

## Experiment NB-3: Membrane Potentials

Allow two lab periods to complete this experiment.

### Background

All living cells maintain a difference in electrical charge between the solutions that are inside and outside the cell membranes. The voltage difference across the cell membrane is usually at a steady level known as the resting membrane potential ( $E_m$ ). The resting membrane potential is produced by the differential distribution of ions on either side of the membrane. In muscle fibers, the potassium concentration in the cytoplasm is over 50 times greater than the ion's concentration in the extracellular fluid. On the other hand, the concentration of sodium is 10 times greater outside the membrane than inside. The concentration gradient of potassium is greater and in the opposite direction to the sodium concentration gradient. The concentrations of the cations, sodium and potassium, and the anions, chloride and large organic anions on either side of the membrane are governed by three factors: the sodium-potassium pump; the relative rates of diffusion of these ions down concentration gradients; and, the attraction and repulsion of opposite and like charges.

The sodium-potassium pump actively moves sodium out of the cell and potassium into the cell. The pump establishes and maintain the concentration gradients of these two cations across the cell membrane. The concentration gradients form an ionic battery across the membrane, and the sodium-potassium keeps that battery charged.

The resting membrane potential, which ranges between -50 to -90 mV in most cells, results from potassium diffusing. Because of its large concentration gradient, potassium diffuses faster across the membrane than any other ion. When potassium diffuses out of a cell, an excess of negative charges occurs inside the cell. The potassium ions flowing out of the cell attract negative ions to follow them, and push positive ions on the outside to move back into the cell. Since these other ions have relatively low permeabilities, they don't move as rapidly as the potassium ions. Therefore, these other ions have only a small effect on the neutralization of the negative potential inside the cell. The net movement of the potassium is also limited by the potential difference that builds between the inside and the outside of the cell. The negativity inside the cell holds back the positive potassium ions. At equilibrium, a potassium ion is pushed out of the cell through a channel by a chemical force (concentration gradient) that has the same magnitude as the electrical force (negative potential) pulling the ion back into the cell. The magnitude of the

electrical potential that offsets the concentration gradient at equilibrium is defined by the **Nernst equation**:

$$E_K = 58 \log \frac{[K]_O}{[K]_i}$$

where  $[K]_i$  is 140 mM  $K^+$ .

The membrane potential depends upon the concentrations of the different ions across the membrane and the relative permeability of the membrane to these ions. The **Goldman equation** can be used to predict the membrane potential ( $E_M$ ) from the concentrations and permeabilities of potassium, sodium, and chloride:

$$E_M = 57.7 \log \frac{(P_K [K]_O + P_{Na} [Na]_O + P_{Cl} [Cl]_i)}{(P_K [K]_i + P_{Na} [Na]_i + P_{Cl} [Cl]_O)}$$

### Glass Microelectrodes

Resting membrane potentials, action potentials, and slow receptor and synaptic potentials are measured using fine-tipped, fluid-filled glass micropipettes known as microelectrodes. Glass microelectrodes permit the acquisition of quantitative information about cell membrane potentials and signal integration between single nerve cells and muscle fibers. The measurement of the transmembrane potentials with microelectrodes has become a standard procedure in research. For example, the technique is frequently used to track changes in the potential during synaptic activation or chemical treatment. With the addition of pharmacological techniques, intracellular recording methods have advanced the understanding of membrane properties and mechanisms.

Microelectrodes are formed from glass capillary tubes that are melted in the center and pulled apart on a device known as an electrode puller. Modern electrode pullers can be programmed to control the temperature used to melt the capillary tubes and the forces used to pull the ends of the tubes apart. Once an electrode puller is programmed to make a microelectrode with certain tip diameter and taper, the puller is capable of producing matching electrodes time after time. The glass tubes used for microelectrodes have a fine glass filament fused to the inside wall. Even after the tube is pulled to a fine point, the inside filament is continuous down to the open end of the pipette where the opening of the tip is less than a micron in diameter. When the filling solution is injected into the large end of the pipette, the solution is drawn down to the tip by capillary action created by the filament. In this way, microelectrodes can be made, filled and used in a matter of minutes.

