Moisten the chest cavity with room temperature Ringer's solution.

### **Exercise 3: Effects of Warm Temperature on the ECG and Heart Rate**

Aim: To record changes in the ECG and heart rate after the heart is bathed in warm Ringer's solution.

Approximate Time: 20 minutes

#### **Procedure**

1. Type **Room Temp Ringer's** in the Mark box.
2. Click the Record button. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the ECG and heart rate for thirty seconds.
4. Apply ten drops of Ringer's solution (at room temperature) to the heart. Click the Mark button when the Ringer's solution is dropped on the heart.
5. Place the beaker with warm Ringer's solution near the preparation.
6. Type **Warm Ringer's** in the Mark box.
7. Twenty seconds after the addition of room temperature Ringer's to the heart, apply five drops of warm Ringer's solution to the heart. Click the Mark button when the warm Ringer's solution is dropped on the heart.
8. Record until the heart has recovered from the effects of warm Ringer's solution.

**Note:** Recovery is when the ECG amplitudes and rate of the heart contraction have returned to the resting values.

9. Click Stop to halt the recording. Select Save in the File menu.
10. Moisten the chest cavity with room temperature Ringer's solution.

### **Exercise 4: Effects of Drugs on the Frog Heart**

Aim: To monitor the effects of Epinephrine and Acetylcholine on the ECG amplitudes and rate of heart contraction.

Approximate Time: 15 minutes each

#### **Procedure-Epinephrine**

1. Type **Resting** in the Mark box.

2. Click the Record button. Click the Mark button. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the ECG and heart rate for thirty seconds.
4. Type **Epinephrine** in the Mark box.
5. Apply two drops of Epinephrine solution (at room temperature) to the heart. Click the Mark button when the Epinephrine solution is dropped on the heart. Continue recording.
6. After recording the effects of Epinephrine for sixty seconds, rinse the heart with room temperature Ringer's solution until the ECG and heart rate return to the resting state.
7. Click Stop to halt the recording.
8. Select Save in the File menu.
9. Moisten the chest cavity with Ringer's solution.

### ***Procedure-Acetylcholine***

1. Type **Acetylcholine** in the Mark box.
2. Click the Record button. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the ECG and heart rate for thirty seconds.
4. Apply one drop of Acetylcholine solution (at room temperature) to the heart. Click the Mark button when the Acetylcholine solution is dropped on the heart. Continue recording.

***Warning: If the heart goes into cardiac arrest, rinse the Acetylcholine solution off the heart with fresh, room temperature Ringer's solution. If the heart is still in cardiac arrest after 10 seconds, add two drops of Epinephrine solution to the heart.***

5. After recording the effects of Acetylcholine for sixty seconds, rinse the heart with room temperature Ringer's solution until the ECG and heart rate return to the resting rate.
6. Click Stop to halt the recording. Select Save in the File menu.
7. Moisten the chest cavity with Ringer's solution.

### **Data Analysis**

#### ***Exercise 2: Effect of Cold Temperature***

1. Scroll to the data recorded from the heart fifteen seconds before cold Ringer's solution was added to the heart. Click the AutoScale button to maximize the size of the ECG and heart rate channels on the window.



- Use the Display Time icons to adjust the Display Time of the Main window to show ten complete cardiac cycles on the Main window. The cycles can be selected by:
  - Placing a cursor before the first P Wave, and a cursor after the tenth T Wave; and
  - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the ten selected cardiac cycles to the width of the Main window.



Figure AM-6-L1: Frog ECG and heart rate displayed on the Main window.

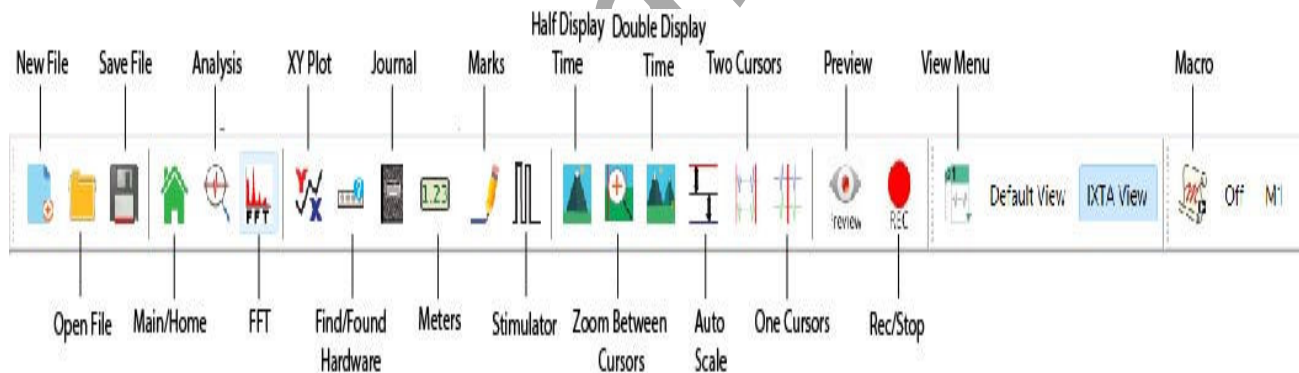


Figure AM-6-L2: The LabScribe toolbar.

