

# Experiment NB-4: Nerve Action Potentials and Synaptic Potentials

## Background

The purpose of this experiment is to demonstrate the effect that electrical activity (action potentials) in a presynaptic cell has on the electrical activity (synaptic potentials) in a postsynaptic cell.

In this experiment, the same technique used to record the resting membrane potentials of frog muscle fibers is used to record the **synaptic potentials** from crayfish muscle fibers. Synaptic potentials will be recorded using the a **microelectrode** and an intracellular electrometer.

At the same time, the same technique used to record compound action potentials from frog nerve is employed to record the **action potentials** of crayfish motor axons. These spontaneously generated action potentials will be recorded using a **suction electrode** and an extracellular amplifier.

## Crayfish Slow Flexor Muscles

The abdomen of the crayfish consists of six segments, each having its own armor plating--a sturdy exoskeleton--that is hinged to the ones anterior and posterior to it. Inside each segment lies the nervous system and the muscles that control the bending of the hinge between the segments. The segments can move only in one dimension, to curl the tail under the thorax (this is called flexion) or straighten the tail out (extension). These movements are controlled by different sets of muscles: flexors and extensors. Fast movements, such as the tail flip used to escape from predators, is controlled by a massive set of muscle fibers whose contractions are very fast; these are the fast flexor muscles. The slower, postural adjustments that crayfish use to balance themselves are controlled by a smaller sheet of more slowly contracting muscle fibers; these are the slow flexor muscles. These slow flexors are located just inside the exoskeleton on the ventral surface, very close to the ventral nerve cord. By removing the exoskeleton and some overlying soft tissue, the superficial muscles and the nerves to them are exposed and readily recorded.

Crustacean muscles show many interesting differences from vertebrate muscle. In most vertebrate muscles, each fiber is innervated by only one motor neuron, and that connection is always excitatory. In crustaceans, a given muscle fiber may be innervated by as many as six motor neurons, and the synaptic connections may be either excitatory or inhibitory. A vertebrate muscle fiber usually generates a propagated action potential in response to a single action potential in its motor neuron; crustacean muscles, on the other hand, rarely produce action potentials. Instead, impulses from crustacean

motor neurons produce decremented responses, excitatory and inhibitory junction potentials (EJPs or IJPs), that are identical in all respects to postsynaptic potentials in neurons. Thus, crustacean muscle provides good material to study integrative physiology, such as summation of synaptic potentials and facilitation.

## Equipment Required

- PC Computer
- IWX/214 data acquisition unit
- USB cable
- IWX/214 power supply
- C-STIM-BNC-P2 BNC-dual pinjack stimulator cable
- Double male banana-female BNC adapter
- Model 3000 AC/DC extracellular differential amplifier
- Model 3000 input cable
- Suction electrode
- Audio monitor
- BNC T-connector
- Model 3100 Intracellular electrometer and headstage
- Microelectrode holder (adapter)
- Glass microelectrodes
- Micromanipulator with base (2 each)
- 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
- Crayfish
- Crayfish saline (See appendix)
- Electrode filling solution (See appendix)

## 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.

## 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 **SynapticPotentials-A-NBK** settings file.
- 5 LabScribe will appear on the computer screen as configured by the **SynapticPotentials-A-NBK** settings.
- 6 For your information, the settings used to configure the LabScribe software and the IWX/214 unit for this experiment are listed in Table NB-4-1 on page NB-4-2. 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.

**Table NB-4-1: Settings on the Channel Window of the Preferences Dialog Used to Configure the iWorx Recording System for Experiment NB-4.**

Parameter	Units/Title	Setting	Mode/Function
Acquisition Mode		Scope	Multiple
Sweeps	Number	1	
Delay between Sweeps	Sec	0.000	
Start		User	
Stop	Sec	Timed - 1.000	
Display Time	Sec	1.000	
Speed	Samples/Sec	10000	
Channel A1	Nerve AP	✓	BNC
Channel A2	Synaptic Potentials	✓	BNC

## Model 3000 Amplifier and Audio Monitor Setup

- 1 Place the Model 3000 AC/DC extracellular amplifier (Figure NB-4-1 on page NB-4-2) inside the Faraday cage and its AC power supply on the bench outside the cage.
- 2 Place a T-connector (BNC) on the Channel 1 BNC Input of the IWX/214.

