Experiment AN-11: Leech CNS - Physiological Identification of Neurons

Allow two lab periods to complete this experiment.

Equipment Required

PC or Macintosh computer

IXTA, USB cable, IXTA power supply

Model 3100 intracellular amplifier and headstage probe

Microelectrode holder (adapter)

Glass microelectrodes

Audio monitor (optional)

BNC T-connector

Double male banana-female BNC adapter

Micromanipulator with base

Faraday cage

Steel base plate floating on a bicycle inner tube

Headstage tester (18 megohm resistor with clip)

Preparation dish

Dissection microscope and light source

Bath ground electrode with cable

Pasteur pipettes and bulbs

Assorted banana cables and alligator clips

Leech

Leech saline

Electrode filling solution (see Appendix)

Model 3100 Amplifier and Audio Monitor Setup

1. Place the Model 3100 intracellular amplifier inside the Faraday cage and its AC power supply on the bench outside the cage.

Warning: *Before connecting the headstage to the probe input of the Model 3100, turn off the Model 3100 amplifier.*

2. Place a T-connector (BNC) on the Channel 1 BNC Input of the IXTA.

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3. Use a BNC-BNC cable to connect the BNC output of the Model 3100 to an open end of the T-connector (BNC) on the Channel 3 BNC input of the IXTA.
4. Attach the BNC connector on the input cable of the audio monitor (if using one) to the remaining open end of the T-connector on the Channel 3 BNC input of the IXTA.
5. Locate the headstage probe in the NBK kit. Attach the connector on the cable of the probe into the Mini-DIN6 probe socket of the Model 3100 amplifier.
6. Mount the intracellular probe in the micromanipulator and place it near the dissection microscope and the preparation dish.
7. Place the knobs and switches on the front of the Model 3100 amplifier in positions that configure the amplifier for this experiment. The settings used on the Model 3100 for this experiment are listed in Table AN-11-S1.
8. Position the preparation dish on the microscope stage, so that the center of the dish is visible through the microscope. Orient the light so that it shines on the center of the dish.
9. Plug the output of the AC power supply into the power input on the back of the Model 3100 amplifier. Plug the power cord of the AC power supply into the electrical outlet.
10. Flip the power switch of the Model 3100 to the ON position and allow the amplifier to warm up for 5 minutes.

Table AN-11-S1: Settings on the Model 3100 Amplifier for Recording Cell Potentials

Function	Setting
CURRENT COMPENSATION DC BAL	Counterclockwise
CURRENT COMP. TRANSIENTS (2)	Counterclockwise
CURRENT COMPENSATION SWITCH	OFF
DC OFFSET	Counterclockwise
DC OFFSET SWITCH	OFF
CAPACITY COMPENSATION	Counterclockwise
ΔA	Counterclockwise
RESISTANCE (Ω) TEST	OFF
NOTCH FILTER	OFF
LOW PASS FILTER (kHz)	10
CURRENT INJECTION LEVEL (μA)	Counterclockwise
CURRENT INJECTION SWITCH	OFF
OUTPUT	X1

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Note: *The settings listed in this table are suggested for use in ideal recording conditions. If noise is present in the recording environment, the notch filter may need to be used to block noise caused by AC line voltage used to power the equipment in the lab.*

Headstage Probe Test

Warning: *Before testing the headstage probe, turn off the Model 3100 amplifier.*

1. Obtain an 18 megohm resistor (1/4 watt) with an alligator clip attached to one end and a banana jack on the other end.
2. Place a pin in the socket on the end of the headstage. Fasten the alligator clip on the end of the resistor to the pin in the socket of the headstage.

Warning: *Make sure nothing touches the gold ring around the probe input or the body of the headstage.*