- Click on the Analysis window icon in the toolbar to transfer the data displayed in the Main window to the Analysis window.

**Note: Data analysis can also be performed right from the Main window. V2-V1 and Mean will be on the right side of each channel. T2-T1 will be on the upper right of the main toolbar.**

4. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The mathematical functions, V2-V1, T2-T1, and Mean should appear in this table. Values for V2-V1, T2-T1, and Mean on each channel are seen in the table across the top margin of each channel.
5. Once the cursors are placed in the correct positions for determining the amplitude and period of each cardiac cycle, the values of the parameters in the Function Table can be recorded in the on-line notebook of LabScribe by typing their names and values directly into the Journal, or on a separate data table.
6. The functions in the channel pull-down menus of the Analysis window can also be used to enter the names and values of the parameters from the recording to the Journal. To use these functions:
  - Place the cursors at the locations used to measure the amplitude and period of each heart contraction.
  - Transfer the names of the mathematical functions used to determine the amplitude and times to the Journal using the Add Title to Journal function in the Frog ECG Channel pull-down menu.
  - Transfer the values for the amplitude and period to the Journal using the Add Ch. Data to Journal function in the Frog ECG Channel pull-down menu.
7. On the Frog ECG Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
  - The R wave amplitude. To measure the R wave amplitude, place one cursor on the Q wave that precedes the R wave and the second cursor on the peak of the R wave. The value for V2-V1 on the ECG channel is this amplitude. Measure the amplitudes of four additional R waves.
  - The P wave amplitude. To measure the P wave amplitude, place one cursor on the baseline before the P wave and the second cursor on the peak of the P wave. The value for V2-V1 on the ECG channel is this amplitude. Measure the amplitudes of four additional P waves.
  - The T wave amplitude. To measure the T wave amplitude, place one cursor on the baseline after the T wave and the second cursor on the peak of the T wave. The value for V2-V1 on the ECG channel is this amplitude. Measure the amplitudes of four additional T waves.
  - Beat Period, which is the time between the peaks of two adjacent R waves. To measure this parameter, place one cursor on the peak of an R Wave, and the other cursor on the peak of an adjacent R Wave. The value for the T2-T1 function on the Frog ECG Channel is the beat period.
  - Mean Heart Rate, is the average heart rate calculated from the ECG Channel. To measure this parameter, place one cursor at the beginning of the first of 10 cardiac cycles selected and the second cursors at the end of the cycles selected. The value for Mean on the Heart Rate (ECG) channel is this mean heart rate for that 10 cycle period.

8. Record the values in the Journal using the one of the techniques described in Steps 6 or 7, and on Table 1.
9. Scroll to the section of data recorded when cold Ringer's solution was added to the heart. Click AutoScale to maximize the size of the response on the window.
10. Repeat Steps 8, 9 and 10 to measure and record the various amplitudes and beat period of the heart at the time the cold Ringer's solution was added to the heart and at 10 second intervals for the first minute after the addition of the cold Ringer's.

