Experiment AM-9: Crayfish Gut Pharmacology

Equipment Required

PC or Macintosh Computer

IXTA, USB cable, IXTA power supply

FT-302 Force transducer

Thread

Ring stand and clamp

Dissection dish deep enough to submerge a crayfish abdomen

Dissecting tools and #2 insect pins

Crayfish Ringer's Solution (See appendix)

10^{-3}M Acetylcholine in Ringer's (See appendix)

10^{-3} M Epinephrine in Ringer's (See appendix)

10^{-3} M GABA in Ringer's (see appendix)

FT-302 and Stimulus Electrode Setup

1. Locate the FT-302 force transducer and plug it into the Channel A5.

Figure AM-9-S1: The FT-302 force transducer.

Figure AM-9-S2: The FT-302 force transducer connected to the IXTA.

Calibration of the FT-302 Force Transducer

1. Type **No Weight** in the Mark box. Click Record, and press the mark button to attach the comment to the recording. Record for ten seconds with no weight hanging from the arm or hook of the transducer.
2. Type **5 grams** in the Mark box. Hang a 5 gram weight on the arm or hook of the transducer. Press the mark button. Record for ten more seconds.
3. Click Stop to halt the recording.
4. Select Save As in the File menu, and name the file. Choose a destination on the computer in which to save the file. Click on the Save button to save the data file.

Unit Conversion

1. Scroll to the beginning of data when no weight was attached to the force transducer.
2. Use the Display Time icons on the LabScribe toolbar to adjust the display time of the Main window to show the complete calibration data on the Main window.

3. Click the Double Cursor icon. Place one cursor on the flat section of data collected when no weight was attached to the force transducer, and the second cursor on the flat section of data collected when the 5 gram weight was attached to the transducer.
4. To convert the voltages at the positions of the cursors to correct values, use the Simple Units Conversion dialogue window. Click V2-V1 on the force channel, then select Units, and select Simple.
    - Put a check mark in the box next to Apply units to all blocks. Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
    - Enter "Zero" in the corresponding box to the right of the voltage recorded when no weight was attached to the transducer. Enter "5" in the box to the right of the corresponding voltage recorded when the 5 gram weight was hung on the hook of the transducer.
    - Enter the name of the units, grams, in the box below the weights. Click on the OK button in the lower right corner of the window to activate the units conversion.

In the 10 gram range, the FT-302 will deliver approximately 75 mV/gram at x1 gain and approximately one tenth of that in the 100 gram range. The FT-302 is now ready for use.

Experiment AM-9: Crayfish Gut Pharmacology

The Dissection

1. In a dissecting pan, cover a crayfish with ice for 5 minutes.
2. With scissors, remove the entire crayfish abdomen (tail).
3. Place the crayfish cephalothorax in the freezer.
4. Use small dissecting scissors to remove the ventral surface of the abdomen.
5. Use the scissors to remove the musculature from the abdomen, taking care to leave the intestine intact.
6. Free the length of the intestine from any nerves and connecting tissue.
7. Cover the abdomen with 100 ml of room temperature Ringer's. It is important to keep track of exactly how much saline is in the dish.
8. Pin the abdomen down with several insect pins.

The Preparation

1. Clamp the force transducer to a ring stand or manipulator base. Adjust its angle and height such that a thread tied to the free end of the intestine can effectively transmit intestinal contractions.
2. Tie a piece of thread to the 10g connector on the FT-302 Force Transducer.
3. Tie the other end of the thread to the free end of the crayfish midgut.
4. Adjust the dish so that there is slight tension in the thread and gut.

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

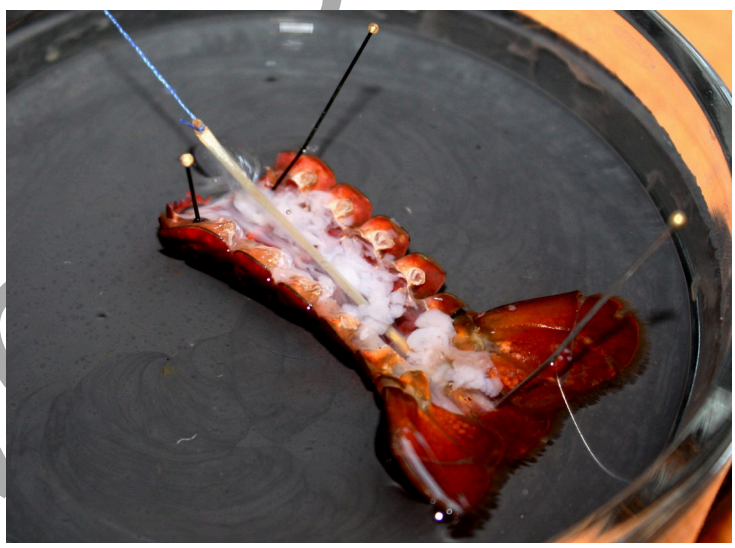

Figure AM-9-L1: Thread tied to the free end of the crayfish intestine.