3. Connect the banana jack on the other end of the resistor to the ground (GND) input on the front of the Model 3100 amplifier with a male banana-banana cable.
4. Type Probe Test in the Mark box.
5. Click Record and click the Mark button. The trace should appear near the center of the screen. Use the DC OFFSET knob of the Model 3100 to set the baseline equal to zero.
6. Locate the ΩTEST switch on the front of the Model 3100 amplifier. Flip up the switch for a few seconds. The trace on the computer screen should deflect to a higher amplitude and return to the baseline when the switch is returned to the OFF position. Click Stop to halt recording.
7. Select Save As in the File menu and type a name for the file. Click on the Save button to save the data file.
8. Click the Double Cursor icon, so that two blue vertical lines appear over the recording window.
9. Click and drag one cursor to the baseline prior to the electrode test being run. Place the second cursor on the plateau recorded while the test was running.
10. On the upper right margin on the Cell Potential channel, the voltage difference between the positions of the two cursors, V2-V1, is the output of the Model 3100 and its probe. The output of the amplifier is 10mV for every megohm of load applied
11. If an 18 megohm resistor is used to test the probe and the X1 output of the Model 3100 is recorded, the output of the Model 3100 during the test is 180 millivolts (mV):

    $$18\ M\Omega \times 10mV/M\Omega = 180\ mV$$

    If an 18 megohm resistor is used to test the probe and the X10 output of the Model 3100 is recorded, the output of the Model 3100 during the test is 1800 millivolts (mV):

    $$(18\ M\Omega)\ (10mV/M\Omega)\ (10X\ gain) = 1800\ mV$$

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12. If the probe and amplifier test to the level that is appropriate for the gain of the unit, the probe, the electrometer, and the recording unit are working properly.

If there is electrical noise in the recording, please see the Appendix.

Ganglia

Isolated ganglia are prepared and provided to students by the laboratory staff. Students will need to mount their own ganglia in the prep dish before recording.

1. Obtain a small, plastic prep dish which has a thin layer of Sylgard elastomer on the bottom. Fill the prep dish with chilled leech saline.
2. Place the isolated ganglion in the prep dish filled with chilled leech saline.
3. Pin the anterior end of the nerve cord, ventral side up, onto the Sylgard surface of the dish. Use pins that are cut from 0.002" diameter tungsten wire.
4. Stretch the nerve cord tightly and pin the posterior end of the preparation to the Sylgard layer.
5. Pin out the lateral nerve roots through the connective tissue that lies between the roots. Cut away any excess connective tissue from the roots.
6. Exchange the saline in the preparation dish with fresh, chilled leech saline:
    - Every 10 or 15 minutes to prevent the isolated ganglion preparation from overheating and desiccating.
    - Whenever electrodes are changed.
    - Whenever a microelectrode tip breaks to prevent the electrolyte leaking from the broken electrode from desiccating any cells.

Microelectrode Preparation

1. Fill a glass microelectrode with 3M potassium acetate using a fine syringe needle (Hamilton type) on a 3cc syringe.
    - Potassium acetate is used as the electrolyte in electrodes to prevent changes to potentials in the cells. If chloride ions were used in the electrolyte, chloride would leak into the neurons from the electrode and change some of the characteristics of the inhibitory postsynaptic potentials (IPSPs) in the cells. Acetate ions are too large to leak into cells, and they do not affect IPSPs.
    - The microelectrode is filled from the back. Gently lower the needle into the lumen of the microelectrode until the tip of the needle is close to the taper in the microelectrode.
    - As you slowly inject the 3M potassium acetate into the microelectrode, pull the needle up the microelectrode to allow space for the fluid. Keep the tip of the needle under the meniscus of the fluid as you withdraw the needle from the microelectrode. This will prevent bubbles from forming in the microelectrode.

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- Make sure the microelectrode is filled to the top. You do not want a bubble at the end of the microelectrode when it is put in the microelectrode holder.

2. Fill the microelectrode holder with the same 3M Potassium Acetate solution used for the microelectrode. This solution completes the electrical connection between the glass microelectrode and the intracellular probe. Make sure the gold-plated socket (pin jack) on the back of the plastic microelectrode holder is screwed tightly into the holder. If the pin jack is loose, the 3M potassium acetate solution can leak out of the electrode holder and make contact with both the pin jack and the pin of the intracellular probe. If the different metals used to make the pin jack, the pin on the intracellular probe, and the pellet or wire in the electrode holder are all in contact with 3M potassium acetate, then, reactions can take place among the different metals. These reactions can cause an off-scale shift in the baseline of the recording.
3. If you are using an microelectrode holder with a silver wire in place of a Ag-AgCl pellet, the holder does not need to be filled with 3M potassium acetate. However, the pin jack needs to be screwed tightly into the holder. In this type of holder, the silver wire goes into the back of the glass microelectrode and makes contact with the solution in the microelectrode. This electrolyte can migrate up the wire and into the back of the holder. If the pin jack on the holder is loose, the 3M potassium acetate can move past the rubber gasket that normally seals the lumen of the holder from the gold-plated socket, and create the same problems mentioned in Step 2.
4. If you are using a microelectrode holder with a Ag-AgCl pellet, fill the holder with 3M potassium acetate.
    - Loosen the plastic cap of the microelectrode holder to take pressure off the rubber gasket behind the cap.
    - Gently lower the needle of the filling syringe into the lumen of the cap, through the gasket, and into the lumen of the holder until the tip of the needle is close to the Ag-AgCl pellet.
    - Fill the microelectrode holder with 3M potassium acetate, until a drop forms on top of the cap.