- 3 Use a BNC-BNC cable to connect the BNC output of the Model 3000 to an open end of the T-connector (BNC) on the Channel 1 BNC input of the IWX/214.
- 4 Attach the BNC connector on the input cable of the audio monitor to the remaining open end of the T-connector on Channel 1 BNC input of the IWX/214.



Figure NB-4-1: The front panel of the Model 3000.

**Warning:** Before connecting the input cable to the probe input of the Model 3000, turn off the Model 3000 amplifier.

- 5 Locate the C-DB15-P3 input cable (Figure NB-4-2 on page NB-4-2) in the NBK/214 kit. Attach the DB15 connector on the end of the input cable to the DB-15 probe socket of the Model 3000 amplifier.

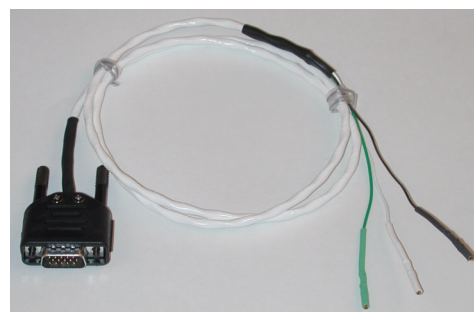


Figure NB-4-2: The C-DB15-P3 recording cable.

- 6 Attach the three connectors of the suction electrode assembly to the matching connectors of the input cable so that:
  - the recording electrode, which is the wire inside the lumen of the suction tubing, is connected to the black (+) connector.
  - the indifferent (reference) electrode, which is the wire wrapped around the suction tubing down to the glass micro-electrode tip, is connected to white (-) connector.
  - the ground electrode, that is in the solution in the bath chamber, is connected to the green (driven shield) connector.
- 7 Place the barrel of the suction electrode on a micromanipulator placed close the crayfish preparation dish.
- 8 Place the knobs and switches on the front of the Model 3000 amplifier in positions that configure the amplifier for this experiment. The settings used on the Model 3000 for this experiment are listed in Table NB-4-2 on page NB-4-3.
- 9 Plug the output of the AC power supply into the power input

on the back of the Model 3000 amplifier. Plug the power cord of the AC power supply into the electrical outlet.

- 10 Flip the power switch of the Model 3000 to the ON position and allow the amplifier to warm up for 5 minutes.

**Table NB-4-2: Settings on the Model 3000 Amplifier for Recording Motor Nerve Potentials.**

Function	Setting
HIGH PASS FILTER (Hz)	100
NOTCH FILTER	OFF
LOW PASS FILTER (kHz)	10
CAPACITANCE COMPENSATION	Counterclockwise
COARSE DC OFFSET	Midrange
FINE DC OFFSET	Midrange
DC OFFSET	OFF
INPUT MODE	DIFF
GAIN	100
RESISTANCE ( $\Omega$ ) TEST	OFF
MODE	REC

**Note:** The filter settings listed in this table are suggested for use in ideal recording conditions. If noise is present in the recording environment, the high and low pass filters can be set at different levels to create a recording with less noise. If noise is caused by AC line voltage used to power the equipment in the lab, the notch filter can be used.

### Model 3100 Amplifier Setup

- 1 Place the Model 3100 AC/DC extracellular amplifier inside the Faraday cage and its AC power supply on the bench outside the cage.

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

- 2 Connect the BNC output of the Model 3100 to the Channel 2 BNC input of the IWV/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.
- 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-4-3 on page NB-4-3.

- 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 and allow the amplifier to warm up for 5 minutes.

