Experiment AN-1: Membrane Potentials

Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

Preparation dish

Dissection microscope and light source

IC-200 Intracellular probe and indifferent electrode

Micromanipulator

Glass microelectrodes

Microelectrode holder (adapter)

Headstage tester (18 megohm resistor with clip)

Pasteur pipettes and bulbs

Assorted banana cables and alligator clips

Crayfish

Crayfish salines

Electrode filling solution

Equipment Setup

1. Locate the IC-200 intracellular probe and the microelectrode holder..
2. Mount the intracellular probe in the micromanipulator and place it near the dissection microscope.
3. 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.
4. Plug the DIN connector of the IC-200 intracellular probe cable into Channel A5.

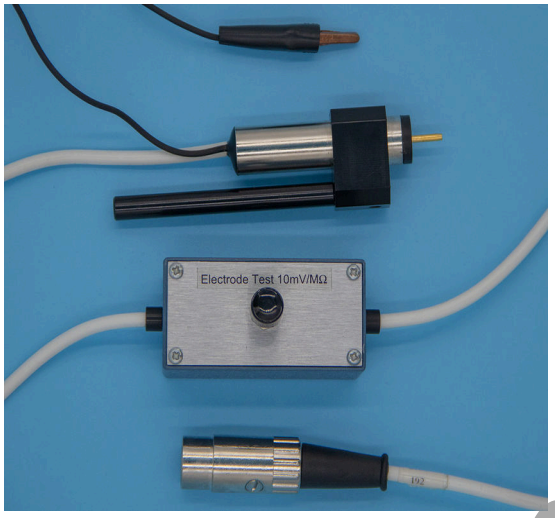

Figure AN-1-S1: The IC-200 intracellular probe.

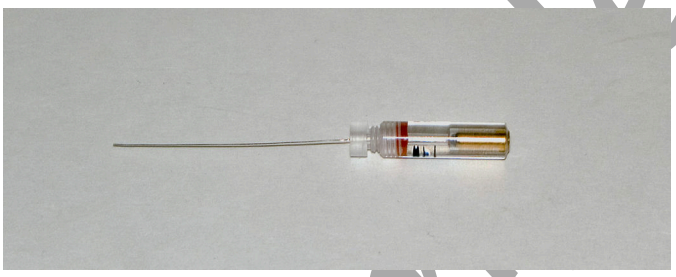

Figure AN-1-S2: The microelectrode holder for the IC-200.

Figure AN-1-S3: The IC-200 intracellular probe with a microelectrode holder connected to an IXTA.

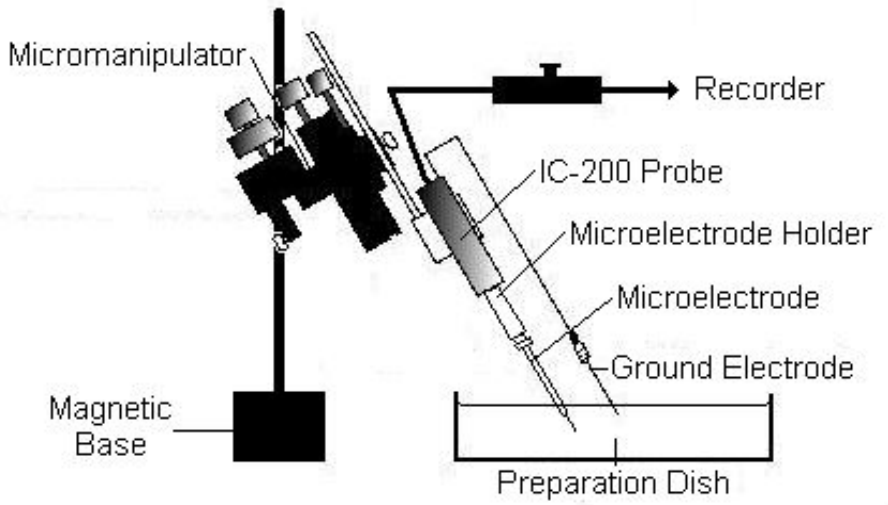

Figure AN-1-S4: Diagram showing the arrangement of equipment used to record resting membrane potentials from fast abdominal extensor muscles.

