

Experiment AM-11: Earthworm Smooth Muscle Contractions

Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

FT-302 force transducer

STB-125 Student tissue bath

Suture thread and needle

Pasteur pipets and bulbs

Non-toxic modeling clay

Dissection pan and instruments

5% Ethanol solution

250 ml beakers

Cylinder with a mixture of 95% O₂ & 5% CO₂

Regulator, valve, and tubing for oxygenation setup

Worm Saline (See appendix)

Various reagents in Worm Saline (See appendix)

Transducer Setup

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



Figure AM-1-S1: The FT-302 force transducer.



Figure AM-11-S2: The FT-302 force transducer connected to an IXTA.

Tissue Bath Setup

1. Assemble the tissue bath. The components of the student tissue bath are listed in Table 1. The completed tissue bath is pictured below.
2. Thread the 50cm stainless steel rod into the matching threaded hole on the back of the base. With the rod in this position, the base will provide a stable support for the components aligned above it.
3. Place a brass parallel frame clamp on the stainless steel rod. This clamp should be about 20cm above the surface of the base and aligned.
4. Place the arm of the glassware extension clamp in the clamping hole on the side of the parallel frame clamp. Position the extension clamp so its prongs can hold the tissue chamber vertically over the front half of the base.
5. Place a second parallel frame clamp on the stainless steel rod. Align this clamp above the first clamp. The second clamp will eventually hold the glass tissue support that will hold the lower end of the earthworm gut in the chamber.
6. Place the transducer positioner on the stainless steel rod with the adjustment knob of the positioner on top. Clamp the positioner on the rod so the bottom of the positioner is about 30cm above the base.

7. Clamp the FT-302 force transducer in the holder on the threaded rod of the positioner. Align the positioner and the transducer so the hook on the transducer is directly over the center of the tissue chamber.
8. Turn the adjustment knob on the positioner to move the transducer and its holder to the middle of the threaded rod.
9. Determine the best location on the lab bench for the tissue bath setup. The setup should be convenient to a sink or a drain flask, the mixed gas supply used for aeration, and the data acquisition system used to record the responses of the earthworm gut.

Table AM-11-2: Components of the STB-125 Student Tissue Bath Needed for this Experiment

Part Number	Part Description
A-TB-NMB-125	Base
A-TB-SSR-125	50cm Stainless Steel Rod
A-TB-BATH-125	25ml Tissue Bath
A-TB-BPF-125	Brass Parallel Frame Clamp
A-TB-GEC-125	Glassware Extension Clamp
A-TB-ST15-125	Silicone Tubing, 0.153"OD X 0.106"ID
A-TB-O2C-125	Oxygen Connector
A-TB-ST25-125	Silicone Tubing, 0.250"ID X 0.438"OD
A-TB-PPC-125	Polypropylene Pinch Clamp
A-TB-GTS-125	Glass Tissue Support
A-TB-TP-125	Transducer Positioner

10. Feed about 8cm of the drain line through the two circular holes on the plastic pinch clamp. Carefully put the end of the drain line with the pinch clamp on the drain port of the tissue bath. Leave the drain open.
11. Carefully put the ends of the additional fluid lines on the appropriate ports of the tissue bath.
12. Put the oxygen connector on the aeration port of the tissue bath.
13. Carefully clamp the assembled tissue bath between the prongs of the glassware extension clamp already on the stand.
14. If the tubing needed on the tissue chamber has not been prepared, find the two coils of silicone tubing.

15. Attach one end of the smaller diameter tubing to the oxygen connector on the tissue chamber. Connect the other end of the tubing to the valve on the cylinder containing 95% O₂ and 5% CO₂.
16. Place the end of the drain line in the sink or a flask used to collect waste buffer. Connect the tubing on the water inlet, at the bottom of the tissue bath, to the water supply at the sink or to the outlet of a flask. Place the end of the tubing on the water outlet, at the top of the tissue bath, in the sink or on the inlet of the flask.