## Microelectrode Amplifier

The small cross-sectional area of the microelectrode tip greatly reduces the flow of current through the electrode. So, it is said that this type of electrode has a high impedance. If the membrane has the same high impedance as the electrode and the recording device has a low impedance, the voltage measured by the recorder would be a very small percentage of the actual membrane potential. The signal that is recorded would be a poor representation of the membrane potential. The loss in the amplitude of the signal is caused by the membrane potential being distributed across resistances at three locations in the recording circuit (Figure NB-3-1 on page NB-3-2): the electrode ( $R_e$ ), the cell membrane ( $R_m$ ), and the input of the recorder ( $R_o$ )

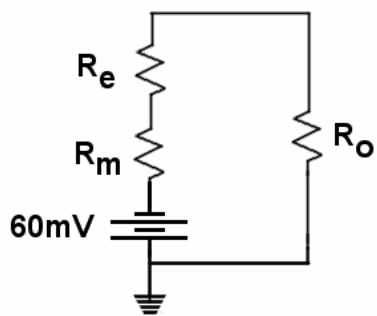


Figure NB-3-1: Schematic diagram of the recording circuit for membrane potentials.

The voltage across a resistor in series with other resistors can be determined using an equation. The following equation would be used to measure the voltage of the membrane at the recorder ( $V_o$ ):

$$V_o = \frac{R_o}{R_e + R_m + R_o} V_{\text{Total}}$$

If the resting membrane potential is 60 mV and the electrode impedance ( $R_e$ ) and the membrane impedance ( $R_m$ ) are  $10^7$  ohms and the recorder impedance ( $R_o$ ) is  $10^6$  ohms, the voltage at the input of the recorder would only be 2.9 millivolts (mV):

$$V_o = \frac{10^6}{10^7 + 10^7 + 10^6} (60\text{mV}) = \frac{1}{21} (60\text{mV}) = 2.9\text{mV}$$

Because of the difference between the impedances of the recorder and the microelectrode, over 95% of the amplitude of the signal is lost.

To preserve the amplitude of the signal, a high impedance DC amplifier, known as an intracellular electrometer or a microelectrode amplifier, is used in the circuit between the microelectrode and the recording unit. This type of amplifier matches the impedance of the microelectrode to the input impedance of the recording unit through a circuit known as a **voltage follower**. Electrometers usually have input impedances that are equal to or greater than  $10^{10} \Omega$ . As a rule, the input impedance of an electrometer should be at least 100 times greater than the impedance of the microelectrode being used for recording. This means that measuring potentials from smaller cells using microelectrodes with smaller tips and higher impedances requires the use of electrometers with higher input impedances.

In addition to having high input impedances, electrometers have low output impedances that more closely match the low input impedances of recording units. This means that the actual amplitudes of the membrane potentials recorded using electrometers are the amplitudes displayed by recorders. Since these signals require little or no amplification, electrometers only need output gains of 1X or 10X. Also, electrometers do not pick up noise from electrical radiation as easily as other types of amplifiers.

In this experiment students will record the resting membrane potentials across the membranes of fast extensor muscle fibers in the tail of crayfish. 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. Recordings will be made from the muscle fibers that contract to rapidly extend the tail. Students will test the hypothesis that all the fibers within the extensor muscles are the same by measuring membrane potentials from several fibers in the same muscle, and from several fibers in muscles from different abdominal segments.

## IWX/214 Setup

- 1 Place the IWX/214 on the bench, close to the computer.
- 2 Check Figure NT-3-1 in the Tutorial chapter for the location of the USB port and the power socket on the IWX/214.
- 3 Check Figure NT-3-2 in the Tutorial chapter for a picture of the IWX/214 power supply.
- 4 Use the USB cable to connect the computer to the USB port on the rear panel of the IWX/214.
- 5 Plug the power supply for the IWX/214 into the electrical outlet. Insert the plug on the end of the power supply cable into the labeled socket on the rear of the IWX/214. Use the power switch to turn on the unit. Confirm that the red power light is on.