Intracellular Probe Test

1. Obtain a 18 megohm resistor (1/4 watt), and attach a small alligator clip to one of its ends.
2. Attach the end of the resistor with the alligator clip to the pin on the intracellular probe. Fasten the alligator clip on the black wire of the indifferent input to the other end of the resistor.

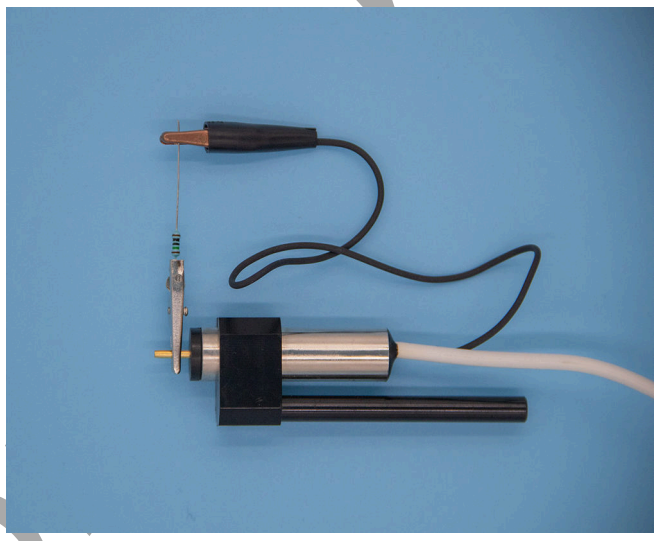

Figure AN-1-S5: Circuit for testing the output of the IC-200 probe. The pin of the probe input is connected to the ground electrode with an 18 megohm resistor that simulates the cell membrane.

3. Type Probe Test in the Mark box.

4. Click Record and click the mark button. The trace should appear near the center of the screen.
5. Locate the test module on the cable of the intracellular probe. Depress and hold the electrode test button for a few seconds. The trace should deflect to a higher amplitude as the button is depressed, and return to the baseline when the button is released. Click Stop to halt recording.
6. Select Save As in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder. Designate the file type as *.iwxdata. Click on the Save button to save the data file.
7. Click the Double Cursor icon so that two vertical lines appear over the recording window.

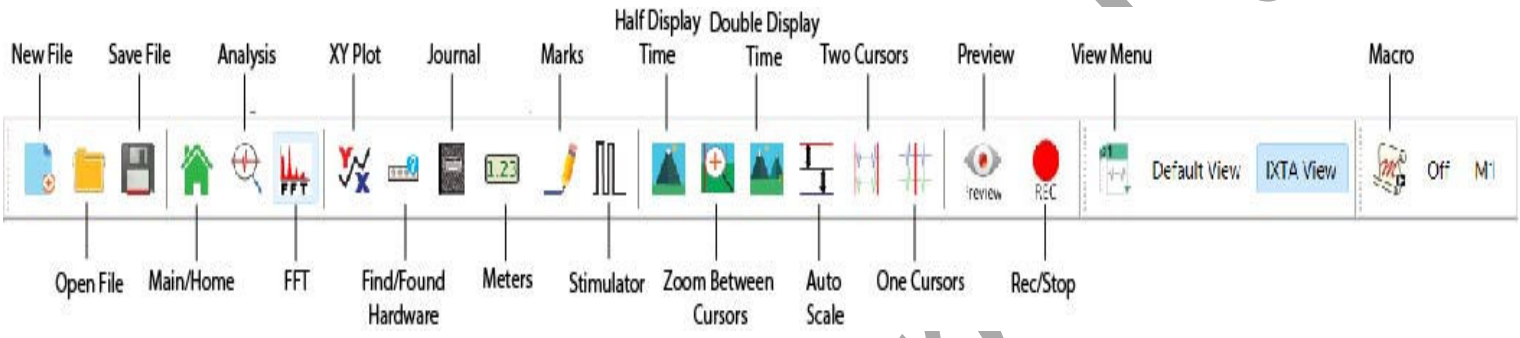

Figure AN-1-S6: The LabScribe toolbar.