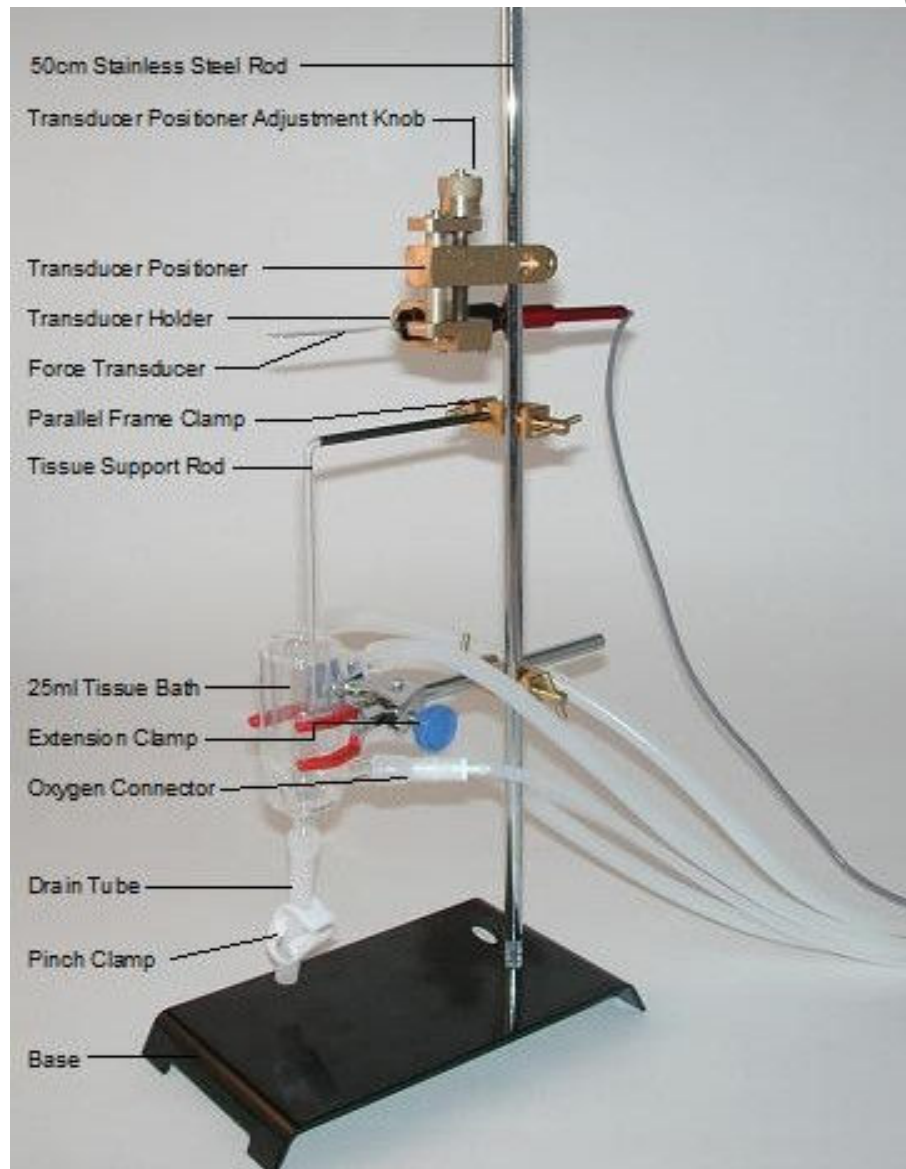


Figure AM-11-S3: STB-125 student tissue bath with a FT-302 force transducer.