## Equipment Required

PC Computer  
 IWX/214 data acquisition unit  
 USB cable  
 IWX/214 power supply  
 Preparation dish  
 Dissection microscope and light source  
 Model 3100 Intracellular electrometer and headstage  
 Micromanipulator  
 Glass microelectrodes  
 Microelectrode holder (adapter)  
 Headstage tester (18 megohm resistor with clip)  
 Bath ground electrode with cable  
 Pasteur pipettes and bulbs  
 Assorted banana cables and alligator clips  
 Crayfish  
 Crayfish salines (See appendix)  
 Electrode filling solution (See appendix)

- 2 Connect the BNC output of the Model 3100 to the Channel 1 BNC input of the IWX/214 with a BNC-BNC cable.
- 3 Locate the headstage probe in the NBK/214 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.

**Table NB-3-1: Settings on the Channel Window of the Preferences Dialog Used to Configure the iWorx Recording System for Experiment NB-3.**

Parameter	Units/Title	Setting	Mode/Function
Acquisition Mode		Chart	
Start		User	
Stop		User	
Display Time	Sec	10	
Speed	Samples/Sec	50	
Channel A1	Membrane Potential (mV)	√	BNC

## Start the Software

- 1 Click on the LabScribe shortcut on the computer's desktop to open the program. If a shortcut is not available, click on the Windows Start menu, move the cursor to **All Programs** and then to the listing for **iWorx**. Select **LabScribe** from the **iWorx submenu**. The LabScribe Main window will appear as the program opens.
- 2 On the **Main window**, pull down the **Settings menu** and select **Load Group**.
- 3 Locate the folder that contains the settings group, **INBLMV1.iwxgrp**. Select this group and click **Open**.
- 4 Pull down the **Settings menu** again. Select the **Membrane Potentials-NBK** settings file.
- 5 LabScribe will appear on the computer screen as configured by the **MembranePotentials-NBK** settings.
- 6 For your information, the settings used to configure the LabScribe software and the IWX/214 unit for Exercises 1 and 2 are listed in Table NB-3-1 on page NB-3-3. These settings are programmed on the **Preferences Dialog window** which can be viewed by selecting **Preferences** from the **Edit menu** on the LabScribe Main window.

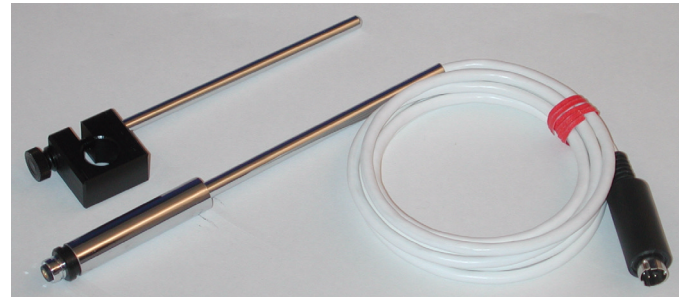


Figure NB-3-2: The head stage of the Model 3100 electrometer.



Figure NB-3-3: The front panel of the Model 3100.

## Model 3100 Setup

- 1 Place the Model 3100 AC/DC extracellular amplifier on the bench near the IWX/214.

- 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 NB-3-2 on page NB-3-4.

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

**Table NB-3-2: 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

**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 is caused by AC line voltage used to power the equipment in the lab.

- 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.
- 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.
- Flip the power switch of the Model 3100 to the ON position

## Headstage Probe Test

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

- Obtain a 18 megohm resistor (1/4 watt) with an alligator clip attached to one end and a banana jack on the other end.
- Place a pin in the socket on the end of the headstage (Figure NB-3-2 on page NB-3-3). Fasten the alligator clip on the end of the resistor to the pin in the socket of the head stage.

- 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.