8. Click and drag one cursor to the baseline prior to the electrode test button being depressed. Place the second cursor on the plateau recorded while the test button was depressed.
9. On the upper right margin on the Membrane Potential channel, the voltage difference between the positions of the two cursors, V2-V1, is the output of the IC-200 intracellular probe during the electrode test. The output of the IC-200 is 10mV for every megohm of load applied to the circuit by the resistor used to connect the two poles of the IC-200. If an 18 megohm resistor is used on an IC-200 with a 1X gain, the output of the IC-200 during the test is 180 millivolts (mV):

   18 megohm x 10mV/megohm = 180 mV

   If an 18 megohm resistor is used on an IC-200 with a 10X gain, the output of the IC-200 during the test is 1800 millivolts (mV):

   (18 megohm) (10mV/megohm) (10X gain) = 1800 mV

10. If the IC-200 intracellular probe tests to the level that is appropriate for the gain of the unit, the IC-200 probe and the iWorx unit are working properly.

The Dissection

1. Place a crayfish in ice water for 10 minutes. Remove the crayfish from the ice water and quickly cut off its head.
2. Remove the tail (abdomen) from the thorax by cutting around the joint (seam) connecting those two parts.
3. Observe the hinge ridge that runs along each side of the abdomen; only cut on the ventral side of the hinge ridge in order to preserve the hinges that hold the segments of the tail together.
4. Hold the tail and make a longitudinal cut along each side of the abdomen (below the hinge ridge) to loosen the ventral shell, swimmerets, and flexor muscles from the dorsal shell. Leave the tail fins attached to the dorsal exoskeleton.
5. Begin at the anterior end of the abdomen and separate the ventral and dorsal halves of the shell from each other. It may be necessary to cut (use small forceps) the connections that the segmental flexor muscles make to the dorsal shell.
6. Discard the ventral portion of the shell.

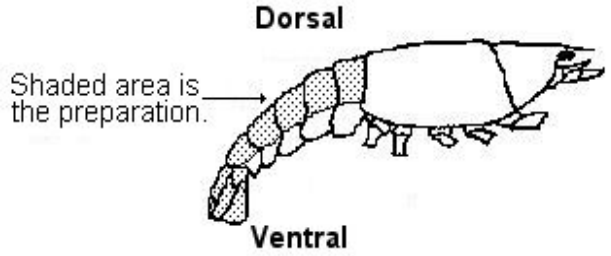

Figure AN-1-S7: Diagram showing the region of the crayfish tail used in the experiment.

7. Place the dorsal shell in the preparation dish and quickly fill the dish with crayfish saline.
8. Push one pin through the shell in the first abdominal segment and a second pin through the telson.
9. Place the dish under the dissection microscope, position the light for optimal illumination and focus on the preparation. Use small forceps to remove the gut (the green tube in the midline) and any connective tissue from the preparation.
10. Examine the preparation and identify:
    - The six abdominal segments.
    - The paired fast extensor muscles in each segment—one muscle group on either side of the mid-line.
    - The medial and two lateral bundles in the fast extensor muscle group on each side of a segment.

