

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.

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Exercise 1: Spontaneous Contractile Activity

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

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 to the right of the Mark button. Click the Record button, and press the Enter key 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 ([Figure AM-11-L1](#)).
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.

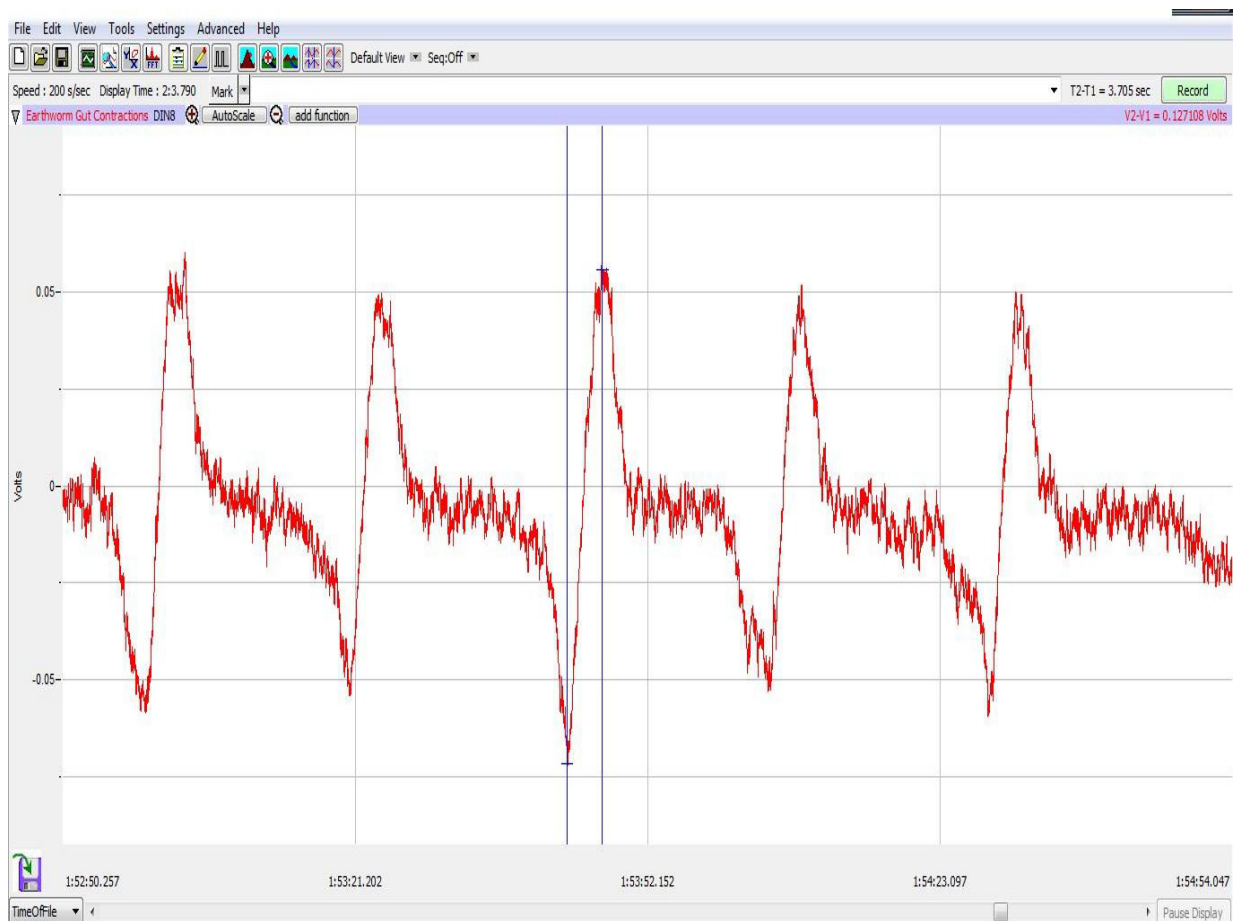


Figure AM-11-L1: The main window of the Earthworm Smooth Muscle lab showing 5 contractions in a period of 2 minutes. The cursors are also shown in position to measure the contraction amplitude.

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.

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 to the right of the Mark button. Click the Record button, and press the Enter key 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.
4. Add 0.25 ml of the 10⁻⁸ aliquot of Acetylcholine to the smooth muscle chamber. Press the Enter key on the keyboard to mark the recording at the same time the drug is added to the chamber. Click AutoScale to maximize the height of the recording to the window.
5. Record for at least 5 minutes.
6. Remove the solution from the tissue bath chamber. Carefully rinse the gut preparation and the tissue bath chamber with fresh, room temperature Worm saline.
7. Refill the chamber containing the gut tissue with fresh, room temperature Worm saline.
8. Record for 5 minutes while the gut preparation returns to near baseline levels.
9. Type Acetylcholine 10⁻⁷ in the Mark box.
10. Add 0.25 ml of the 10⁻⁷ aliquot of Acetylcholine to the smooth muscle chamber. Press the Enter key on the keyboard to mark the recording at the same time the drug is added to the chamber. Click AutoScale to maximize the height of the recording to the window.
11. Record for at least 5 minutes.
12. Remove the solution from the tissue bath chamber. Carefully rinse the gut preparation and the tissue bath chamber with fresh, room temperature Worm saline.
13. Refill the chamber containing the gut tissue with fresh Worm saline.
14. Record for 5 minutes while the gut preparation returns to near baseline levels.
15. Repeat steps 9 through 13 with the remaining aliquots of Acetylcholine: 10⁻⁶, 10⁻⁵, 10⁻⁴, and 10⁻³.
16. Click Stop to halt the recording when the gut response to the drug appears consistent and predictable.
17. Select Save in the File menu.
18. Remove the solution from the tissue bath chamber. Carefully rinse the gut preparation and the tissue bath chamber with fresh, room temperature Worm saline.
19. 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 Enter key on the keyboard to mark the recording.
 - This may take as long as 5 minutes.
3. Type Serotonin 10-8 in the Mark box. Click the Record button. Press the Enter key 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 Enter key on the keyboard to mark the recording.
 - This may take as long as 5 minutes.
3. Type Epinephrine 10-8 in the Mark box. Click the Record button. Press the Enter key 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 to the right of the Mark button. Click the Record button, and press the Enter key to attach the comment to the recording.
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 AM-11-L1](#).
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.

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}						
Acetylcholine 10^{-7}						
Acetylcholine 10^{-6}						
Acetylcholine 10^{-5}						
Acetylcholine 10^{-4}						
Acetylcholine 10^{-3}						
Control 2						
Serotonin 10^{-8}						
Serotonin 10^{-7}						
Serotonin 10^{-6}						
Serotonin 10^{-5}						
Serotonin 10^{-4}						
Serotonin 10^{-3}						

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

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.

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⁺.