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

- Type **Probe Test** in the **Mark box** to the right of the **Mark button**.
- Click **Record** and press the **Enter key** on the keyboard. The trace should appear near the center of the screen.
- 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.
- 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.
- Click the **Double Cursor icon** (Figure NB-3-4 on page NB-3-4), so that two blue vertical lines appear over the recording window.
- 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.

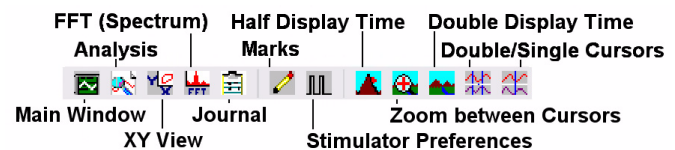


Figure NB-3-4: The *LabScribe* toolbar.

- 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 electrometer is 10mV for every megohm of load applied
- 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):

$$18 \text{ megohm} \times 10\text{mV/megohm} = 180 \text{ mV}$$

If an 18 megohm resistor is used to test the probe and the 10X output of the Model 3100 is recorded, the output of the Model 3100 during the test is 1800 millivolts (mV)

$$(18 \text{ megohm}) (10\text{mV/megohm}) (10\text{X gain}) = 1800 \text{ mV}$$

- 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.

## Electrical Noise

Electrical noise is the most common problem associated with the recording of bioelectric signals. It radiates through the air and comes from electrical devices in the lab room or building: lights, power outlets, computers, monitors, and the power supplies. Since the source of power for these devices is 60Hz alternating current (AC), this electrical noise appears as a distorted sine wave with a repeating period of 16.7 milliseconds (msec).

There are two major sources of electrical noise: **pickup** and **ground loops**.

### Pickup

**Pickup** is caused by electrical radiation that produces currents in the electrodes and wires leading to the amplifiers in the recording system. Because the resistance in the electrodes is high, small currents produce large voltages that may be greater than the biopotential being recorded. The major ways to reduce **pickup** are:

- **Faraday Cage:** Put a grounded, screened enclosure, known as a Faraday cage, around the preparation and the electrodes. The enclosure separates the source of the radiation from the electrodes. The person operating the equipment might also be a source of noise, and he or she may need to be grounded.
- **Shielded Cables:** Use shielded cables to carry the signals from the electrodes to the amplifier and the recorder; this puts a protective ground around the wires carrying the bioelectric signal.
- **Differential Recording:** Record using both a positive and a negative recording electrode placed on the nerve. The noise signals that are equal in magnitude, but opposite in polarity, will cancel each other out and leave a flat baseline.
- **Short Cables:** Use the shortest cables available to reduce the length of wiring exposed to electrical noise.
- **Direct Current Equipment:** Use equipment, like preamplifiers and illuminators, that are powered by batteries or direct current (DC) transformers.
- **Equipment Removal:** Unplug or remove unused alternating current (AC) equipment from the area.

### Ground Loops

**Ground loops** are a troublesome source of electrical noise caused by the ground cable itself serving as an antenna for the noise radiating in the room. Using a Faraday cage to shield the preparation and the recording electrodes does not remove the electrical noise caused by **ground loops**. To avoid **ground loops**, use the following techniques:

- **Ground Hub:** Ground all the equipment around the preparation to a common grounding point (hub). This includes all the items that are electrically powered or are made of metal, like illuminators or microscopes. Use simple cables, like banana cords equipped with alligator clips, to connect each device directly to the common grounding point. The common ground point is connected to the ground of the recording device with a single cable. The recording device is connected to the building ground.

- **Simple Chain:** Ground the devices to the common grounding point using the simplest route that links the first device to the second device, the second device to the third device, and so on. Start the chain at the device that is the farthest from the common grounding point. End the chain by connecting the last device to the common grounding point, which is connected to the ground of the recording device.
- **Free-Floating:** In addition to using one of the grounding techniques described earlier, plug all devices powered by alternating current (AC), like illuminators, amplifiers, and recording units to power outlets using three-two prong adapters.