Note: *Pushing the glass microelectrode into the holder too vigorously, or while the cap is tight, may result in the microelectrode breaking.*

- Place the back of the microelectrode into the lumen of the cap. Carefully push the microelectrode through the rubber gasket that sits behind the cap, and into the lumen of the microelectrode holder. The lumen of the microelectrode holder steps down to a smaller diameter, so the back of the glass microelectrode will stop on the edge of this smaller lumen.
- Gently tighten the cap of the holder. Tightening the cap too much will crack the glass microelectrode.

5. Push the microelectrode holder into the socket of the headstage probe.
6. Carefully position the microelectrode tip over the preparation.

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7. Use the micromanipulator's vertical controls to move the microelectrode until its tip is in the saline overlying the preparation.
8. To complete the circuit, a ground electrode needs to be attached to the ground (GND) input of the Model 3100. The best material for the indifferent electrode is a coil of Ag-AgCl wire. Place the coil of wire in the bath solution surrounding the ganglion. Make sure no other metal, only the Ag-AgCl coil is in contact with saline solution.
9. Check that both electrodes are in the leech saline in the prep dish.
10. Test the resistance of the microelectrode before impaling the cell.
    - Type Electrode Test in the Mark box to the right of the Mark button.
    - Click Record to begin recording. Use the DC OFFSET knob of the Model 3100 to set the baseline equal to zero. Press the Enter key on the keyboard to mark the recording.
    - Use the ΩTEST switch on the Model 3100 amplifier to send a pulse through the microelectrode to test its resistance. Examine the recording to determine the voltage recorded and the resistance of the microelectrode.

Note: *When the 1X output is used, a defection of 10 mV is equal to 1 MΩ.*

- The ideal microelectrode is one with a resistance between 10 MΩ and 20 MΩ. If the resistance is below 5 MΩ, discard the microelectrode. If the resistance is above 30 MΩ, try vibrating the microelectrode by pressing the RINGER button on the Model 3100. If the resistance remains above 30 MΩ, discard the microelectrode.

11. Click Stop to halt the recording. The trace on the screen may have a small wave or ripple through it. This is noise from devices in the room that operate on 60Hz AC current. The probe is picking up these currents, but they can be reduced by grounding metal objects like the microscope or light source to any grounded point on the iWorx unit. Turning off and unplugging the light source, when not needed also reduces this noise.

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Experiment AN-11: Leech CNS - Physiological Identification of Neurons

Allow two lab periods to complete this experiment.

Exercise 1: Recording Potentials from Neurons in a Central Nervous System

Aim: To learn the techniques for recording membrane, synaptic, and action potentials from neurons in a leech CNS ganglion.

Approximate Time: 30 minutes

Preparation

1. Position the prep dish holding a leech ganglion in the field of view of the dissecting microscope.
2. Position the micromanipulator, the headstage probe, and the microelectrode and its holder so that these items and the microscope can be used easily.
3. Use the micromanipulator and the dissecting microscope to guide the tip of the microelectrode to a position in the bath solution and just above the ganglion.
4. Align the beam of light from the illuminator and focus the microscope so that both the tip of the microelectrode and the outline of the neuron are clearly visible. Illuminating the preparation from below or from the side can provide the best view of these objects against the background.
5. Make sure only the Ag/AgCl ground electrode is in the bath solution.

