Experiment AM-12: Crayfish Electrocardiogram (ECG)

Equipment Required

PC or Mac computer

IXTA, USB cable, IXTA power supply

iWire-B3G - ECG recording cable

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

Large wax or Sylgard coated dissection dish

Large dissecting scissors

Insect pins (#2 or greater)

Insulin syringe or a one-milliliter syringe with a fine needle (23 gauge or finer)

Crayfish Ringer's Solution (See Appendix)

Reagent Solutions (See Appendix)

ECG Recording Cables and Stimulus Electrode Setup

1. Locate the following items: iWire-B3G ECG cable and the C-ISO-F3 lead wires with flexible silver electrodes.
2. Plug the iWire-B3G cable into the iWire1 input. Attach the color coded C-ISO-F3 lead wires into the appropriate sockets on the iWire-B3G.

Note – You must plug the iWire-B3G cable into the IXTA before turning it on.

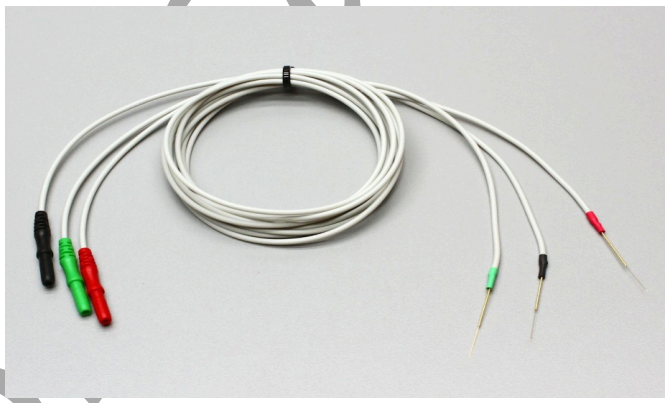

Figure AM-12-S1: The C-ISO-F3 lead wires with flexible silver electrodes.

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Figure AM-12-S2: The iWire-B3G and C-ISO-F3 leads attached to the IXTA.

The Dissection

1. Bury a crayfish in ice for 5-10 minutes.
2. Once the crayfish has stopped moving, use the large scissors to remove its abdomen ("tail"), its claws and its legs. If there is a concern about possible centrally mediated sensation, the crayfish "head" can also be removed. Although the head is part of the cephalothorax and is fairly ill-defined, the crayfish brain is located directly behind the eyes, and the most anterior section of the cephalothorax can be cut cleanly off.
3. Use four large insect pins to secure the crayfish thorax to the dissecting dish. Pin through the sides of the thorax downward into the wax or Sylgard at the four corners of the thorax.
4. The crayfish heart is located directly beneath the dorsal posterior part of the thorax. Use a large insect pin to poke holes through the carapace a little less than a centimeter to either side of the midline, and about a centimeter anterior to the end of the thorax. Poke a third hole at the midline slightly anterior to these two holes. Poke a fourth hole anterior to this, off to one side. Avoid penetrating too deeply to avoid unnecessary damage to the heart.

The Preparation

1. Bend all three silver electrodes at a 90 degree angle with about one centimeter below the bend. Place the "red" and the "black" electrodes though the holes on either side of the heart. Place the "green" ground electrode into the most anterior hole. See below for an illustration of the preparation.
2. It is important that the carapace remain dry to avoid shorting the electrodes.

Warning: The heart preparation used in this experiment is functional for a limited period of time. To conserve time, complete all the exercises in the experiment before analyzing the data.