### High Frequency Noise

High frequency noise can also be a problem when recording bioelectric potentials. This type of noise is seen as the thickening of the recorded line. This noise contains many frequencies, and the amplitude of the noise is proportional to the resistance of the electrode. Therefore, intracellular electrodes, with high resistances, pick up a greater amplitude of high frequency noise than extracellular electrodes, with low resistances.

### Mechanical Noise

Mechanical noise, like vibrations from the ventilation system in the room, can cause the electrodes to vibrate and produce voltage changes with each vibration cycle. To alleviate this problem, isolate the platform holding the preparation with foam pads or bicycle inner tubes. Also, avoid bumping the table when the recording electrodes are in place.

### Grounding

The recording circuit of the Model 3100 intracellular electrometer has only one grounding point. Place the reference electrode in the bath solution of the preparation. Plug the connector on the end of the cable of the reference electrode into the GND input on the front panel of the Model 3100.

### Signal Improvement

- 1 To improve recordings of the membrane potentials, move the prep dish away from sources of 60Hz noise. These sources include outlets, computers, monitors, lights, refrigerators, water baths, and other AC powered devices.
- 2 If the recording still contains a great deal of electrical noise, apply the digital filtering function to the data. Click on the **add function button** in the upper margin of the **Compound Action Potential channel**. Select **Filter** from the menu of computed functions.
- 3 In the **Filter Setup Dialog window**, the **Filter Mode** is set to the **Hamming Window** and the **Filter Order** is set to **51**. These are the default settings that should be used.
- 4 Set the **Low Cutoff filter value** to **65** and the **High Cutoff filter value** to **8000**. The values for the filter cutoffs can be set by:

- Typing the values for the filter cutoffs in the boxes to the right of the names of the filter cutoffs.
- Clicking on the **up or down arrows** to the right of the boxes displaying the values of the filter cutoffs.
- Clicking on the margins of the colored area in the graphic display of the filter and dragging the margins to the values required.

## 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 NB-3-5 on page NB-3-6).

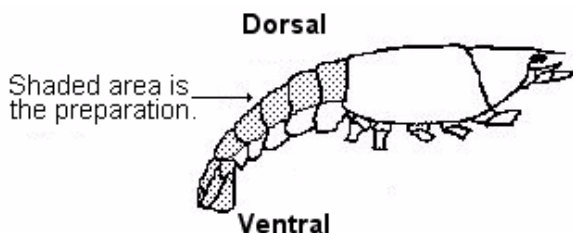


Figure NB-3-5: Diagram showing the region of the crayfish tail used in the experiment.

- 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, compare with Figure NB-3-6 on page NB-3-6 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.

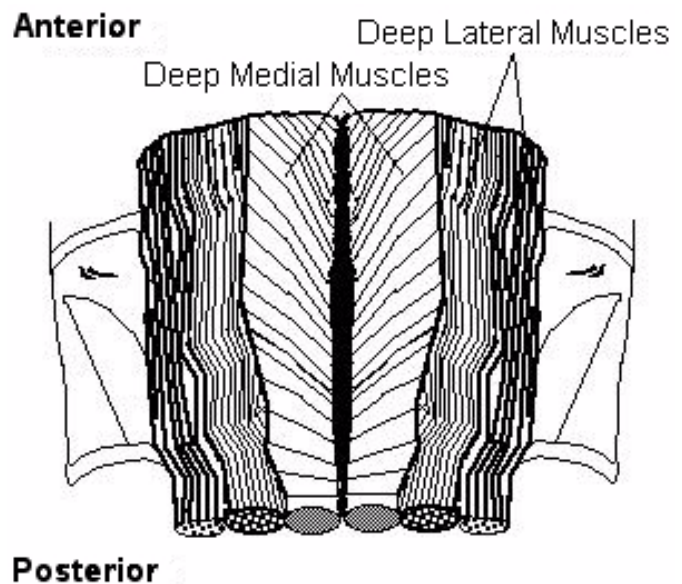


Figure NB-3-6: Fast extensor muscles inside the dorsal surface of second abdominal segment of crayfish.