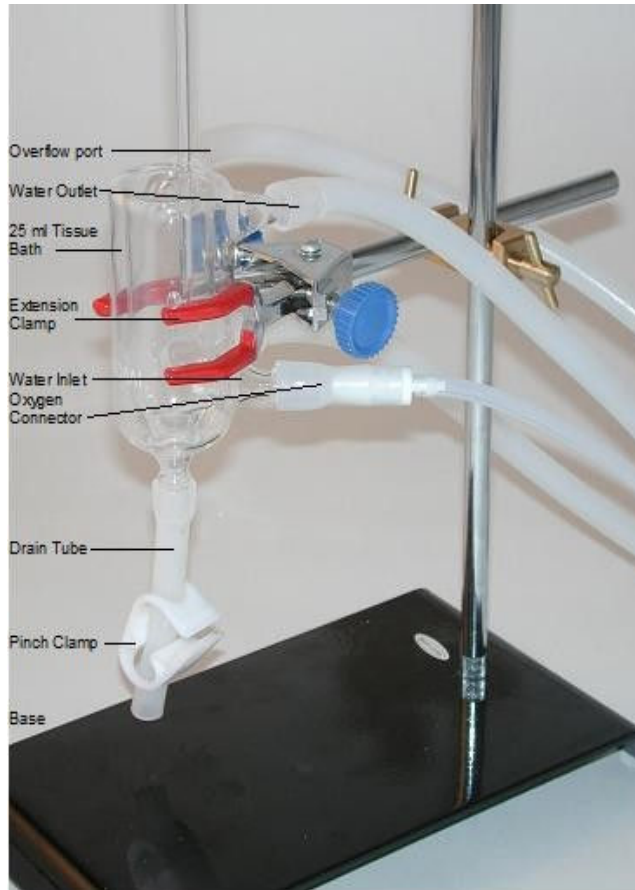


Figure AM-11-S4: 25 ml tissue bath in position for recording tissue tension.

Calibration of FT-302 Force Transducer

Aim: To calibrate the force transducer used to measure earthworm gut tension.

Procedure

1. Make sure the data acquisition unit is turned on and the FT-302 force transducer is connected to the DIN8 input for ten minutes before the calibration is performed.
2. Type **No Weight** in the Mark box to the right of the Mark button. Click the Record button, and click the mark button. Record for ten seconds with no weight hanging from the hook of the transducer.
3. Type **5 grams** in the Mark box. Hang a 5 gram weight on the hook of the transducer. Click the mark button. Record for ten more seconds.
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.

Units Conversion

1. Scroll to the beginning of data when no weight was attached to the FT-302 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 so that two blue cursors appear on the Main window. Place one cursor on the flat section of data collected when no weight was attached to the FT-302, 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 output of the FT-302 force transducer from a voltage to the grams of force:
 - Click on V2-V1 to the right of the Earthworm Gut Contractions channel.
 - Select Simple.

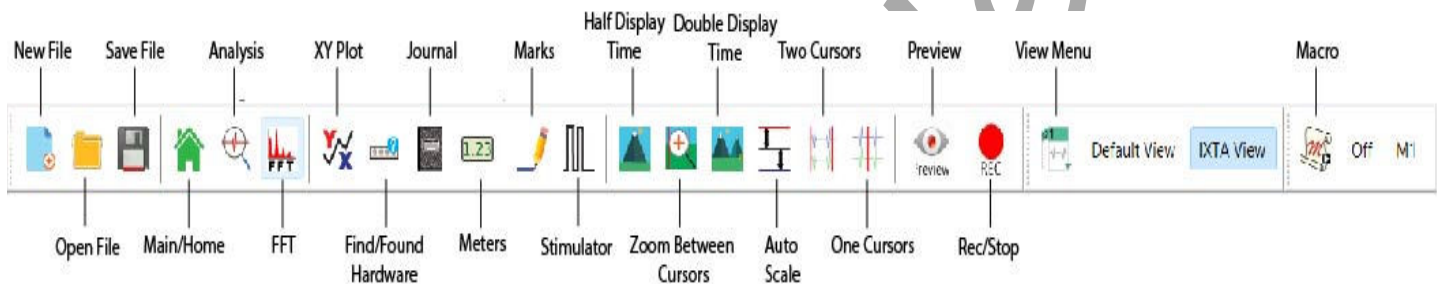


Figure AM-11-S5: The LabScribe toolbar.

5. The Simple Units Calibration window will appear. On this window:
 - Select 2 point calibration from the pull-down menu in the upper-left corner of the window.
 - 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 weight was hung on the hook of the transducer.
 - Enter the name of the units, grams, in box below the weights.
 - Click on the OK button in the lower right corner of the window to activate the units conversion.
6. Click Save in the File menu to save your file.

