Experiment AN-9: Membrane Potentials

Background

The aim of this laboratory exercise is to record resting potentials across the membranes of fast extensor muscle fibers in the tail of crayfish. Microelectrodes are glass capillary tubes which have been melted and then pulled to produce a very fine (<0.5 μm diameter) tip at one end. The tip is placed through the membrane and is so fine that the membrane seals around the tip. The microelectrode filled with potassium chloride acts as a "saline bridge" between the inside of the cell and the recording equipment.

The Goldman equation can be used to predict the membrane potential:

$$E_M = 57.7 \log 10 \frac{(PK_{[K+]out} + PNa_{[Na+]out} + PCl_{[Cl-]in})}{(PK_{[K+]in} + PNa_{[Na+]in} + PCl_{[Cl-]out})}$$

According to this equation, the membrane potential depends upon the concentration of the different ions across the membrane and the relative permeability of the membrane to these ions. Recordings will be made from muscle fibers that are functionally identical; they all contract to rapidly extend the tail. You will test the hypothesis that all the fibers within this muscle are the same by measuring membrane potentials from several fibers in the same muscle and from fibers in muscles from different abdominal segments.

The Goldman equation indicates that the membrane potential is dependent upon the concentration gradients of the different ions. Since the permeability of the resting membrane is highest to potassium, changing the potassium gradient across the membrane might have a great effect on the membrane potential. This hypothesis will be tested by recording the membrane potential from preparations bathed in crayfish salines with different concentrations of potassium.

Experiment AN-9: Membrane Potentials

Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

Preparation dish

Dissection microscope and light source

Model 3100 intracellular amplifier and headstage

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 (see Appendix)

Electrode filling solution (see Appendix)

Start the Software

1. Click on LabScribe
2. Click Settings → Animal Nerve → MembranePotentials-3100
3. Once the settings file has been loaded, click the **Experiment** button on the toolbar to open any of the following documents:
    - Appendix
    - Background
    - Labs
    - Setup (opens automatically)

Equipment Setup

1. Place the Model 3100 AC/DC extracellular amplifier on the bench near the IXTA.
2. Connect the BNC output of the Model 3100 to the Channel 3 BNC input of the IXTA with a BNC-BNC cable.
3. 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.

4. Mount the intracellular probe in the micromanipulator and place it near the dissection microscope and the preparation dish.
5. 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-9-S1.
6. 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.
7. 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.
8. Flip the power switch of the Model 3100 to the ON position.

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.*

Table AN-9-S1: Settings on the Model 3100 Amplifier for recording membrane 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

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.
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 banana-banana cable.

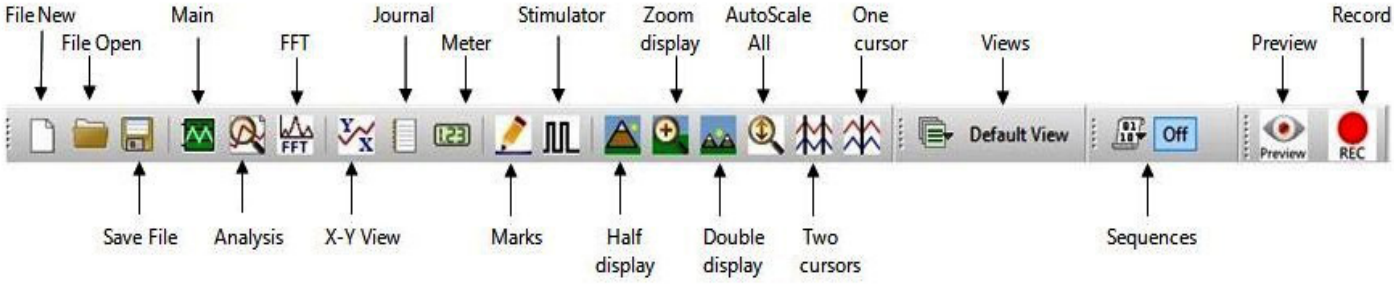

Figure AN-9-S1: The LabScribe toolbar.

4. Type Probe Test in the Mark box to the right of the Mark button.
5. Click Record and press the mark button. The trace should appear near the center of the screen.
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. 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.
8. Click the Double Cursor icon in the Toolbar (Figure AN-9-S1), 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 Membrane 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 1X output of the Model 3100 is recorded, the output of the Model 3100 during the test is 180 millivolts (mV):

    18 megohm x 10mV/megohm = 180 mV

12. If an 18 megohm resistor is used with the 3100 set to 10X gain, the output of the 3100 during the test is 1800 millivolts (mV):

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

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

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

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 the connections that the segmental flexor muscles make to the dorsal shell.
6. Discard the ventral portion of the shell (Figure AN-9-S2).