Warning: *Neither the copper wire in the ground cable nor the solder joint between the Ag/AgCl ground electrode and the copper wire should be in contact with the solution in the prep dish. Having all these metals in contact with each other through an electrolytic solution can cause an offset of the recording baseline that cannot be negated by the controls on the Model 3100 amplifier.*

6. Record another electrode test before impaling the first neuron to measure the resistance of the microelectrode:
    - If the resistance has increased, try ringing the microelectrode to lower the resistance.
    - If the resistance is lower by more than 20%, the tip has probably broken off, and the microelectrode should be replaced to avoid damaging the neurons being penetrated.
    - If the recording drifts wildly or if the electrodes have resistances that are higher than expected, check the position and condition of the bath ground. Make sure the bath ground is in the proper location and that there is no bath solution on the solder joint or wire of the ground cable.

Procedure

1. Select one of the cells in the ganglion, and manipulate the tip of the microelectrode as close as possible to the center of the cell body.
2. Turn on the audio monitor and set the volume to a low, but audible level.

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Warning: *Before removing the electrode from the bath solution or ringing the electrode, turn off the audio monitor to avoid damaging the speaker.*

3. Click the Record button. Use the DC OFFSET knob of the Model 3100 to set the baseline equal to zero. Mark the recording to indicate the type of cell from which the recording is made.
4. Lower the tip of the microelectrode towards the cell until the cell membrane dimples under the pressure of the electrode tip. The diameter of the cell also expands when pressure is placed on the membrane.
5. Gently tap the base, or the back of the micromanipulator, to create a vibration that will drive the tip of the microelectrode into the cell. Watch the recording on the screen and not the cell. When a microelectrode is inside a cell, a sharp negative deflection accompanied by action potentials and synaptic potentials is seen on the recording.
6. The proper penetration of a cell can also be heard as a sharp click on the audio system. The action potentials from cells also generate sharp clicks. The patterns and tones of these action potentials can be used to recognize different cells.
7. If the first attempt is unsuccessful, advance the microelectrode slightly and try again. If this does not succeed, reposition the microelectrode and try again. Each time the microelectrode is repositioned, the resistance of the electrode should be measured to make sure that the tip is neither broken nor clogged.
8. Click Stop to halt recording.
9. Impale and record from at least five different cells in the ganglion. Record long enough to obtain information on the membrane potential and any action potentials.
10. Select Save in the File menu.

Data Analysis: Resting Membrane Potential Entering

1. Scroll through the data file and locate the section of the recording made when the microelectrode penetrated the membrane of the first cell.
2. Use the Display Time icons to adjust the Display Time of the Main window to display a 5-second section of the recording at the time when the microelectrode penetrated the membrane on the Main window. This section of data can also be selected by:
    - Placing the cursors on either side of the 5-second section of the recording, and
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand or contract the 5-second section of the recording to the width of the Main window.

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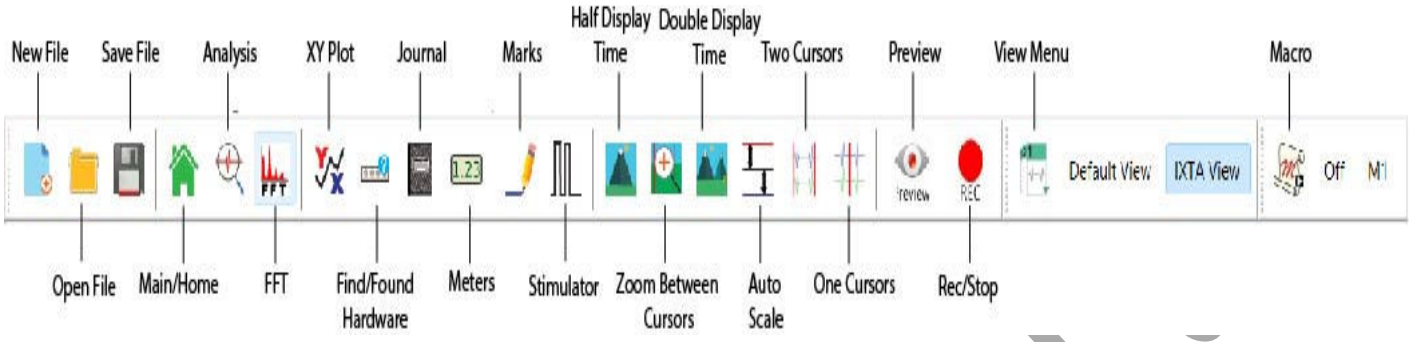

Figure AN-11-L1: The LabScribe toolbar.