Precautions

1. Keep the earthworm in a cool environment until ready to use.
2. Earthworm gut tissue is very hardy, however we suggest that all the lab exercises be completed before taking time to analyze any of the data. Completing the exercises quickly improves the chances of completing the experiment with the same earthworm preparation.
3. The temperature of the worm saline used to rinse the gut preparation and replace the saline in the chamber should be at room temperature. Keep flasks of fresh saline on the lab bench to be used as needed.
4. Start the experiment as quickly as possible after the isolation of the earthworm gut. Designate members of the lab group to perform different parts of the equipment setup: opening and setting up the LabScribe software; assembling the tissue chamber, calibrating the transducer; and so on.

Tissue Preparation

Dissection

1. Add 25 ml of 5% ethanol to a 250 ml beaker. This should be just enough to cover an earthworm.
2. Rinse a large earthworm with worm saline to clean it and place it in the beaker for approximately 5 minutes. This will anesthetize the worm.
3. Remove the earthworm from the ethanol and place it on a dissecting pan. Pin the earthworm at each end to secure it to the tray. Moisten the earthworm with saline. During dissection it is important to keep the worm moist.
4. Locate the clitellum. Using small dissecting scissors carefully make a shallow incision slightly off center of the clitellum. Be careful not to cut too deep.
5. Continue making this incision, upwards towards the anterior end of the earthworm until a section of intestine, the gizzard and crop are exposed.
6. Pin the skin to the dissecting pan to expose the organs as the incision is made. The incision should expose a 3-5 cm section of gut.
7. Remoisten the earthworm with saline making sure to keep the exposed gut moist.
8. Carefully use a blunt probe to separate the gut from the body wall being sure not to cut or pierce the gut wall.
9. Using suture thread, tie a ligature just above the crop at the anterior end of the gut. Leave some thread to anchor the tissue in the chamber and to the force transducer.
10. Tie another ligature at the posterior end of the gizzard. If some intestine is included, that will not affect the outcome of the experiment.
11. Using the scissors, carefully remove the gut section from the earthworm.

Note: Remember to leave some extra suture thread on both ligatures so the gut preparation can be attached to both the transducer and tissue support rod.

Placement of the Tissue in the Chamber

1. Use a clean beaker to obtain about 100 ml of Worm saline. Take only as much saline as you need for each rinse. Reserve this beaker for transferring clean saline throughout the exercise.

Note: Avoid contamination! Do not return any Worm saline solution taken from the supply flask back to the supply flask!

2. Rinse the tissue chamber thoroughly, three or four times, with saline.
3. Close the drain of the tissue chamber and fill the chamber with about 20ml of Worm saline. Open the valve on the aeration line and adjust the flow of the oxygen/carbon dioxide mixture through the aeration frit to create a plume of small bubbles.
4. Obtain the dissected section of earthworm gut to use in the experiment. Keep the gut moist in the worm until you are ready to attach it to the support rod.

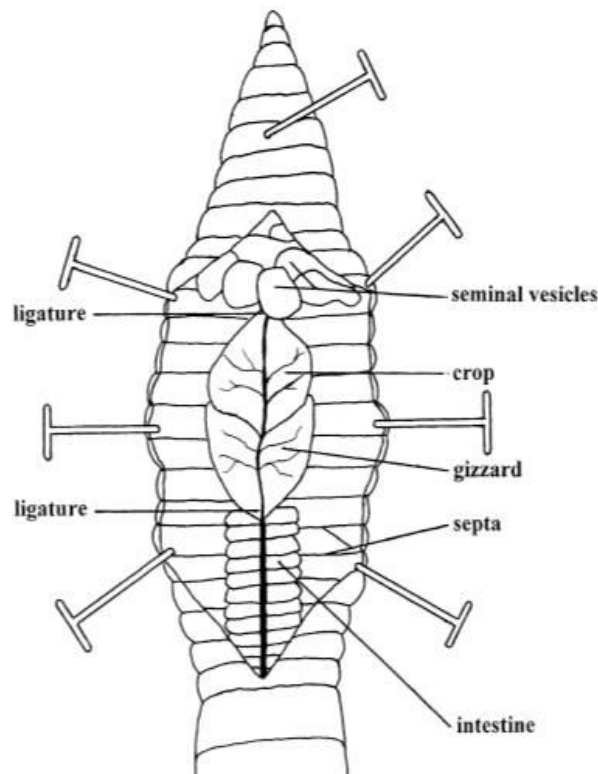


Figure AM-11-S6: Diagram showing the relative position of the earthworm gut.