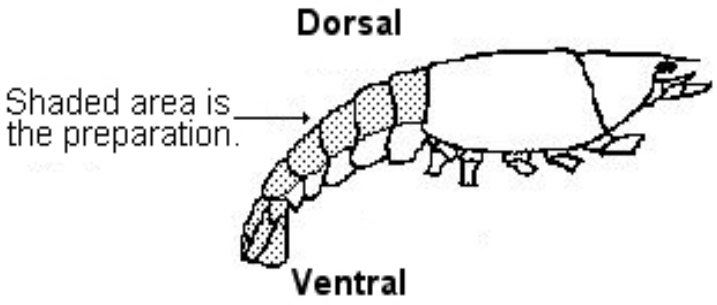

Figure AN-9-S2: Diagram showing the region of the crayfish tail used in the experiment.

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.

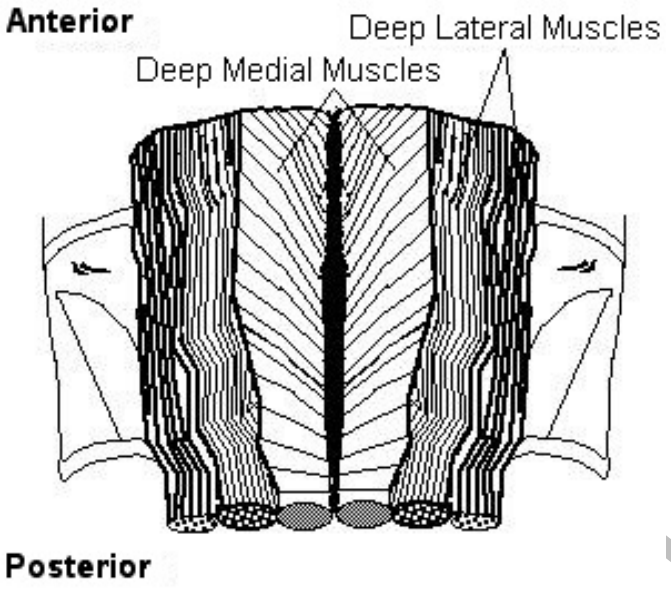

Figure AN-9-S3: Fast extensor muscles inside the dorsal part of second abdominal segment of crayfish.

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, compare with Figure AN-9-S3 and identify:
    - The six abdominal segments.
    - The paired fast extensor muscles in each segment—one muscle group on either side of the midline.
    - The medial and two lateral bundles in the fast extensor muscle group on each side of a segment.

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 into the socket of the headstage 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, 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 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. Test the resistance of the microelectrode before impaling the muscle fiber.

    • Type Electrode Test in the Mark box to the right of the Mark button and click Record to begin recording. Press the mark button 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 pulse voltage recorded and the resistance of the microelectrode. A good microelectrode for this preparation should have a resistance between 10 and 20 megohms.
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.

Experiment AN-9: 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 to the right of the Mark button.
3. Click Record to begin 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.
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 (Figure AN-9-L1). 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 the 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.