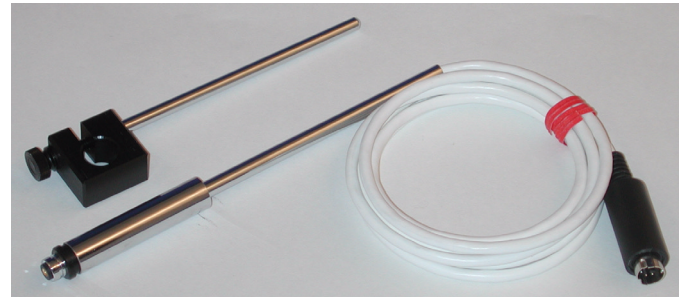


Figure NB-4-3: The head stage of the Model 3100 electrometer.



Figure NB-4-4: The front panel of the Model 3100.

**Table NB-4-3: Settings on the Model 3100 Amplifier for Recording Synaptic 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
$\Delta A$	Counterclockwise
RESISTANCE ( $\Omega$ ) TEST	OFF
NOTCH FILTER	OFF
LOW PASS FILTER (kHz)	10
CURRENT INJECTION LEVEL ( $\mu A$ )	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.

## Headstage Probe Test

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

- 1 Obtain a 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 head stage.

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

- 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** to the right of the **Mark button**.
- 5 Click **Record** and press the **Enter key** on the keyboard. The trace should appear near the center of the screen.
- 6 Locate the  $\Omega$ **TEST 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**, 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** (Figure NB-4-5 on page NB-4-4), 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.

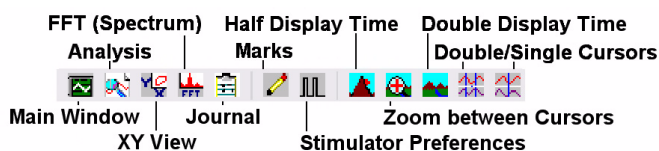


Figure NB-4-5: The *LabScribe* toolbar.

- 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 electrometer 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):

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

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)

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

- 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.

## 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

When the Model 3000 extracellular amplifier and Model 3100 intracellular electrometer are used on the same preparation, a common ground electrode should be used for the two amplifiers.

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**Warning:** *The driven shield of the Model 3000 input cable should not be connected to any electrode when an Model 3100 is used on the same preparation. If the driven shield of the Model =3000 is connected to an electrode that is in the same bath solution as the ground electrode of the Model 3100, the driven shield is connected to ground which is a condition that should not exist.*

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- 1 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 cable of the reference electrode into the GND input on the front panel of the Model 3100.
- 2 Place a cable between the ground input of the Model 3100 and the ground input of the Model 3000 to complete the connection of the ground electrode to the Model 3000.

### Signal Improvement

- 1 To improve recordings of the compound action and synaptic 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 **Nerve 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.

### Dissection

- 1 Immerse a crayfish in ice water for 10 minutes, to anesthetize it. When the crayfish is fully limp, quickly pith it.
- 2 Separate the tail (abdominal segments) from the thorax. These techniques will be demonstrated in the laboratory.
- 3 Securely pin the abdomen on its back in a wax-filled chamber. Cover the preparation with cold crayfish saline. Cut off the swimmerets which are the paired appendages on the ventral surface of each segment. The movements of the swimmerets interfere with the recordings if they are not removed.

- 4 With sturdy scissors, make a midline incision along the ventral surface, starting at the cut end (abdominal segment 1) and continuing to the most posterior end (segment 6).

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**Warning:** *Lift up the tissue with the tips of the scissors to avoid damaging the underlying nerve cord and muscles.*

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- 5 Cut through both the stout ribs (sternites) and the leathery **ventral membrane** tissue in between the ribs.
- 6 Make two more incisions, each one a few millimeters to either side of the midline. Remove the loose tissue. This will expose the ventral nerve cord, including the ganglia, the connectives running between the ganglia, and the three nerves (I, II, and III) coming out of each side of each ganglia.
- 7 The slow flexor muscles should be visible to the side of the opening made to expose the ventral nerve cord. These muscles are attached to the **cuticle** on the ventral membrane in each segment of the tail. Each segment of cuticle is nearly 1 cm long and starts about a third of the way back from sternite and ends at the following sternite.
- 8 Remove the ventral membrane that covers the tail from the posterior of the cuticle to the following sternite to create a window that exposes the **slow flexor muscle** as well as the superficial branch of the third nerve (SBIII) that innervates slow flexor muscle.