5. Work quickly and carefully when mounting the gut section in the chamber.
 - Attach the one end of the gut to the glass tissue support using a loop of suture thread securely tied to the end of the crop and looped under the hook of the tissue support rod.

- Use the extra thread from the gizzard ligature to connect the preparation to the transducer.
 - Once the lower end of the gut is attached to the hook of the tissue support rod, lower the gut and its support rod into the tissue chamber. Keep tension on the upper suture thread as the assembly is lowered into the chamber. This will prevent the gut from coming off the hook on the support rod.
6. Attach the suture thread on the upper end of the gut to the appropriate hook on the arm of the transducer. The length should be no greater than its in situ length.

Note: Be very careful not to overstretch the gut. The preparation is delicate and the gut will tear if stretched too far.

7. Align the transducer, the tissue bath, and the tissue support rod. The suture thread and gut preparation should be vertical, and should not be touching the inside of the tissue bath.
8. Start recording the tension in the earthworm gut. Click on the Record button in the upper right corner of the LabScribe2 Main window.
 - Click the AutoScale button on the upper margin of the recording channel.
 - Observe the position of the trace on the screen as you gradually raise the transducer by turning the adjustment knob on the positioner.
 - Turn the knob until the trace on the screen visibly moves from its initial level. The amount of adjustment required depends on the initial slack in the dissection section of earthworm gut and the ligature threads.
9. If necessary, adjust the flow of bubbles from the aeration frit to prevent the gut from being moved around by the bubbles.

Experiment AM-11: Earthworm Smooth Muscle Contractions

Adapted from: The Use of an Isolated Earthworm Crop-Gizzard Preparation as a Model for Teaching Smooth Muscle Physiology.

Author(s): Kevin G. Krajniak, PhD and Ryan W. Klohr, PhD; Source: The American Biology Teacher, Vol. 63, No. 1 (Jan., 2001), pp. 59-65 ~ National Association of Biology Teachers

Exercise 1: Spontaneous Contractile Activity

Aim: To measure the frequency and amplitude of spontaneous contractions in the smooth muscle of the earthworm gut.

Approximate Time: 15 minutes

Note: *If contractions in the tissue are visible, but do not produce a noticeable movement in the recording, check the tension of the suture threads holding the tissue in place and the operation of the transducer and the recording system.*

Procedure

1. Type **Normal** in the Mark box. Click the Record button, and click the mark button to attach a comment to the recording.
2. Record until the contraction cycles are consistent and predictable. It may take a few minutes for the gut to have a consistent rhythm as the anesthesia wears off.
3. Record for 5 minutes once the contraction rate and amplitude are consistent.
4. Click AutoScale to expand the recording to the window and record for 5 more minutes. This will be the baseline data for your experiment.
5. Click Stop to halt the recording.
6. Select Save in the File menu.