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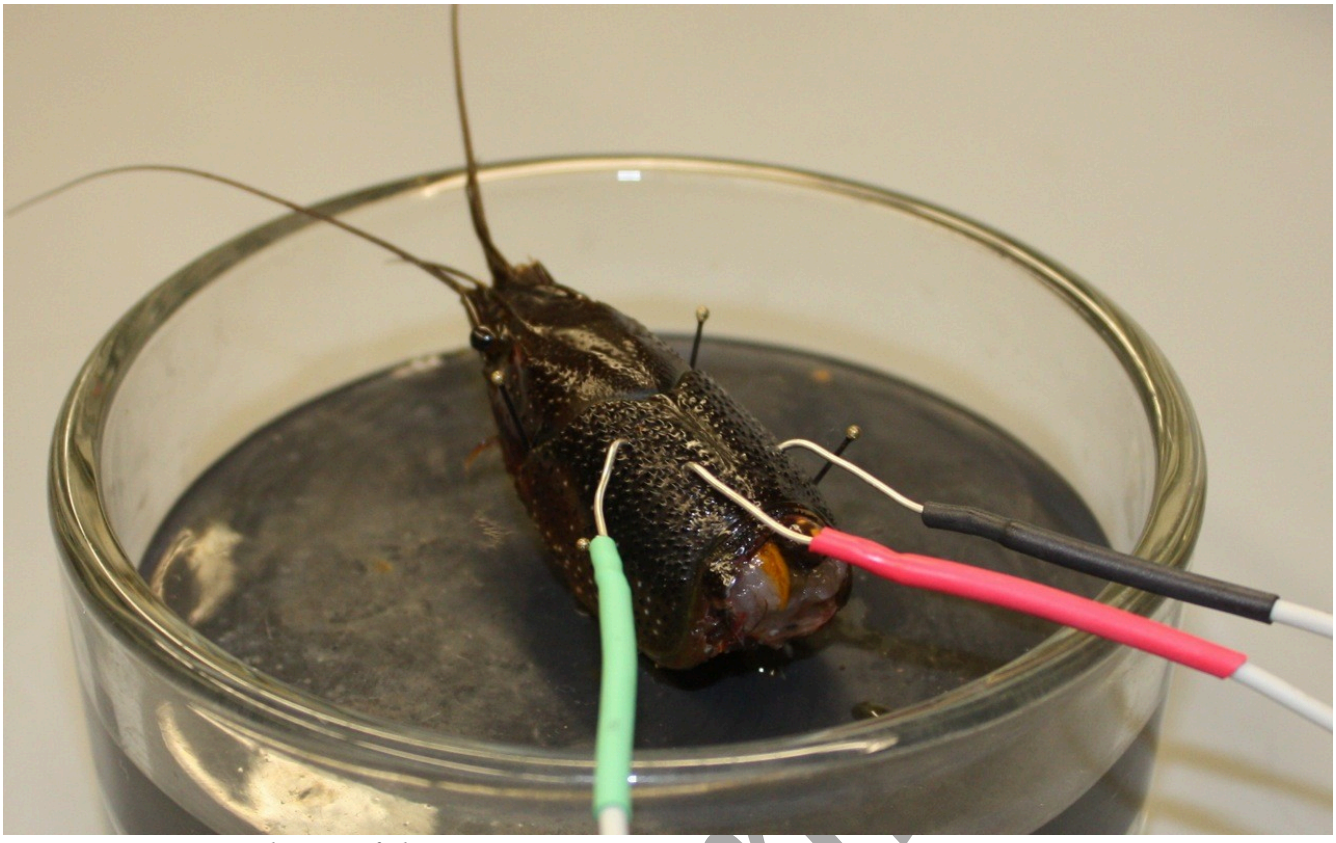

Figure AM-12-S3: The Crayfish ECG Preparation.

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Experiment AM-12: Crayfish Electrocardiogram (ECG)

Exercise 1: The Crayfish 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: 10 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 thirty seconds.
4. Click Stop to halt the recording.
5. Select Save As in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder. Click on the Save button to save the data file.

Note: *If the recording is noisy, or if it seems to be shorted out altogether, try removing the "green" ground and recording with just the two other electrodes.*

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: 15 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. Use the syringe to inject about 0.3 ml of room temperature saline into the hole in the carapace between the "red" and "black" electrodes.

Warning: Avoid putting the tip of the syringe needle too deeply into the hole to avoid damage to the heart tissue.

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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 0.3 ml of the cold Ringer's solution through the hole in the carapace. Repeat two more times, leaving 10 seconds between applications.
8. Click the mark button after the third application of cold Ringer's solution.
9. 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.*

10. Click Stop to halt the recording.
11. Select Save in the File menu.
12. Use the syringe to inject about 0.3 ml of room temperature saline onto the heart.

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: 15 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 0.3 ml of Ringer's solution (at room temperature) to the heart. Click the mark button when the Ringer's solution is injected onto 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 0.3 ml of warm Ringer's solution to the heart. Repeat two more times. leaving 10 seconds between applications.
8. Click the mark button after the third application of warm Ringer's solution.
9. Record until the heart has recovered from the effects of warm Ringer's solution.

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

10. Click Stop to halt the recording.
11. Select Save in the File menu.
12. Use the syringe to inject about 0.3 ml of room temperature saline onto the heart.

Exercise 4: Effects of Drugs on the Crayfish Heart

Aim: To monitor the effects of serotonin and GABA on the ECG amplitudes and rate of heart contraction.