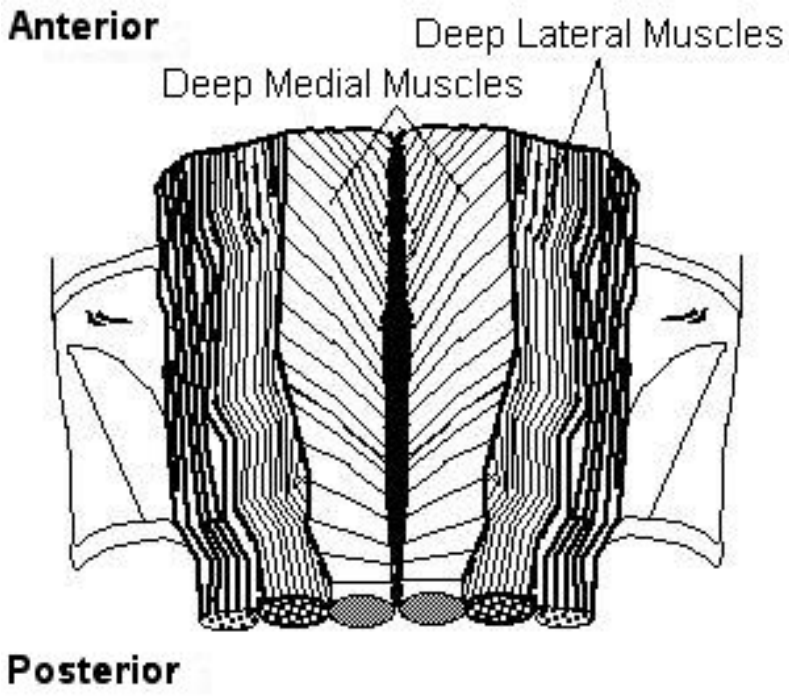

Figure AN-1-S8: Fast extensor muscles inside the dorsal part of second abdominal segment of crayfish.

Microelectrode Preparation

1. Fill a glass microelectrode with 3M KCl using a fine syringe needle (Hamilton type) on a 3cc syringe.
    - 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 KCl 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.
    - 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 KCl 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 KCl 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 KCl, 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 KCl. 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 KCl 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 KCl.
    - 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 KCl, 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.
- Push the gold-plated socket of the microelectrode holder onto the pin of the intracellular probe.

6. Carefully position the microelectrode tip over the preparation.
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, an indifferent electrode needs to be attached to the alligator clip on the black wire of the intracellular probe. 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 crayfish tail. Make sure no other metal, only the Ag-AgCl coil is in contact with saline solution.
9. Check that both electrodes are in the crayfish saline in the prep dish.
10. Check the resistance of your glass microelectrode before you push the microelectrode into muscle fibers. The resistance of the microelectrode tip is a measure of its diameter; the smaller the tip, the greater the resistance. Click the button on the electrode test module, located in the middle of the cable for the intracellular probe, for a few seconds. Measure the deflection of the trace; it will deflect 100mV for every megohm of resistance (10 mV per megohm times the 10X gain of the input amplifier). A good microelectrode for this preparation should have a resistance between 10 and 20 megohms.

11. 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.

Experiment AN-1: Membrane Potentials

Exercise 1: Impaling Muscle Fibers

Aim: To measure the membrane potentials in muscle fibers.

Approximate Time: 20 minutes

Procedure

1. Look through the microscope; you should see the tip of the microelectrode in the bath solution. Use the controls of the micromanipulator to move the tip of the microelectrode over a bundle of muscle fibers.
2. Type Muscle Fiber 1 in the Mark box.
3. Click Record to begin recording. Click the mark button to mark the recording.
4. Use the micromanipulator's controls to move the tip of the microelectrode toward the muscle fibers, gradually. Aim for the center of the fibers. Once the tip of the microelectrode is close to the membrane of any of the muscle fibers in view, begin to watch the recording of the membrane potential on the computer screen.
5. When the microelectrode tip touches a muscle fiber membrane, you will see a small deflection of the recording, either up or down. Click the mark button to mark the recording.
6. At this stage, penetrate the membrane of the muscle fiber by either:
    - Pushing the microelectrode tip through the membrane using the controls of the micromanipulator; or,
    - Gently tapping the base of the micromanipulator to create a small amount of vibration in the microelectrode tip, which will aid in the penetration of the membrane.
7. As the tip of the microelectrode penetrates the membrane, the recording displayed on the computer screen deflects downward rapidly. When this happens, do not touch the manipulator! The tip of the microelectrode is inside the muscle fiber. Click AutoScale to view the voltage levels before and after the microelectrode penetrated.
8. Use manipulator to remove the electrode. When analyzing data, it is often easier to Max-Min the removal rather than the insertion. It's often cleaner and faster.
9. Click Stop to halt recording.
10. Select Save in the File menu.