## Microelectrode Preparation

Cells and fibers that are impaled by microelectrodes sustain a negligible amount of damage because the tips of these electrodes are very fine, usually less than 0.5  $\mu\text{m}$  in diameter. Because a microelectrode tip is fine, the membrane can easily seal the area around the tip and prevent the leakage of ions and currents into and out of the cell. Tips are too fine to be measured accurately using the types of microscopes typically used during intracellular recordings. So, another method is used. The size of the tip is determined by measuring the resistance of the microelectrode during an electrode check procedure. The higher the resistance of the tip, the finer the tip.

To record membrane potentials through a fine tip, the microelectrode is filled with a concentrated potassium chloride solution that acts as a saline bridge between the inside of the cell and the recording equipment. The fine glass filament fused to the inside wall of the microelectrode draws the potassium chloride solution down to the tip.

- 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.

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

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- 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.
  - 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**.

- Click **Record** to begin recording. 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 pulse voltage recorded and the resistance of the microelectrode.

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**Note:** The resistance should be between 10 and 20 MΩ. If the resistance is not within this range, replace the microelectrode with a new one.

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- 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.

## Exercise 1: Impaling Muscle Fibers

**Aim:** To measure the membrane potentials in different muscle fibers.

### 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. 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 NB-3-7 on page NB-3-8). 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 Click **Stop** to halt recording.
- 9 Select **Save** in the **File** menu.

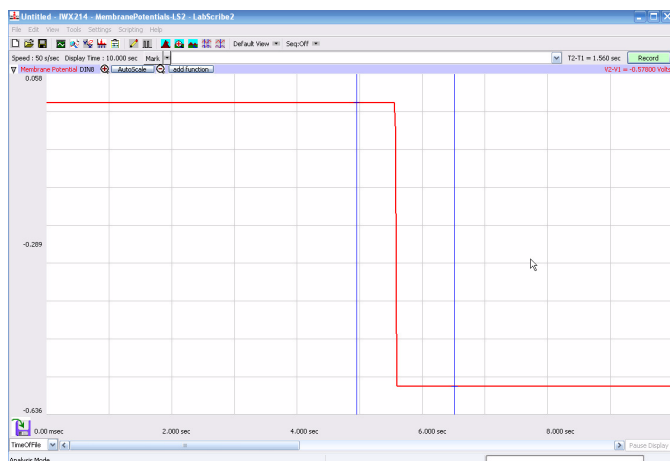


Figure NB-3-7: Recording of the downward deflection when a muscle fiber is impaled. The cursors are used to measure a voltage difference, ( $V_2-V_1$ ) of 0.578 Volts or 578 millivolts.

### 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**. This section of data can also be selected by:
  - Placing the cursors on either side of the seventy-second section of the recording, and
  - Clicking the **Zoom between Cursors** button on the **LabScribe** toolbar (Figure NB-3-4 on page NB-3-4) to expand or contract the seventy-second recording to the width of the **Main window**.
- 3 Click on the **Analysis window** icon in the toolbar (Figure NB-3-4 on page NB-3-4) 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 **Membrane Potential channel** in the **Analysis window**. The mathe-

tical function,  **$V_2-V_1$** , should appear in this table. The value for  **$V_2-V_1$**  are displayed in the table across the top margin of the **Membrane Potential channel**.

- 5 Once the cursors are placed in the correct positions for determining the change in voltage ( **$V_2-V_1$** ) as the tip of the microelectrode enters the muscle fiber, the value for  $V_2-V_1$  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 microelectrode penetrated the fiber. Drag the other cursor to the voltage level that results from the microelectrode being in the fiber.
- 8 Record the value for the voltage change ( **$V_2-V_1$** ) in the **Journal** using the one of the techniques described in Steps 5 or 6, and in Table NB-3-3 on page NB-3-9.
- 9 Divide the value for  **$V_2-V_1$**  by the gain of the Model 3100 intracellular electrometer to obtain the membrane potential. For example, a voltage difference ( **$V_2-V_1$** ) of 620 millivolts recorded by an Model 3100 with a gain of 10X is equal to a membrane potential of 62 millivolts:

$$620 \text{ millivolts} / 10X \text{ Gain} = 62 \text{ millivolts}$$

### Exercise 2: Membrane Potentials from Different Fibers

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

#### 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.

- 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.
- Enter the data into the **Journal** using either of the two techniques described in Exercise 1. Enter the membrane potentials into Table NB-3-3 on page NB-3-9.