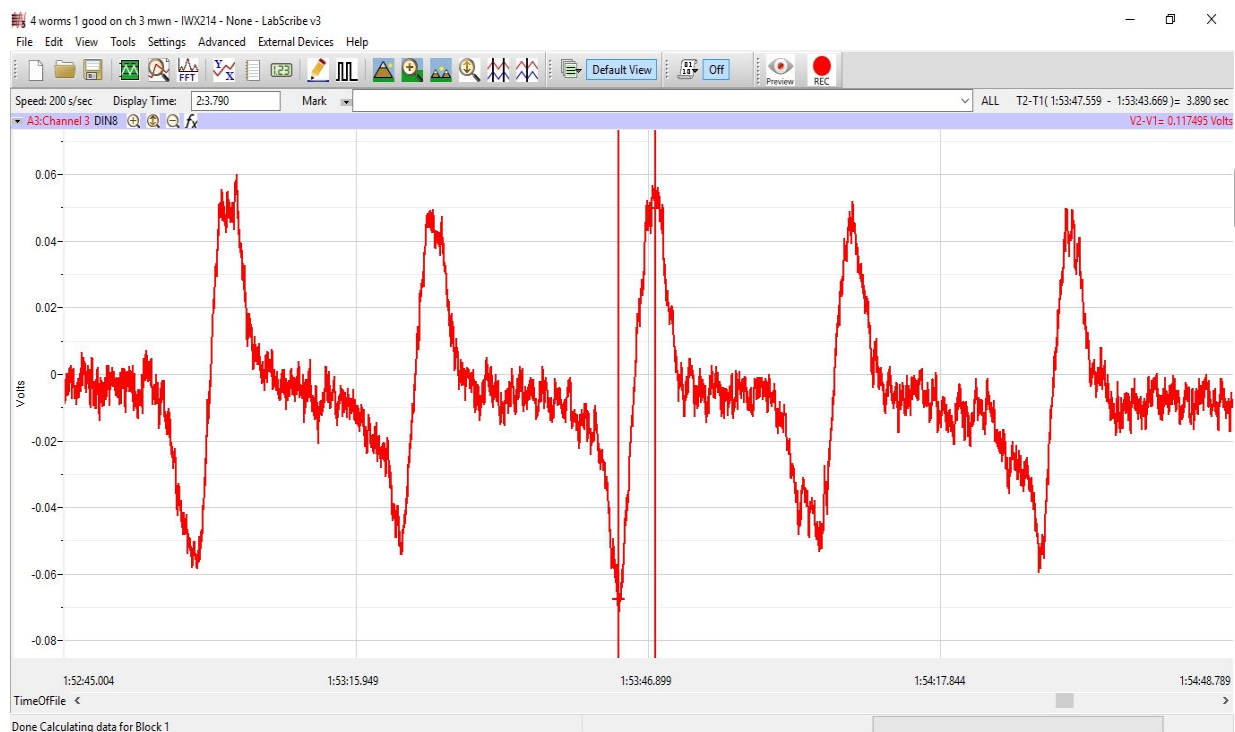


Figure AM-11-L1: The main window of the Earthworm Smooth Muscle lab showing 5 contractions in a period of 2 minutes. The cursors are also shown in position to measure the contraction amplitude.

Exercise 2: Effects of Neurotransmitters on Earthworm Smooth Muscle

Aim: To examine the effects of different neurotransmitters on amplitude and frequency of contractions in earthworm gut tissue.

Approximate Time: 20 minutes per neurotransmitter

Note: Serial dilutions of the neurotransmitter with concentrations from 10^{-3} to 10^{-8} M are prepared from a 10^{-2} molar stock. A 0.25 ml aliquot of each dilution of neurotransmitter is added to the bath beginning with the lowest concentration. The concentration reported is the final molar concentration in the bath which can be calculated by dividing the concentration of the neurotransmitter added to the bath by 100, since the 0.25 ml of neurotransmitter added is one hundredth of the tissue bath volume of 25 ml.

Warning: Each dose of neurotransmitter is followed by a saline wash once the maximal effect has been recorded.

Procedure

1. Type **Control 1** in the Mark box. Click the Record button, and then the mark button to attach a comment to the recording.

2. Before adding acetylcholine to the gut preparation, make sure the frequency and amplitude of the contractions are consistent for two or three successive cycles. Continue recording.
3. Type **Acetylcholine 10-8** in the Mark box and add 0.25 ml of the 10^{-8} Molar of Acetylcholine to the smooth muscle chamber. Click the mark button at the same time the drug is added to the chamber. Click AutoScale to maximize the height of the recording to the window.
4. Record for at least 5 minutes.
5. Remove the solution from the tissue bath chamber. Carefully rinse the gut preparation and the tissue bath chamber with fresh, room temperature Worm saline.
6. Refill the chamber containing the gut tissue with fresh, room temperature Worm saline.
7. Record for 5 minutes while the gut preparation returns to near baseline levels.
8. Type **Acetylcholine 10-7** in the Mark box and add 0.25 ml of the 10^{-7} M of Acetylcholine to the smooth muscle chamber. Click the mark button at the same time the drug is added to the chamber. Click AutoScale.
9. Record for at least 5 minutes.
10. Remove the solution from the tissue bath chamber. Carefully rinse the gut preparation and the tissue bath chamber with fresh, room temperature Worm saline.
11. Refill the chamber containing the gut tissue with fresh Worm saline.
12. Record for 5 minutes while the gut preparation returns to near baseline levels.
13. Repeat steps 9 through 13 with the remaining aliquots of Acetylcholine: 10^{-6} , 10^{-5} , 10^{-4} , and 10^{-3} M.
14. Click Stop to halt the recording when the gut response to the drug appears consistent and predictable.
15. Select Save in the File menu.
16. Remove the solution from the tissue bath chamber. Carefully rinse the gut preparation and the tissue bath chamber with fresh, room temperature Worm saline.
17. Continue rinsing of the tissue preparation and the chamber with fresh saline to remove any remnants of acetylcholine from the tissue. Any residue on the tissue or in the chamber could cause multiple drug effects.