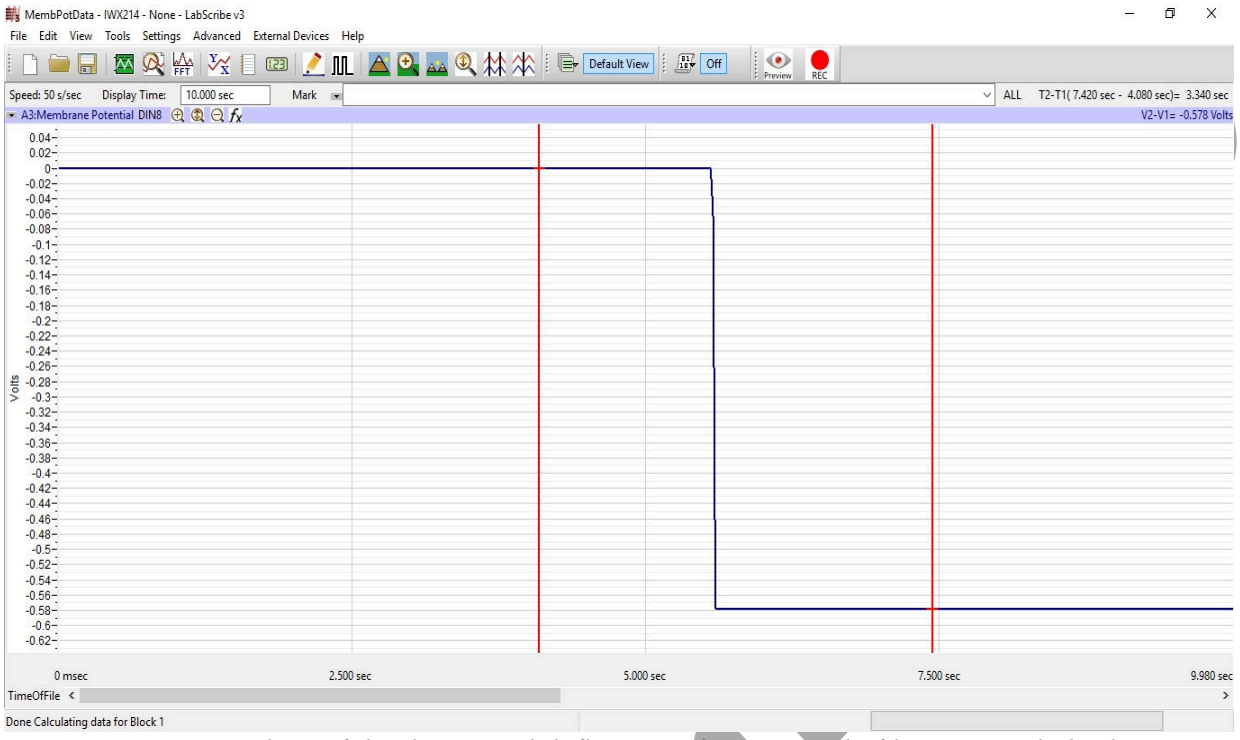

Figure AN-1-L1: Recording of the downward deflection when a muscle fiber is impaled. The cursors are used to measure a voltage difference, (V2-V1) of 0.578 Volts or 578 millivolts.