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. The mathematical functions, V2-V1 and T2-T1 should appear on screen. 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.
5. Once the cursors are placed in the correct positions for determining the change in voltage (V2-V1) as the tip of the microeletrode enters the cell, the value for V2-V1 can be recorded in the on-line notebook of LabScribe by typing the names and values of the parameters directly into the Journal.
6. The functions in the channel menu of the Analysis window can also be used to enter the name and value of the voltage change from the recording to the Journal. To use these functions:
    - Place the cursors at the locations used to measure the membrane potential.
    - Transfer the name of the parameter to the Journal using the Add Title to Journal function in the Cell Potential channel menu.
    - Transfer the value for the parameter to the Journal using the Add Ch. Data to Journal function in the Cell Potential channel menu.
7. On the Cell Potential channel displayed in the Analysis window, use the mouse to click on and drag a cursor to the baseline recorded before the microelectrode penetrated the cell. Drag the other cursor to the voltage level that results from the microelectrode being in the cell.
8. Record the value for the voltage change (V2-V1) in the Journal using the one of the techniques described in Steps 5 or 6, and in Table AN-11-1.
9. Divide the value for V2-V1 by the gain of the Model 3100 amplifier to obtain the resting membrane potential on entering (RMPE). For example, a voltage difference (V2-V1) of 300 millivolts recorded by the Model 3100 with a gain of 10X is equal to a membrane potential of 30 millivolts:

300 mV/10X Gain = 30 mV

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Data Analysis: Action Potentials

1. On the Main window, place the section of data recorded just as the microelectrode entered the first cell.
2. Use the AutoScale and the Display Time functions to zoom in on this section of the recording. Scroll through this section of data and locate an action potential.
3. Use the Display Time icons to adjust the Display Time of the Main window to display the action potential on the Main window. This section of data can also be selected by using the Zoom between Cursors button.
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. The mathematical functions, V2-V1 and T2-T1 should appear on screen. 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. Place the cursors in the appropriate positions on the action potential to measure the following parameters:
    - Amplitude (Amp): Measure the amplitude of an action potential by placing one cursor on the level of the resting membrane potential, and the second cursor on the peak of the action potential. The value for the V2-V1 function on the Cell Potential channel is the amplitude.
    - Duration at Half Peak (Dur-1/2A): Measure this parameter by placing one cursor at the onset of the action potential, and the second cursor on the point that is halfway between the onset of the action potential and the peak. The value for the T2-T1 function on the Cell Potential channel is the duration at the half peak.
    - After-Potential Amplitude (After-P Amp): Measure the amplitude of the after-potential by placing one cursor on the level of the resting membrane potential, and the second cursor on the peak of the after-potential. The value for the V2-V1 function on the Cell Potential channel is the after-potential amplitude.
    - After-Potential Duration (After-P Dur): Measure this parameter by placing one cursor at the onset of the after-potential, and the second cursor on the offset of the after-potential. The value for the T2-T1 function on the Cell Potential channel is the duration of the after-potential.
7. Record the values for the parameters in the Journal using the one of the techniques described earlier and in Table AN-11-1.
8. Divide the value for V2-V1 by the gain of the Model 3100 electrometer to obtain the actual amplitude of the action potential. For example, a voltage difference (V2-V1) of 50 millivolts recorded by the Model 3100 with a gain of 10X is equal to an amplitude potential of 5millivolts:

    50 mV/10X Gain = 5 mV

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Data Analysis: Resting Membrane Potential Exiting

1. On the Main window, place the section of data recorded as the microelectrode was removed from the first cell.
2. Use the Display Time icons to adjust the Display Time of the Main window to display a 5-second section of the recording at the time when the microelectrode was removed from the membrane on the Main window. This section of data can also be selected by using the Zoom between Cursors button.
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. The mathematical functions, V2-V1 and T2-T1 should appear on screen. 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.
5. On the Cell Potential channel displayed in the Analysis window, use the mouse to click on and drag a cursor to the voltage recorded while the microelectrode was in the cell. Drag the other cursor to the baseline recorded after the microelectrode was withdrawn from the cell.
6. Record the value for the voltage change (V2-V1) in the Journal using the one of the techniques described earlier and in Table AN-11-1.
7. Divide the value for V2-V1 by the gain of the Model 3100 amplifier to obtain the resting membrane potential as exiting (RMPX). For example, a voltage difference (V2-V1) of 320 millivolts recorded by the Model 3100 with a gain of 10X is equal to a membrane potential of 32 millivolts:

320 mV/10X Gain = 32 mV

Questions

1. Compare the magnitude of the voltage change that occurs when the cell was first impaled (RMPE) to the magnitude of the voltage change that occurs when the microelectrode is removed from the cell (RMPX). Are these two values the same?
2. Why would the magnitudes of the voltage changes that occurred when the microelectrode was placed in or removed from the cell be different?