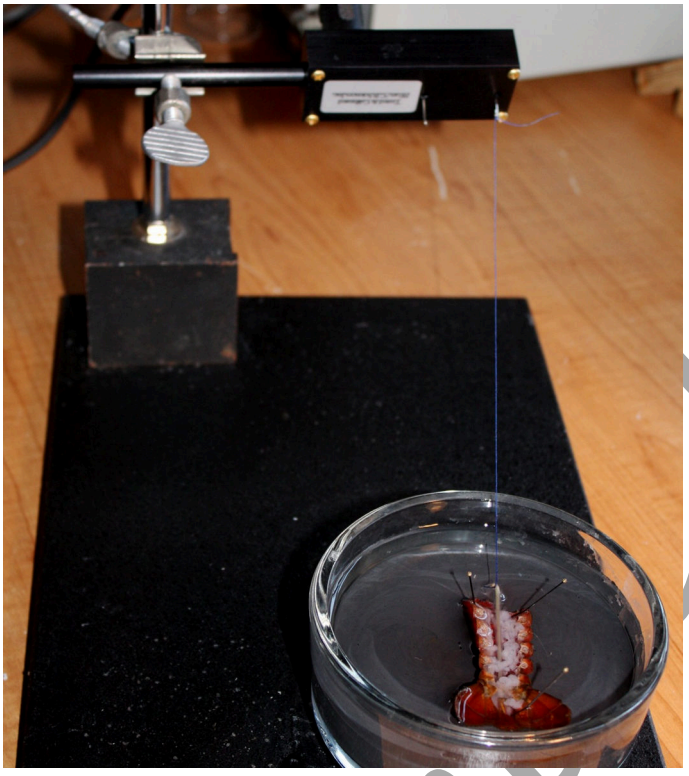

Figure AM-9-L2: The crayfish gut preparation.

Exercise 1: Spontaneous Contractions

Aim: To record the spontaneous contractions of the intestine.

Approximate Time: 15 minutes

Procedure

1. Type **Spontaneous** in the Mark box.
2. Click the Record button and click the mark button to attach the comment to the record. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the gut contractions, if any, for thirty seconds. A sample recording can be seen below.
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.

Note: *It is possible that the gut will not contract without chemical stimulation. In this case, proceed to Exercise 2.*