### Questions

- Were the resting membrane potentials of fibers in the same muscle bundle the same?
- 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?
- 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?
- Why do resting membrane potentials recorded from different fibers vary?

**Table NB-3-3: 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_____	

### Exercise 3: Membrane Potentials and Extracellular Potassium

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

#### Procedure

- Remove the normal crayfish saline solution from the preparation dish.
  - 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.
  - 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.
  - Record the resting membrane potentials from five muscle fibers bathed in the low-sodium saline.
  - Remove the low-sodium crayfish saline solution from the preparation dish.
  - 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.
  - 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.
  - Record the resting membrane potentials from five muscle fibers bathed in the high-potassium saline.
- #### Analysis
- Use the same procedures used in Exercise 1 to measure the voltage changes recorded when the microelectrode penetrated the fibers.
  - 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.
  - Enter the data into the **Journal** using either of the two techniques described in Exercise 1. Enter the membrane potentials into Table NB-3-4 on page NB-3-10.

**Table NB-3-4: 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?

### Appendix

#### Electrode Filling Solution:

3M Potassium Chloride in deionized water.

#### Chloriding silver wire with 9V battery:

- 1 Attach an alligator clip to each lead of the connector for a 9V battery.
- 2 Coil two 16" pieces of silver wire around a large pencil. Clip a coil of silver wire on each lead of the battery connector.
- 3 Connect the battery lead, with the silver wire coils, to the 9V battery. Submerge the wire coils in a beaker of 3M KCl. Make sure the coils do not touch each other.
- 4 Let the current flow through the silver wires for 5 minutes. Remove the wires from the 3M KCl and reverse their position on the alligator clips. Return the coils to the beaker of 3M KCl, and let the current flow through this reversed circuit. For example, one coil will be on the positive battery pole for five minutes, followed by five minutes on the negative battery pole.
- 5 Reverse the position of the coils four more times for a total of 30 minutes.
- 6 After thirty minutes, the silver wire should be evenly chlorided. The wire should be a dull gray.

**Table NB-3-5: Recipe for Crayfish Saline Solution with Tris.**

Concentration (mMolar)	Salt	Grams/Liter DI H <sub>2</sub> O
205.34	Sodium Chloride	12.00
5.36	Potassium Chloride	0.40
13.43	Calcium Chloride*2H <sub>2</sub> O	1.97
2.61	MgCl <sub>2</sub> .6H <sub>2</sub> O	0.53
9.99	Tris	1.21
Adjust pH to 7.4 with 6N HCl		

**Table NB-3-6: Recipe for Low [Sodium] Crayfish Saline Solution with Tris.**

Concentration (mMolar)	Salt	Grams/Liter DI H <sub>2</sub> O
160	Sodium Chloride	9.35
45	Choline Cl	6.28
5.36	Potassium Chloride	0.40
13.43	Calcium Chloride*2H <sub>2</sub> O	1.97
2.61	MgCl <sub>2</sub> .6H <sub>2</sub> O	0.53
9.99	Tris	1.21
Adjust pH to 7.4 with 6N HCl		

**Table NB-3-7: Recipe for High [Potassium] Crayfish Saline Solution with Tris.**

Concentration (mMolar)	Salt	Grams/Liter DI H <sub>2</sub> O
160	Sodium Chloride	9.35
50	Potassium Chloride	3.73
13.43	Calcium Chloride*2H <sub>2</sub> O	1.97
2.61	MgCl <sub>2</sub> .6H <sub>2</sub> O	0.53
9.99	Tris	1.21
Adjust pH to 7.4 with 6N HCl		