Data Analysis

1. Scroll through the data file and locate the section of the recording made when the microelectrode penetrated the muscle fiber membrane.
2. Use the Display Time icons to adjust the Display Time of the Main window to display a seventy-second section of recording with a consistent slope on the Main window.

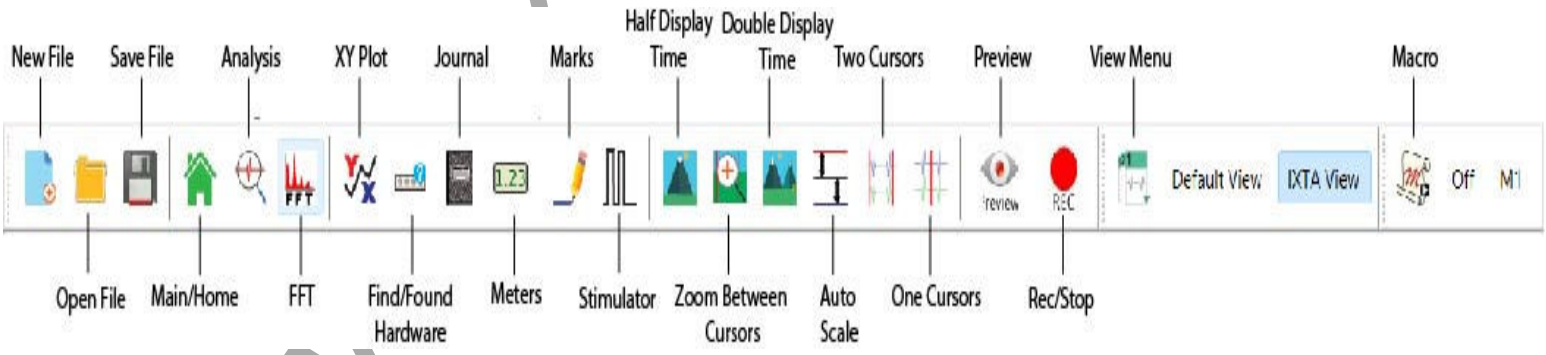

Figure AN-1-L2: LabScribe toolbar.

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 microelectrode enters the muscle fiber, or upon removal (whichever works better), 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 oxygen concentration in a minute.
    - Transfer the names of the parameters to the Journal using the Add Title to Journal function in the Membrane Potential channel menu.
    - Transfer the values for the parameters to the Journal using the Add Ch. Data to Journal function in the Membrane Potential channel menu.
7. On the Membrane Potential channel displayed in the Analysis window, use the mouse to click on and drag a cursor to the baseline recorded before the electrode test button was pushed. Drag the other cursor to the voltage level that results from the electrode test button being pushed.
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-1-L1.
9. Divide the value for V2-V1 by the gain of the IC-200 intracellular probe to obtain the membrane potential. For example, a voltage difference (V2-V1) of 620 millivolts recorded by an IC-200 with a gain of 10X is equal to a membrane potential of 62 millivolts:

    620 millivolts/10X Gain = 62 millivolts

Exercise 2: Membrane Potentials from Different Fibers

Aim: To measure any variations in the membrane potentials between muscle fibers.

Approximate Time: 20 minutes

Procedure

1. Repeat Exercise 1 on two additional muscle fibers in the same segment of the crayfish tail (Location A). Label the recording from each fiber
2. Record from fibers on the contralateral side of the same segment (Location B) and from fibers in other segments (Locations C and D).

Analysis

1. Use the same procedures used in Exercise 1 to measure the voltage changes recorded when the microelectrode penetrated the other fibers.

2. Convert the voltage changes into membrane potentials as performed in Exercise 1 by dividing the voltage change by the gain programmed into the IC-200.
3. Enter the data into the Journal using either of the two techniques described in Exercise 1. Enter the membrane potentials into Table AN-1-L1.