Approximate Time: 45 minutes

Procedure - Serotonin

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 **0.01 mM Serotonin** in the Mark box to the right of the Mark button.
5. Apply 0.3 ml of 0.01 mM serotonin solution (at room temperature) to the heart. Repeat two more times. leaving 10 seconds between applications.
6. Click the mark button after the third application of 0.01 mM Serotonin.
7. If you didn't see a clear response, repeat steps 4-6 with 0.1 mM Serotonin.
8. After recording the effects of serotonin for sixty seconds, inject 0.3 ml of room temperature Ringer's solution onto the heart at 10 second intervals until the ECG and heart rate return to the resting state.
9. Click Stop to halt the recording.
10. Select Save in the File menu.
11. Inject 0.3 ml of room temperature Ringer's solution onto the heart.

Procedure - GABA

1. Type **0.01 mM GABA** 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.

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4. Apply 0.3 ml of 0.01 mM GABA solution (at room temperature) to the heart. Repeat two more times. leaving 10 seconds between applications.
5. Click the mark button after the third GABA application. Continue recording.

Warning: If the heart goes into cardiac arrest, rinse the GABA solution off the heart with fresh, room temperature Ringer's solution. If the heart is still in cardiac arrest after 10 seconds, add 0.3 ml of 0.01 mM Serotonin solution to the heart.

6. If you don't see a clear response to the 0.01 mM GABA, repeat steps 1-5 with 0.1 mM GABA.
7. After recording the effects of GABA for sixty seconds, rinse the heart with room temperature Ringer's solution until the ECG and heart rate return to the resting rate.
8. Click Stop to halt the recording.
9. Select Save in the File menu.
10. Apply 0.3 ml of room temperature saline to the heart.

Data Analysis

Exercise 1: Resting Heart Rate

1. Scroll to the resting heart rate data. Click the AutoScale button to maximize the size of the ECG and heart rate channels on the window.

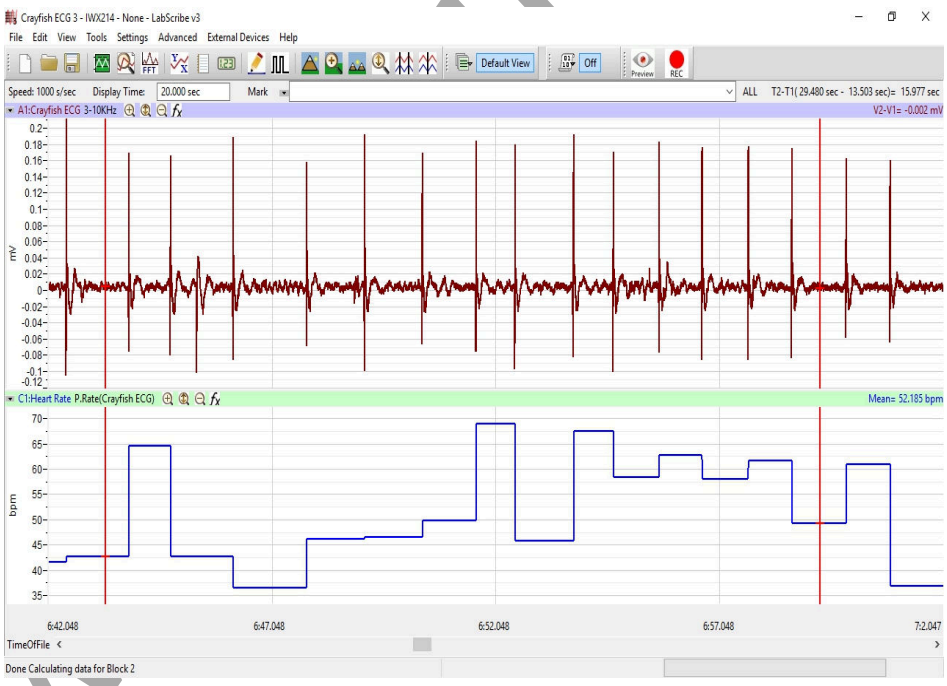

Figure AM-12-L1: Crayfish ECG and heart rate displayed on the Main Window.

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2. Use the Display Time icons to show five complete cardiac cycles on the Main window. The cycles can be selected by:
    - Placing a cursor before the first deflection, and a cursor before the sixth deflection; and
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the five selected cardiac cycles to the width of the Main window.
3. Data can be collected from the Main window or the Analysis window. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.
4. Values for V2-V1 and T2-T1 on each channel are seen in the table across the top margin of each channel, or to the right of each graph. On the heart rate channel, the value for Mean will be shown.