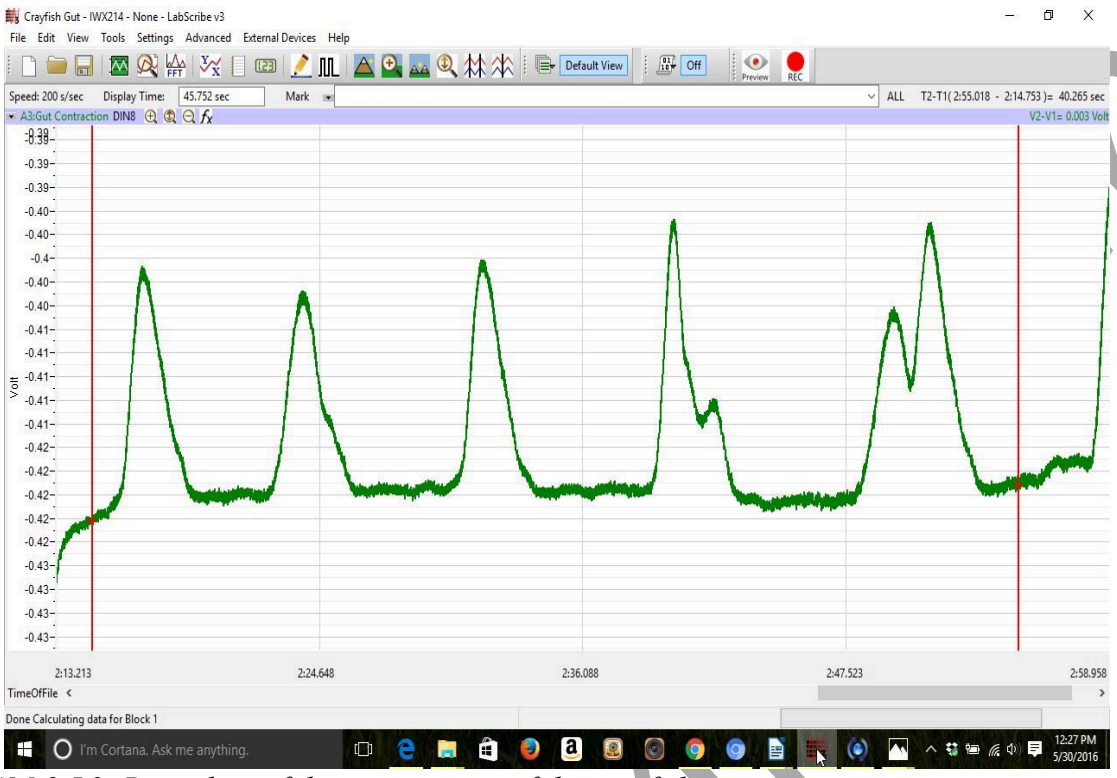

Figure AM-9-L3: Recording of the contractions of the crayfish gut.

Exercise 2: Effects of Acetylcholine

Aim: To record changes in gut contractions after the gut is bathed in acetylcholine.

Approximate Time: 30 minutes

Warning: Keep the saline at the same depth throughout the experiment, since the depth of the saline affects the amplitude of the contractions. The dissecting dish should be firmly affixed to the table with clay so that the dish can't shift accidentally and change the tension on the gut.

Procedure

1. Type **Pre-Acetylcholine** control in the Mark box.
2. Click the Record button. Click the mark button to mark the recording. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the gut contractions, if any, for three minutes.
4. Click Stop to halt the recording.
5. Type **Acetylcholine 10^{-6}M** in the Mark box.

6. Add 100 microliters of the 10^{-3}M stock Acetylcholine solution to the 100 ml of saline in the dish. Gently stir the saline to disperse the Acetylcholine.

Note: *If you used a starting volume of Ringer's other than 100 ml, adjust the amounts of the stock solution accordingly. For example, if you covered the crayfish abdomen with 150 ml, add 150 microliters of the stock Acetylcholine solution to create a 10^{-6}M Acetylcholine solution.*

6. Click Record to start the recording, and click the mark button.
7. Record the effects of 10^{-6}M Acetylcholine for three minutes.

Note:*The response to the transmitter may be delayed. Even if there are no immediate contractions, wait for the entire three minutes before moving on to a higher concentration.*

8. Click Stop to halt the recording.
9. Add an additional 900 microliters of the 10^{-3}M Acetylcholine solution to the saline in the dish to create a 10^{-5}M solution. Gently stir the saline to disperse the Acetylcholine throughout the saline.
10. Type **Acetylcholine 10^{-5}M** in the Mark box.
11. Click Record to start recording and click the mark button. Record for three minutes. Then, click Stop to halt the recording.
12. Add an additional 9 ml of the 10^{-3}M Acetylcholine stock solution to the saline in the dish to create a 10^{-4}M Acetylcholine solution.
13. Type **Acetylcholine 10^{-4}M** in the Mark box.
14. Click Record to start recording and click the mark button to attach the comment to the recording.
15. Record for five minutes. Then, click Stop to halt the recording.
16. Replace the saline with 100 ml of fresh saline, and allow the intestine to recover.

Note: *It is possible that the intestine will not return to pre-treatment conditions. In this case, wait until it has come to a new steady-state, and start the recording at that time. This should occur within ten minutes after the saline change.*

17. Type **Post-Acetylcholine** recovery in the Mark box. Observe the intestinal contractions.
18. Once the gut has recovered, click Record to start the recording and click the Enter key.
19. Record for three minutes. Click Stop to halt the recording.
20. Select Save in the File menu.

Exercise 3: Effects of Epinephrine

Aim: To record changes in gut contractions after the gut is bathed in epinephrine.