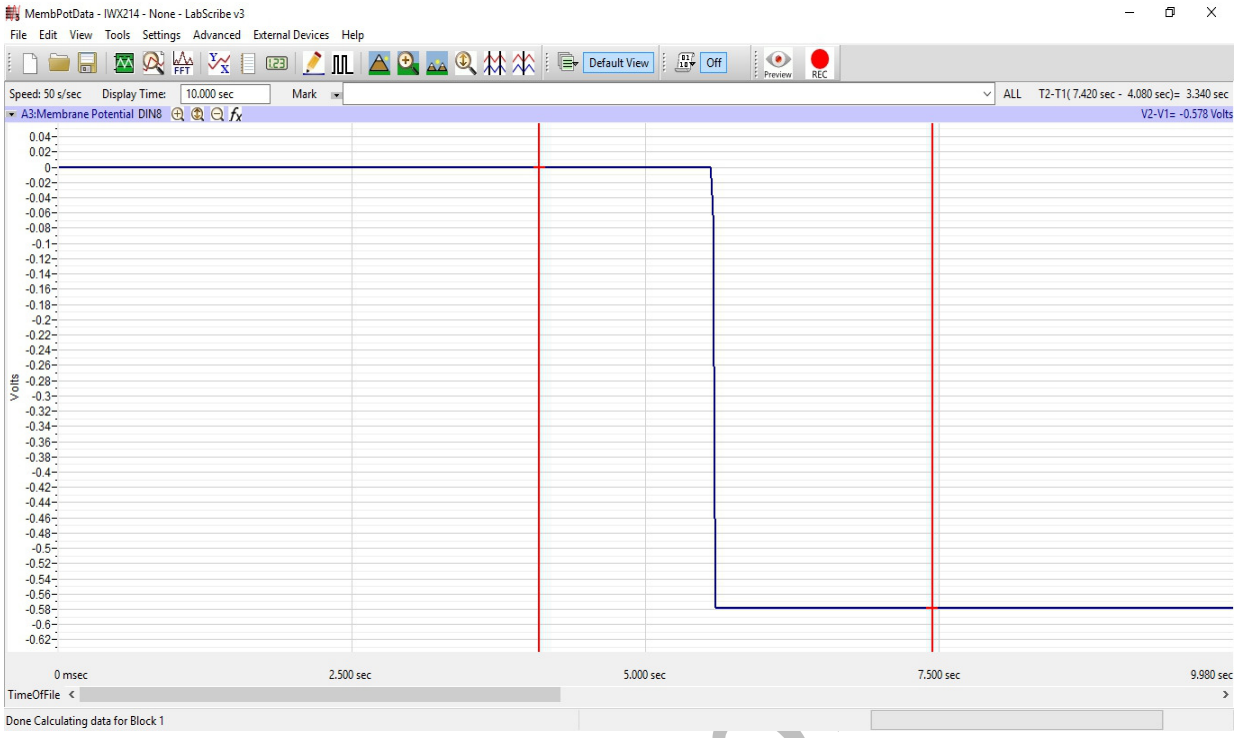

Figure AN-9-L1: Recording of the downward deflection when a muscle fiber is impaled. The cursors are used to measure a voltage difference (V2-V1) of 0.578 Volts or 578 millivolts.

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 70-second section of recording with a consistent slope on the Main window (Figure AN-9-L2).

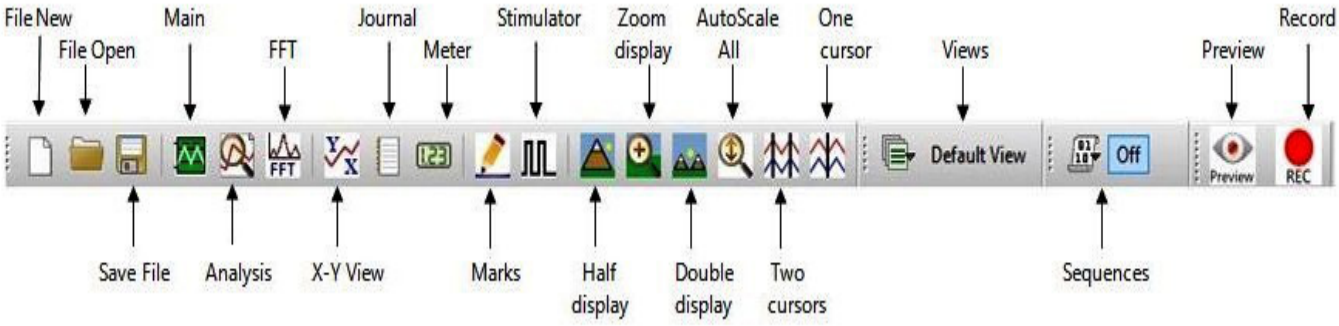

Figure AN-9-L2: LabScribe toolbar.

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 locations before and after the electrode was removed from the cell.
    - 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-9-L1.
9. Divide the value for V2-V1 by the gain of the 3100 amplifier to obtain the membrane potential. For example, a voltage difference (V2-V1) of 620 millivolts recorded 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: 30 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).

Data 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 selected on the 3100 amplifier.

3. Enter the data into the Journal using either of the two techniques described in Exercise 1. Enter the membrane potentials into Table AN-9-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 2 minutes. Remove the low-sodium saline from the preparation dish and refill the dish with fresh low-sodium crayfish saline. Bathe the preparation in this saline for another 3 minutes before recording any membrane potentials. Repeat this procedure once more, and then wait 5 minutes before recording.
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.
6. Refill the preparation dish with a modified crayfish saline containing more potassium chloride than the normal saline:
    - Normal saline contains 5mM KCl and 205mM 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 2 minutes. Remove the high-potassium saline from the dish and refill the dish with fresh high-potassium crayfish saline. Bathe the preparation in this saline for another 3 minutes before recording any membrane potentials.
8. Record the resting membrane potentials from five muscle fibers bathed in the high-potassium saline.

Table AN-9-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_____	

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 selected on the 3100 amplifier.
3. Enter the data into the Journal using either of the two techniques described in Exercise 1. Enter the membrane potentials into Table AN-9-L2.

Table AN-9-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: *Try looking at more than just two levels (at least four) and graph to see it's a logarithmic relationship.*