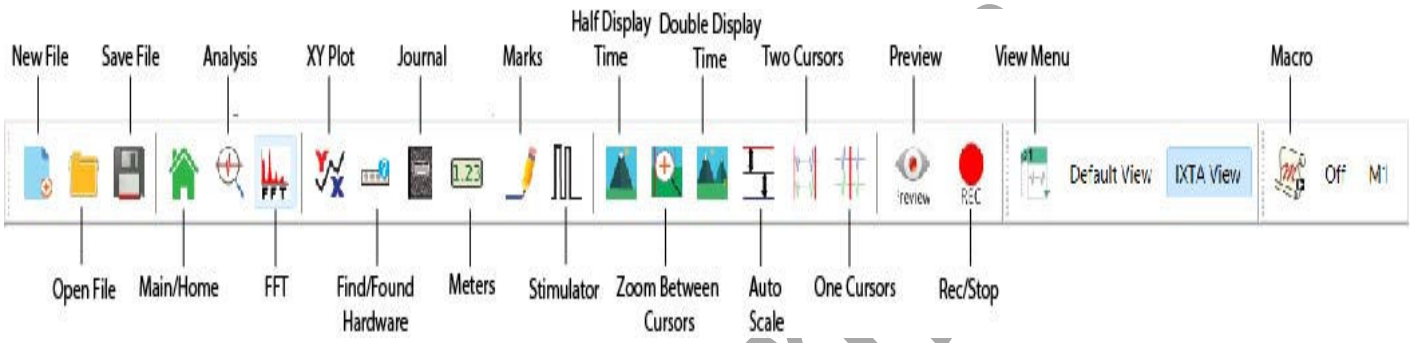

Figure AM-12-L2: The LabScribe toolbar.

Note: *Because the depolarization waves are of fluctuating height and direction, it may be necessary to invert the trace or reconfigure the Periodic setup in order to detect all the heart beats. To invert the trace, select Invert from the Crayfish ECG channel menu. To adjust the Periodic setup, click on P. Rate (Crayfish ECG) in the Heart Rate channel bar and choose Setup Function. Adjust the Threshold and Tolerance lines so that they pass through the Depolarization wave of each cycle.*

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.
2. Use the Display Time icons to adjust the Display Time of the Main window to show five complete cardiac cycles on the Main window.
3. As in Exercise 1, the data can be analyzed in either the Main window or the Analysis window.
4. Values for V2-V1 and Mean will be the data being collected.

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5. Once the cursors are placed in the correct positions for determining the heart rate and the amplitude of each cardiac parameter, 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 heart rate and the amplitude of each cardiac parameter.
    - 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 Crayfish ECG Channel pull-down menu.
    - Transfer the amplitude values to the Journal using the Add Ch. Data to Journal function in the Crayfish ECG Channel pull-down menu.
    - Transfer the average rate over the selected cycles to the Journal using the Add Ch. Data to Journal function in the Heart Rate (Crayfish ECG) Channel pull-down menu.
7. On the Crayfish ECG Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
    - The Depolarization wave amplitude. To measure the amplitude of the Depolarization wave, place one cursor on the positive peak and the second cursor on the negative peak of the Depolarization wave, as illustrated in below. The value for V2-V1 on the Crayfish ECG channel is this amplitude. Measure the amplitudes of four additional depolarization waves.
    - The Repolarization wave amplitude, To measure the amplitude of the Repolarization wave, place one cursor on the baseline before the Repolarization wave and the second cursor on the peak of the Repolarization wave. The value for V2-V1 on the Crayfish ECG channel is this amplitude. Measure the amplitudes of four additional Repolarization waves.
    - Mean Heart Rate, is the average heart rate calculated from the Crayfish ECG Channel. To measure this parameter, place one cursor at the beginning of the first of five cardiac cycles selected and the second cursors at the end of the cycles selected. The value for Mean on the Heart Rate (Crayfish ECG) channel is this mean heart rate for that five 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.

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10. Repeat these steps to measure and record the various amplitudes and heart rate 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.
11. Measure and record the ECG amplitudes and the heart rate at the end of the recovery period from the effects of cold Ringer's.
12. Select Save in the File menu.