Approximate Time: 30 minutes

Procedure

1. Type **Pre-Epinephrine** control in the Mark box.
2. Click the Record button. Click the mark button to mark the recording. Click AutoScale.
3. Record the gut contractions, if any, for three minutes. Click Stop to halt the recording.
4. Type **Epinephrine 10^{-6}M** in the Mark box.
5. Add 100 microliters of the 10^{-3}M stock Epinephrine solution to the saline in the dish. Gently stir the saline to disperse the Epinephrine.
6. Click Record to start the recording, and click the mark button.
7. Record the effects of 10^{-6}M Epinephrine for three minutes.
8. Click Stop to halt the recording.
9. Add an additional 900 microliters of the 10^{-3}M Epinephrine solution to the saline in the dish to create a 10^{-5}M solution. Gently stir the saline to disperse the Epinephrine throughout the saline.
10. Type **Epinephrine 10^{-5}M** in the Mark box.
11. Click Record to start recording and click the mark button. Record for three minutes. Click Stop to halt the recording.
12. Add an additional 9 ml of the 10^{-3}M Epinephrine stock solution to the saline in the dish to create a 10^{-4}M Epinephrine solution.
13. Type **Epinephrine 10^{-4}M** in the Mark box.
14. Click Record to start recording and click the mark button to attach the comment to the recording.
15. Record for three minutes. Click Stop to halt the recording.
16. Replace the saline with 100 ml of fresh saline, and allow the gut to recover. Observe the intestinal activity.
17. Type **Post-Epinephrine** recovery in the Mark box.
18. Once the gut has recovered, click Record to start the recording and click the mark button. Record for three minutes.
19. Click Stop to halt the recording.
20. Select Save in the File menu.

Exercise 3: Effects of GABA

Aim: To record changes in gut contractions after the gut is bathed in GABA.

Approximate Time: 30 minutes

Procedure

1. Type **Pre-GABA** control in the Mark box.
2. Click Record to start the recording and click the mark button to attach the comment to the recording.
3. Record the gut contractions, if any, for three minutes. Click Stop to halt the recording.
4. If the gut is actively contracting, add 100 microliters of the 10^{-3}M stock GABA solution to the saline in the dish. Gently stir the saline to disperse the GABA throughout the saline.
5. If the gut is not contracting, do the following:
    - Add 10 ml of the 10^{-3}M stock solution of the transmitter (either Acetylcholine or Epinephrine) that caused the most contractile activity in Exercise 2 or 3.
    - Wait for contractions to begin.
    - Click Record and record the contractions for one minute.
    - Click Stop to halt the recording.
    - Add 100 microliters of the 10^{-3}M stock GABA solution. Gently stir the saline to disperse the GABA throughout the saline.

Note: *If the effects of acetylcholine and epinephrine are transient and the contractions stop fairly quickly, it may be necessary to add the acetylcholine or the epinephrine at the same time that the GABA is added, to see if the GABA prevents or diminishes the contractions expected to be caused by the excitatory transmitter.*

6. Type **GABA 10^{-6}M** in the Mark box.
7. Click the Record button and click the Enter key. Record the gut contractions for three minutes.
8. Click Stop to halt the recording.
9. If the intestine is still contracting, add an additional 900 microliters of the 10^{-3}M GABA solution to the saline in the dish to create a 10^{-5}M solution. Gently stir the saline to disperse the GABA throughout the saline. If the intestine has stopped contracting, proceed to step 17.
10. Type **GABA 10^{-5}M** in the Mark box.
11. Click Record to start recording and click the mark button. Record for three minutes.
12. Click Stop to halt the recording.
13. If the gut is still contracting, add an additional 9 ml of the 10^{-3}M GABA stock solution to the saline in the dish to create a 10^{-4}M GABA solution. Gently stir the saline to disperse the GABA. If the gut has stopped contracting, proceed to step 17.

14. Type **GABA 10^{-4}M** in the Mark box.
15. Click Record to start recording and click the mark button to attach the comment to the recording. Record for three minutes.
16. Click Stop to halt the recording.
17. Select Save in the File menu.

Data Analysis

Exercise 2: Effects of Acetylcholine

1. Scroll to the beginning of the data from Exercise 2 and find the time at which gut contractions first occurred. If contractions occurred before Acetylcholine was added to the preparation, begin taking measurements in this section of data.

Note: *Enter zeros into Table 1 if no contractions occurred before the addition of Acetylcholine, or after the addition of the lower concentrations of Acetylcholine.*

2. Click the AutoScale button to maximize the size of the gut contractions on the window.
3. Use the Display Time icons to show five contractions on the Main window. The contractions can be selected by:
    - Placing a cursor before the first contraction, and a cursor after the fifth contraction; and
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the five selected contractions to the width of the Main window.

Note: *It is possible that some gut contractions are not well coordinated and difficult to analyze. If this is the case, skip over the more problematic contractions and analyze just those that can be isolated more easily.*

4. 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.
5. 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.
6. Maximize the height of the trace on the Gut Contractions Channel by clicking AutoScale All on the toolbar.