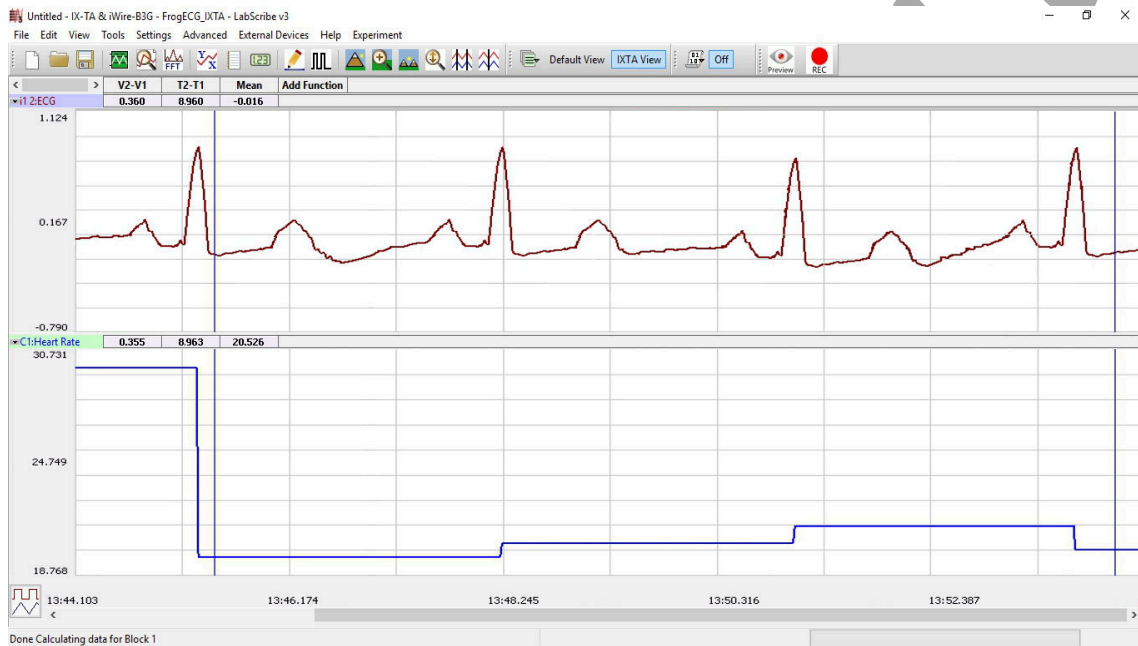


Figure AM-6-L3: Frog ECG and heart rate displayed in the Analysis window. the cursors are placed to measure the mean heart rate over a period of 8.963 seconds.

11. Repeat Steps 8, 9 and 10 to measure and record the ECG amplitudes and beat period of the heart at the end of the recovery period from the effects of cold Ringer's.
12. Determine the mean heart rate for each of the 10 cardiac cycles selected in the Journal and on Table 1.
13. The mean heart rate can also be calculated by converting the beat periods to heart rates using the following equation:

$$\text{Heart Rate (BPM)} = \frac{60 \text{ secs/minute}}{(\times \text{ seconds/beat})}$$

14. Select Save in the File menu.

### ***Exercise 3: Effect of Warm Temperature***

1. Scroll to the data recorded from the heart fifteen seconds before warm Ringer's solution was added to the heart. Click the AutoScale button to maximize the size of the ECG and heart rate channels on the window.
2. Use the same techniques used in Exercise 2 to measure the ECG amplitudes and beat period for the heart during the rest, treatment, and recovery periods when warm Ringer's was applied to the heart.
3. Measure and calculate the mean heart rate during each period.
4. Record your data in Table 1.

### ***Exercise 4: Drug Effects***

1. Scroll to the beginning of the data from Exercise 4 and find the normal heart contractions that occurred before the first drug treatment.
2. Use the same techniques used in Exercise 2 to measure the ECG amplitudes and beat period for the heart during the rest, treatment, and recovery periods for the two drugs applied to the heart.
3. Measure and calculate the mean heart rate during each period.
4. Record the values for the amplitudes and heart rates from this exercise in the Journal and on Table 2 for Epinephrine and Table 3 for Acetylcholine.

### **Questions**

1. What is the effect of cold Ringer's solution on the rate and the amplitude of the ventricular contraction (R wave amplitude and duration)? What mechanism is responsible for this effect?
2. How does warm Ringer's affect the heart? How are the various wave amplitudes different from when cold Ringer's was applied to the heart?
3. What effect does Epinephrine have on the heart rate and the amplitudes of the ECG waves?
4. How does Epinephrine produce its effects on the heart rate and the amplitude of the ventricular contraction specifically?
5. What effect does Acetylcholine have on the heart rate and the amplitude of the ECG waves?
6. How does Acetylcholine produce its effects on the heart rate and the amplitude of the ventricular contraction specifically?
7. Do the time courses for the effect of each drug on the amplitude and the rate of ventricular contraction differ?

**Table AM-6-S1:Amplitudes, Periods, and Rate of Heart Contractions at Different Temperatures.**

	Frog ECG			
Treatment	R Wave (V)	P Wave (V)	T Wave (V)	Mean Heart Rate (BPM)
Room Temp Ringer's				
Cold Ringer's				
10 sec after Cold Ringer's				
20 sec after Cold Ringer's				
30 sec after Cold Ringer's				
40 sec after Cold Ringer's				
50 sec after Cold Ringer's				
60 sec after Cold Ringer's				
Recovered from Cold				
Room Temp Ringer's				
Warm Ringer's				
10sec after Warm Ringer's				
20sec after Warm Ringer's				
30sec after Warm Ringer's				
40sec after Warm Ringer's				
50sec after Warm Ringer's				
60sec after Warm Ringer's				
Recovered from Heat				



**Table AM-6-S2: Amplitudes, Periods, and Rates of Heart Contraction with Epinephrine Treatment.**

	Frog ECG			
Treatment	R Wave (V)	P Wave (V)	T Wave (V)	Heart Rate (BPM)
Resting				
Epinephrine				
10 sec after Epinephrine				
20 sec after Epinephrine				
30 sec after Epinephrine				
40 sec after Epinephrine				
50 sec after Epinephrine				
60 sec after Epinephrine				
Recovered				

**Table AM-6-S3: Amplitudes, Periods, and Rate of Heart Contraction with Acetylcholine Treatment.**

	Frog ECG			
Treatment	R Wave (V)	P Wave (V)	T Wave (V)	Heart Rate (BPM)
Resting				
Acetylcholine				
10 sec after Acetylcholine				
20 sec after Acetylcholine				
30 sec after Acetylcholine				
40 sec after Acetylcholine				
50 sec after Acetylcholine				
60 sec after Acetylcholine				
Recovered				