Questions

1. Were the resting membrane potentials of fibers in the same muscle bundle the same?
2. Was the average resting membrane potential for fibers from one bundle the same as the average potential for fibers from the bundle on the opposite side of the segment?
3. Was the average resting membrane potential for fibers from one segment the same as the average potential for fibers from the same side of another segment? The same as fibers from the opposite side of different segments?
4. Why do resting membrane potentials recorded from different fibers vary?

Exercise 3: Membrane Potentials and Extracellular Potassium

Aim: To measure changes in membrane potential in response to changes in the extracellular concentration of potassium.

Approximate Time: 30 minutes

Procedure

1. Remove the normal crayfish saline solution from the preparation dish.
2. Refill the preparation dish with a modified crayfish saline containing less sodium chloride than the normal saline:
    - Normal saline contains 205mM NaCl, and the modified saline contains 160mM NaCl.
    - The molarity of the low-sodium crayfish saline is adjusted with 45mM choline chloride, or sucrose, to allow for the 45mM NaCl reduction.
    - Neither choline nor sucrose molecules will pass through the membrane or channels.
3. Bathe the preparation in the low-sodium crayfish saline for two minutes. Remove the low-sodium saline from the preparation dish and refill the dish with fresh low-sodium crayfish saline. Bath the preparation in this saline for another three minutes before recording any membrane potentials. Repeat this procedure once more with a sock of five minutes.
4. Record the resting membrane potentials from five muscle fibers bathed in the low-sodium saline.
5. Remove the low-sodium crayfish saline solution from the preparation dish.

Table AN-1-L1: Resting Membrane Potentials (E_m) of Muscle Fibers Bathed in Crayfish Saline.

	Fiber Location-Segment	E_m in Normal Crayfish Saline
Fiber 1		
Fiber 2		
Fiber 3		
Mean	A_____	
Fiber 4		
Fiber 5		
Fiber 6		
Mean	B_____	
Fiber 7		
Fiber 8		
Fiber 9		
Mean	C_____	
Fiber 10		
Fiber 11		
Fiber 12		
Mean	D_____	

6. Refill the preparation dish with a modified crayfish saline containing more potassium chloride than the normal saline:
    - Normal saline contains 5mM KCl and 205 mM NaCl, and the modified saline contains 50mM KCl and 160mM NaCl.
    - Neither choline chloride nor sucrose have been added to this saline since the higher concentration of KCl will give this solution the same osmolarity as normal saline.
7. Bathe the preparation in the high-potassium crayfish saline for two minutes. Remove the high-potassium saline from the dish and refill the dish with fresh high-potassium crayfish saline. Bath the preparation in this saline for another three minutes before recording any membrane potentials.

8. Record the resting membrane potentials from five muscle fibers bathed in the high-potassium saline.

Data Analysis

1. Use the same procedures used in Exercise 1 to measure the voltage changes recorded when the microelectrode penetrated the fibers.
2. Convert the voltage changes into membrane potentials as performed in Exercise 1 by dividing the voltage change by the gain programmed into the IC-200.
3. Enter the data into the Journal using either of the two techniques described in Exercise 1. Enter the membrane potentials into Table AN-1-L2.

Table AN-1-L2: Resting Membrane Potentials (E_m) of Muscle Fibers Bathed in Modified Crayfish Saline.

	Normal Crayfish Saline	Low $[Na^+]$ Crayfish Saline	High $[K^+]$ Crayfish Saline
Fiber 1			
Fiber 2			
Fiber 3			
Fiber 4			
Fiber 5			
Mean			

Questions

1. What happens to the resting potential if you change the level of sodium in the crayfish saline?
2. What happens to the resting potential if you change the level of potassium in the crayfish saline?
3. Why does an increase in $[K^+]_{outside}$ create the observed changes in resting potential?

Note: Look at more than just two levels (at least 4) and graph to see it's a logarithmic relationship.