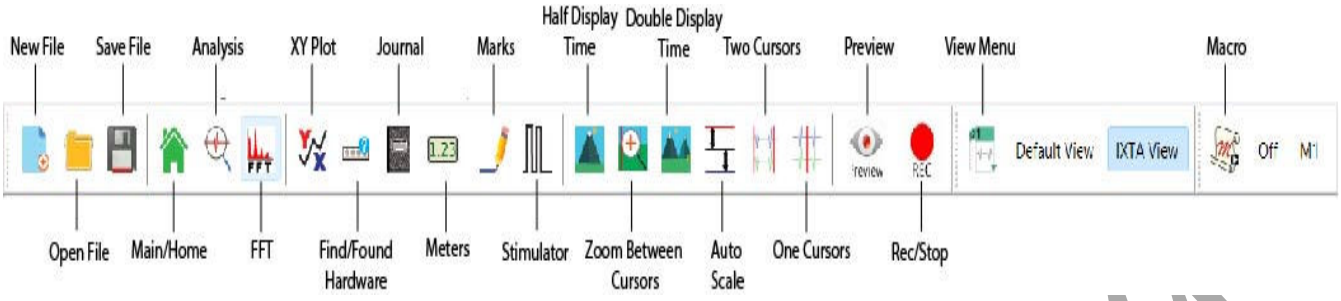

Figure AM-9-L4: LabScribe Toolbar

7. Once the cursors are placed in the correct positions for determining the amplitude and period of each contraction, 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.
8. The functions in the channel pull-down menu 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 gut 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 Gut Contractions 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 Gut Contractions Channel pull-down menu.
9. On the Gut Contractions Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
    - **Contraction Amplitude** is the difference between the baseline level of tension and the tension at the peak of the contraction. To measure this parameter, place one cursor at the peak of the contraction, and the second cursor on the lowest point following the contraction. The value for the V2-V1 function on the Gut Contractions Channel is the contraction amplitude. Determine the average amplitude of five consecutive contractions.
    - **Contraction Period** is the time between the peaks of two adjacent contractions. To measure this parameter, place one cursor on the peak of one gut contraction, and the other cursor on the peak of the adjacent contraction. The value for the T2-T1 function on the Gut Contractions Channel is the contraction period. Determine the average contraction period for five consecutive contractions.
10. Record the values for the contraction amplitudes and periods in the Journal using one of the techniques described in Steps 7 or 8.
11. Determine the average contraction amplitude and period for this section of data. Record the averages in the Journal and in Table 1.

12. Scroll to the next Acetylcholine concentration. Click AutoScale to maximize the size of the response on the window.
13. Repeat Steps 9, 10 and 11 to measure, average, and record the contraction amplitudes and periods of five consecutive contractions in this section of data.
14. Repeat Steps 8, 9 and 10 for each additional concentration of acetylcholine and the recovery period.
15. Determine the frequency of gut contraction for the control, each concentration of Acetylcholine, and the recovery period by dividing 60 secs/minute by the average period from each section of data. Record the frequencies of contraction in the Journal and in on the table.
16. Select Save in the File menu.