Serotonin

1. Refill the chamber containing the gut tissue with fresh Worm saline.
2. Type **Control 2** in the Mark box. Click the Record button. Record the contractions of the gut preparation as it equilibrates to the fresh saline in the chamber.
 - When the contractions of the preparation are consistent and predictable, press the mark button.
 - This may take as long as 5 minutes.

3. Type **Serotonin 10-8** in the Mark box. Click the Record button. Press the mark button as the dose of the new drug is added to the smooth muscle chamber. Record for at least 5 minutes.
4. Click AutoScale to maximize the height of the recording to the window.
5. Follow the same procedure as outlined for Acetylcholine.
6. When all aliquots of Serotonin have been added, click Stop to halt the recording.
7. Select Save in the File menu.
8. Remove the solution from the tissue bath chamber. Carefully rinse the gut preparation and the tissue bath chamber with fresh, room temperature Worm saline.
9. Continue rinsing of the tissue preparation and the chamber with fresh saline to remove any remnants of serotonin from the tissue.

Epinephrine

1. Refill the chamber containing the gut tissue with fresh Worm saline.
2. Type **Control 3** in the Mark box. Click the Record button. Record the contractions of the gut preparation as it equilibrates to the fresh saline in the chamber.
 - When the contractions of the preparation are consistent and predictable, press the mark button.
 - This may take as long as 5 minutes.
3. Type **Epinephrine 10-8** in the Mark box. Click the Record button. Press the mark button as the dose of the new drug is added to the smooth muscle chamber. Record for at least 5 minutes.
4. Click AutoScale to maximize the height of the recording to the window.
5. Follow the same procedures as outlined for Acetylcholine and Serotonin.
6. When all aliquots of Epinephrine have been added, click Stop to halt the recording.
7. Select Save in the File menu.

Recovery

1. Refill the chamber containing the gut tissue with fresh Worm saline.
2. Type **Recovery** in the Mark box. Click the Record button and then the mark button.
3. Record the contractions of the preparation for 10 minutes to make sure the earthworm smooth muscle has recovered. Click AutoScale as needed during recording.
4. Click Stop to halt the recording and Save to save your file.

Data Analysis

Exercise 1-Spontaneous Contractile Activity

1. Scroll through the data file and locate a section near the beginning of the recording where the amplitude and period of the gut contraction cycle is consistent after the anesthesia has worn off.
2. Use the Display Time icons to adjust the Display Time of the Main window so that five gut contraction cycles are displayed on the Main window. This section of data can also be selected by:
 - Placing the cursors on either side of five gut contraction cycles of the recording, and
 - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand or contract the five earthworm gut contraction cycles to the width of the Main window.
3. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window.
4. Look at the Function Table that is above the Earthworm Gut Contractions in the Analysis window. The mathematical functions, Value1, Value2, V2-V1, and T2-T1, should appear in this table. The values for these parameters are displayed in the table across the top margin of the Earthworm Gut Contractions channel.
5. Maximize the height of the trace on the Earthworm Gut Contractions channel by clicking on the arrow to the left of the channel's title to open the channel menu. Select Scale from the menu and AutoScale from the Scale submenu to increase the height of the data on that channel.
6. Once the cursors are placed in the correct positions for determining the amplitude and period of each muscle twitch, 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.
7. 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 times of each of the 5 muscle twitches.
 - 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 Earthworm Gut Contractions channel pull-down menu.
 - Transfer the values for the amplitude and times to the Journal using the Add Ch. Data to Journal function in the Earthworm Gut Contractions channel pull-down menu.
8. On the Earthworm 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, which is the active tension, or phasic response, developed in the earthworm gut during its contraction. To measure this parameter, place one cursor at the beginning of the contraction, and the second cursor on its peak. The value for the V2-V1 function on the Earthworm Gut Contractions channel is the contraction amplitude.