Warning: *If the ganglion appears opaque, or if the cells in the ganglion are shrunken, obtain a new ganglion before beginning the next exercise.*

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Table AN-11-1: Properties Recorded from Selected Neurons in a Leech Ganglion.

Neuron I.D.	RMP_E (mV)	Amp (mV)	Dur-1/2 A (mV)	After P Amp	After P Dur	RMP_X (mV)

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Exercise 2: Identify Specific Neurons

Aim: To record the membrane potentials and action potentials of specific neurons, and to use the location of a neuron and parameters of its potentials to establish the identity of the neuron.

Approximate Time: 60 minutes

Stimulator Setup

1. Locate the double male banana-female BNC adapter in the NB kit.
2. If using an IWX/214, plug the male double banana-female BNC adapter into the positive (red) and ground (green) banana jacks of the IXTA stimulator. The banana plug that goes into the ground (green) stimulator output is identified by a tab, embossed with the letters GND (ground), on that side of the adapter
3. Connect a BNC-BNC cable between the S1 BNC connector on the IXTA, or the BNC connector of the adapter on the stimulator outputs of the IWX/214, and the EXTERNAL BNC current input on the Model 3100 amplifier.
4. Rotate the µA knob on the Model 3100 to zero.

Preparation

1. Use the map of the ventral surface of the ganglion and your observations of the ganglion through the dissecting microscope to identify some of the more prominent neurons in the ganglion.
2. As you examine the ganglion, plan to record from the neurons on this list:
    - Rz (Retzius), in the anterior ventromedial packet. These neurons have large, relatively broad action potentials that do not overshoot. Because the two Rz cells are strongly coupled electrically, a large coupling potential is often seen in an RZ cell every time the other Rz neuron fires.
    - T1 (TL), T2 (TV), or T3 (TD), in either of the anterolateral packets.
    - P1 (PD) or P2 (PV), in either of the posterolateral packets.
    - N1 or N2 in either of the anterolateral packets.
3. To attain the next level and become the envy of your friends and the joy of your family, plan to record from the neurons on this list:
    - AP (Anterior Pagoda), in either of the anterolateral packets. These are large cells near the center of these packets. Their action potentials have a characteristic shape similar to the roof of a Japanese pagoda.
    - AE (Annulus Erector), in the posterior ventromedial packet. These are motor neurons to the annulus erector muscles and are medium-sized cells at the posterior edge of the ganglion. Their action potentials are similar to those of the AP cells. AE cells were called Posterior Pagodas until their function was discovered.
4. To attain the top level and become a leech neuron superstar, plan to record from the S cell, in the anterior ventromedial packet. This cell is unpaired and much smaller than the other neurons listed. It is the only neuron in this packet, besides the Rz cells, that has an action potential greater than 10 mV in amplitude.

Procedure - Resting Membrane Potentials

1. Test the resistance of the microelectrode while its tip is in the bath solution.
2. Set the baseline of the recording to zero using the DC OFFSET of the Model 3100 amplifier while the microelectrode is outside the neuron.
3. Impale the cell and record the voltage change that occurs as the microelectrode enters the cell. Mark the recording with the cell type.
4. Click on the Stop button.
5. Select Save in the File menu.
6. Repeat these steps to measure the resting membrane potentials of the other neurons on the list.

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Procedure - Prolonged Depolarization

1. Click the Stimulator Preferences icon on the LabScribe toolbar to open the stimulator control panel if it does not open automatically.