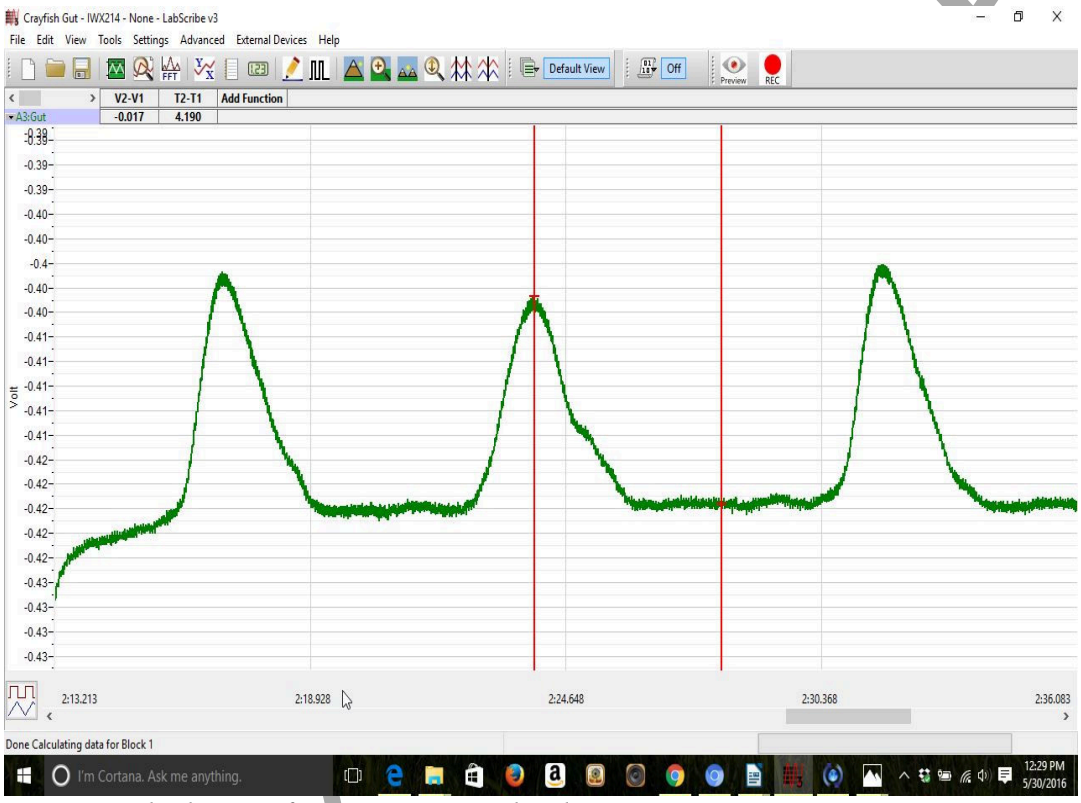

Figure AM-9-L5: Calculation of contraction amplitude using two cursors.

Table AM-9-L1: Amplitudes, Periods, and Rate of Gut Contractions with Acetylcholine treatment.

	Contraction		
Treatment	Average Amplitude (V)	Average Period (sec)	Frequency (BPM)
Pre-Acetylcholine control			
10^{-6}M Acetylcholine			
10^{-5}M Acetylcholine			
10^{-4}M Acetylcholine			
Recovered			

Exercise 3: Effects of Epinephrine

1. Scroll to the beginning of the data from Exercise 3 and find the time at which gut contractions first occurred. Enter zeros in Table 2 for the epinephrine concentrations for which no contractions occurred.
2. Use the same techniques used in Exercise 2 to measure the contraction amplitudes and periods for five consecutive contractions, the averages of these values, and the frequency for all epinephrine concentrations that caused contractions.
3. Record the average contraction amplitudes and periods, and the frequency of contraction, in the Journal and on the table.

Table AM-9-L2: Amplitudes, Periods, and Rate of Gut Contractions with Epinephrine treatment.

	Contraction		
Treatment	Average Amplitude (V)	Average Period (sec)	Frequency (BPM)
Pre-Epinephrine control			
10^{-6}M Epinephrine			
10^{-5}M Epinephrine			
10^{-4}M Epinephrine			
Recovered			

Exercise 4: Effects of GABA

1. Scroll to the beginning of the data from Exercise 4 and find the time at which gut contractions first occurred. Enter zeros into Table 3 for the GABA concentrations for which no contractions occurred.
2. Use the same techniques used in Exercise 2 to measure the contraction amplitudes and periods for five consecutive contractions, the averages of these values, and the frequency for all GABA concentrations that caused contractions.
3. Record the average contraction amplitudes and periods, and the frequency of contraction, in the Journal and on the table.

Table AM-9-57: Amplitudes, Periods, and Rate of Gut Contraction with GABA Treatment.

	Contraction		
Treatment	Average Amplitude (V)	Average Period (sec)	Frequency (BPM)
Pre-GABA control			
10^{-6}M GABA			
10^{-5}M GABA			
10^{-4}M GABA			

Questions

1. You may have noticed that gut contractions are not as discrete as heart contractions. Can you suggest a functional reason why this may be the case? Can you suggest a mechanistic difference between heart contractions and gut contractions that may be responsible for the difference?
2. What effect does Acetylcholine have on the contraction rate and the amplitude of the contractions? Does the effect vary by dose?
3. What effect does Epinephrine have on the contraction rate and the amplitude of the contractions? Does the effect vary by dose?
4. Describe any qualitative differences in the contractions produced by Acetylcholine and Epinephrine. Can you suggest a mechanism for any differences?
5. What effect does GABA have on the contraction rate and the amplitude of the contractions? Does the effect vary by dose?
6. How does GABA produce its effects on the gut contraction rate and amplitude?