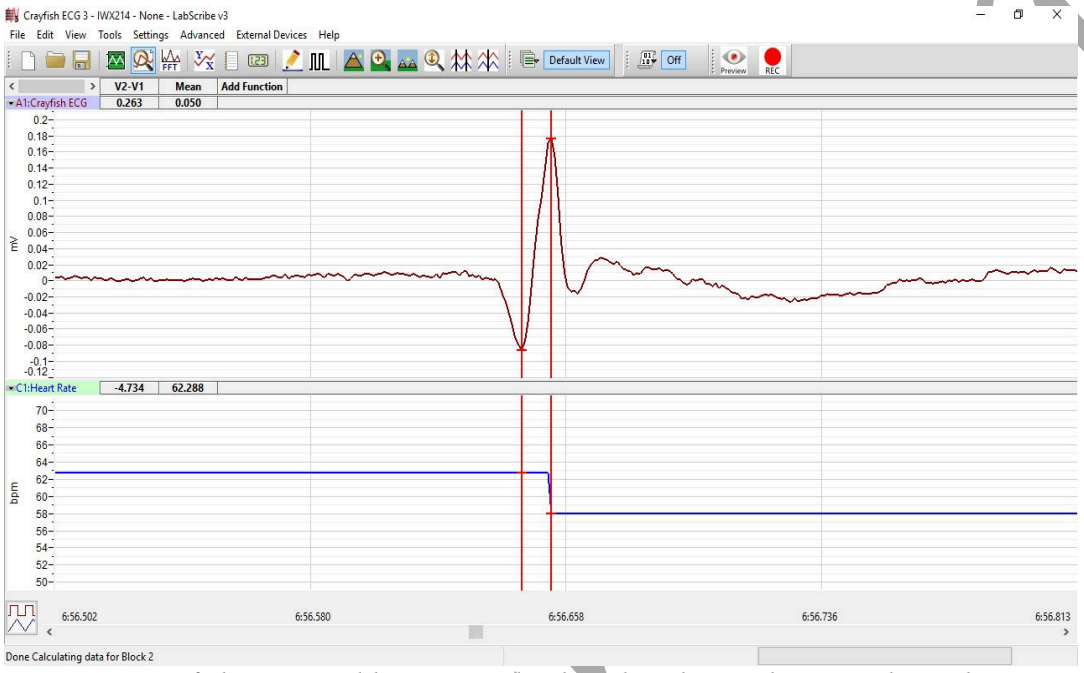

Figure AM-12-L3: Crayfish ECG and heart rate displayed in the Analysis window. The cursors are placed to measure the amplitude of the depolarization wave.

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 heart rates during the rest, treatment, and recovery periods when warm Ringer's was applied to the heart.
3. 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 heart rates during the rest, treatment, and recovery periods for the two drugs applied to the heart.

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3. Record the values for the amplitudes and heart rates from this exercise in the Journal and on Table 2 for Serotonin and Table 3 for GABA

Table AM-12-L1: Amplitudes and Rate of Heart Contractions at Different Temperatures.

	Crayfish ECG		
Treatment	Depolarization Wave (V)	Repolarization 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			

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Table AM-12-L2: Amplitudes and Rate of Heart Contraction with Serotonin Treatment.

	Frog ECG		
Treatment	Depolarization Wave (V)	Repolarization Wave (V)	Heart Rate (BPM)
Resting			
Serotonin			
10 sec after Serotonin			
20 sec after Serotonin			
30 sec after Serotonin			
40 sec after Serotonin			
50 sec after Serotonin			
60 sec after Serotonin			
Recovered			

Table AM-12-L3: Amplitudes and Rate of Heart Contraction with GABA Treatment.

	Frog ECG		
Treatment	Depolarization Wave (V)	Repolarization Wave (V)	Heart Rate (BPM)
Resting			
GABA			
10 sec after GABA			
20 sec after GABA			
30 sec after GABA			
40 sec after GABA			
50 sec after GABA			
60 sec after GABA			
Recovered			

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Questions

1. What is the effect of cold Ringer's solution on heart rate and the amplitude of the ventricular depolarization? What mechanism is responsible for this effect?
2. How does warm Ringer's affect the heart? How do the wave amplitudes and heart rate differ from when cold Ringer's was applied to the heart?
3. What effect does Serotonin have on the heart rate and the amplitudes of the ECG waves?
4. How does Serotonin produce its effects on the heart rate and the amplitude of the ventricular contraction specifically?
5. What effect does GABA have on the heart rate and the amplitude of the ECG waves?
6. How does GABA 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?

Suggested Variations

1. Although Serotonin and GABA are more appropriate to crustaceans, what are the effects of the vertebrate cardiac transmitters, Epinephrine and Acetylcholine?
2. Use a force transducer to measure and record the heart rate and force of contraction under the same conditions using the methods in Experiment AM-7.

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