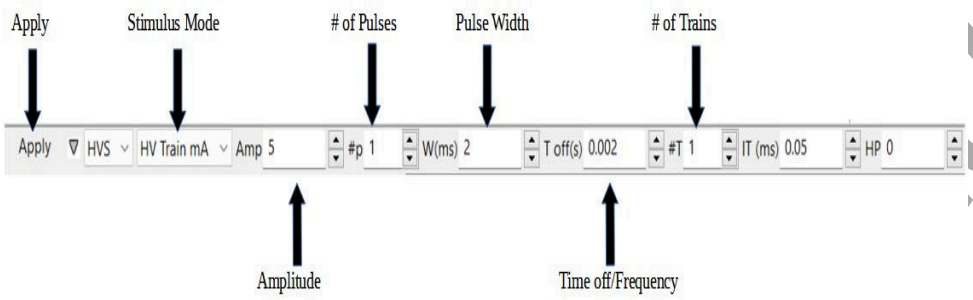

Figure AN-11-L2: The stimulator control panel.

2. Check the settings on the stimulator control panel against the settings for this exercise that are listed in Table AN-11-2.

Table AN-11-2: Stimulator Settings for Determining the Effects of Prolonged Depolarization.

Function	Units/Title	Setting
Stimulus Mode		Constant
Stimulator Start		With Recording
Time Resolution	msec	0.01
Toolbar Step Frequency	Hz	1
Toolbar Step Amplitude	Volts	0.01
Toolbar Step Time	Variable	0.01
Delay	Sec	0.000
Amplitude (Amp)	Volt	0.020

3. Make sure the stimulus amplitude on the stimulator control panel is set to 0.020V. An amplitude of 0.020V sets the current injected into the neuron at 2nA.

Note: *The conversion factor for the EXTERNAL input of the Model 3100 is 100nA of current for each volt sent to the input from a voltage source like a stimulator. A positive voltage generates a current with a positive polarity.*

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4. Click the Apply button to finalize any changes that were made to the stimulator control panel.
5. Set the μA knob on the amplifier to zero.
6. Test the resistance of the microelectrode while its tip is in the bath solution.
7. Set the baseline of the recording using the DC OFFSET of the Model 3100 amplifier while the microelectrode is outside the neuron.
8. Injected current causes a voltage drop across the microelectrode. Before impaling a cell and measuring its response, use the DC BAL knob to eliminate the voltage drop across the electrode:
    - Send a pulse of current through the electrode sitting in the bath solution by holding down the CURRENT INJECTION switch to MOMEM (momentary) position.
    - Adjust the position of the DC BAL knob until the current pulse is eliminated from the recording.
    - The only indications of the current pulse on the recording are the two transient artifacts at the onset and offset of the pulse.
    - Once the current compensation is set, return the CURRENT INJECTION switch to the OFF position.
9. Record the voltage drop as the neuron is impaled.
10. Use the CURRENT INJECTION switch to inject current into the neuron. Determine if the neuron is generating action potentials. Action potentials from leech neurons are less than 10mV in amplitude.

Note: *A depolarizing current injected into a cell moves the membrane potential of the neuron closer to its threshold level. If the added current depolarizes the membrane potential (makes it less negative) to a level above the threshold, the neuron generates an action potential.*

11. If the neuron does not generate action potentials with the present level of current injection, increase the amplitude of the pulse from the stimulator by 10mV. Remember to cancel the effect of the stimulus current by using the DC BAL knob.
12. Once the neuron begins generating action potentials, continue to record for long enough to determine if the neuron is firing tonically or phasically.
    - In a tonic response, a neuron responds as long as current is being passed into the neuron. There are degrees of tonic firing. Some cells accommodate to a constant stimulus very slowly and others accommodate very quickly.
    - In a phasic response, the neuron fires only transiently, at the start of the depolarizing pulse.
13. Repeat these steps to measure the effects of prolonged depolarization in the other neurons on the list.

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Data Analysis- Resting Membrane Potentials

Use the same techniques described in Exercise 1 to measure and record the properties of the entering resting membrane potentials of specific neurons in the Journal and Table AN-11-3.

Data Analysis- Accommodation

By qualitative means, determine if each type of neuron has a phasic or tonic response to prolonged depolarization. Record the Accommodation (Accom) response of the neuron in the Journal and Table AN-11-3.

Data Analysis- Action Potentials

Use the same techniques described in Exercise 1 to measure and record the properties of the action and after-potentials of specific neurons in the Journal and Table AN-11-3.

Table AN-11-3: Properties Recorded from Selected Neurons in a Leech Ganglion.

Neuron I.D.	RMP_E (mV)	Amp (mV)	Dur-1/2 A (mV)	After P Amp	After P Dur	Accom.

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Bibliography

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