- Contraction Time, which is the time between the beginning and the peak of the contraction. To measure this parameter, keep the cursors in the same positions used to measure the contraction amplitude. The value for the T2-T1 function on the Earthworm Gut Contractions channel is the contraction time.
 - Relaxation Time, which is the time between the peak and the end of the contraction. To measure this parameter, keep the cursor on the peak of the contraction and place the other cursor at the end of the contraction. The value for the T2-T1 function on the Earthworm Gut Contractions channel is the relaxation time.
 - Contraction Period, which is the time between the beginnings of adjacent contractions. To measure this parameter, place one cursor at the beginning of one contraction and the other cursor at the beginning of the adjacent contraction. The value for the T2-T1 function on the Earthworm Gut Contractions channel is the contraction period.
 - Muscle Tone, which is the passive tension, or tonic response, present in the gut tissue before or after the contraction. To measure this parameter, keep the cursors in the same positions used to measure the contraction period. Value1 on the Earthworm Gut Contractions channel is the tone of the gut at the beginning of a contraction, and Value2 is the tone at the beginning of the adjacent contraction.
9. Record the mean values in the Journal using the one of the techniques described in Steps 6 or 7, and on Table 1.
 10. Repeat Steps 2 through 9 to find the contraction amplitude, contraction time, relaxation time, contraction period, and muscle tone of five other gut contractions recorded in this exercise. Record the values in the Journal and on the table.
 11. Select Save in the File menu.
 12. Determine the average contraction period of the five gut contractions measured. Determine the average frequency of contraction by finding the inverse of the contraction period.

Exercise 2 - Effects of Neurotransmitters

1. Scroll to the section of data recorded for the control and the activity of each drug at each aliquot dosage.
2. Use the techniques explained in the data analysis section of Exercise 1 to measure the contraction amplitude, contraction time, relaxation time, contraction period, and muscle tone for each drug and aliquot.
3. Enter the mean data in the Journal using one of the techniques explained in the data analysis section of Exercise 1, and on the data table.
4. Follow the same procedures for the Recovery period.

Questions

1. What is the effect of each drug on the amplitude of the earthworm gut contraction?
2. What is the effect of each drug on the frequency of gut contractions?

3. What is the effect of each drug on tone of the smooth muscle?
4. For one of the drugs, hypothesize a mechanism by which the drug affects the contractility of the earthworm gut smooth muscle.

Table AM-11-L1: The Contraction Amplitude, Contraction Period, and Tone of Earthworm Gut Tissue Exposed to Acetylcholine, Serotonin, and Epinephrine.

Treatment	Contraction Amplitude (g)	Contraction Time (sec)	Relaxation Time (sec)	Contraction Period (sec)	Muscle Tone (g) at Beginning	Muscle Tone (g) at End
Normal Contraction						
Control 1						
Acetylcholine 10^{-8} M						
Acetylcholine 10^{-7} M						
Acetylcholine 10^{-6} M						
Acetylcholine 10^{-5} M						
Acetylcholine 10^{-4} M						
Acetylcholine 10^{-3} M						
Control 2						
Serotonin 10^{-8} M						
Serotonin 10^{-7} M						
Serotonin 10^{-6} M						
Serotonin 10^{-5} M						
Serotonin 10^{-4} M						
Serotonin 10^{-3} M						

Control 3						
Epinephrine 10 ⁻⁸ M						
Epinephrine 10 ⁻⁷ M						
Epinephrine 10 ⁻⁶ M						
Epinephrine 10 ⁻⁵ M						
Epinephrine 10 ⁻⁴ M						
Epinephrine 10 ⁻³ M						
Recovery						

Additional Exercises

Additional exercises using the earthworm gut preparation can include:

- examining the effects of changes in temperature on smooth muscle contractions. Use room temperature, warm and cold Worm saline.
- examining the effects of extracellular ions of smooth muscle contractions. Use high Ca⁺ and K⁺ solutions, and solutions containing no Ca⁺ or K⁺.