## 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 to prevent bubbles 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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- 5 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.
  - 6 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: Spontaneous Synaptic Potentials

Aim: To record spontaneous synaptic potentials from the fibers of a slow flexor muscle (SFM).

### Procedure

- 1 Look through the microscope as you use the controls of the micromanipulator to move the tip of the microelectrode over a bundle of muscle fibers. Position the tip above a healthy-looking fiber.

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**Note:** Unhealthy fibers are opaque, beaded, or twitching.

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- 2 Type **SFM Fiber 1** in the **Mark box** to the right of the **Mark button**.
- 3 Click **Record** to begin recording.
- 4 Once the tip of the microelectrode is close to the membrane of a healthy muscle fiber, watch the recording of the membrane potential on the computer screen. When the microelectrode tip touches a muscle fiber membrane, a small deflection of the recording, either up or down, may occur.
- 5 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.

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**Warning:** Do not lower the tip into the muscle fiber more than is necessary to avoid breaking the tip

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- 6 As the tip of the microelectrode penetrates the membrane, the recording displayed on the computer screen deflects downward rapidly. When this happens, **stop moving the electrode!** The tip of the microelectrode is inside the

muscle fiber.

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**Note:** Sometimes, the initial drop in voltage will not be abrupt. The voltage may drift down to a lower level as the tissue seals around the tip of the electrode. Count this more negative value as the resting potential, if it is at a steady level.

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- 7 Click **AutoScale** to view the voltage levels before and after the microelectrode penetrated the membrane.
- 8 Repeat Steps 5 through 10 to record the resting membrane potentials from nine additional muscle fibers. Check the resistance of the electrode frequently; a low resistance means that the tip is broken and it will damage the muscle fibers.
- 9 Click **Stop** to halt recording.
- 10 Select **Save** in the **File** menu.

### 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 fifteen-second section of the recording 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 fifteen-second section of the recording, and
  - Clicking the **Zoom between Cursors button** on the **LabScribe** toolbar (Figure NB-4-5 on page NB-4-4) to expand or contract the fifteen-second recording to the width of the **Main window**.
- 3 Click on the **Analysis window** icon in the toolbar (Figure NB-4-5 on page NB-4-4) 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 mathematical functions, **V2-V1** and **T2-T1**, should appear in this table. The values for **V2-V1** and **T2-T1** 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 (**V2-V1**) as the tip of the microelectrode enters the muscle fiber, 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 **Membrane Potential channel menu**.

- Transfer the value for the parameter 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 (**V2-V1**) in the **Journal** using the one of the techniques described in Steps 5 or 6, and in Table NB-4-4 on page NB-4-8.

9 Divide the value for **V2-V1** by the gain of the Model 3100 electrometer to obtain the membrane potential. 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:

$$320 \text{ millivolts} / 10X \text{ Gain} = 32 \text{ millivolts}$$

10 Place a section data recorded while the microelectrode was in a fiber on the **Main window**.

11 Use the **AutoScale** and the **Display Time** functions to zoom-in on the voltage recorded while the microelectrode was in the fiber.

12 Examine this section of the recording to determine if any synaptic potentials are present. These potentials usually have actual amplitudes of 5mV or less.

**Table NB-4-4: Resting Membrane Potentials ( $E_m$ ) of Slow Flexor Muscle Fibers.**

	Voltage Change ( $\Delta V$ ) Recorded, in mV	$\Delta V / \text{Gain} = E_m$ , in mV
Fiber 1		
Fiber 2		
Fiber 3		
Fiber 4		
Fiber 5		
Fiber 6		
Fiber 7		
Fiber 8		
Fiber 9		
Fiber 10		
Mean		

**Questions**

1 What was the range of values measured for the resting membrane potentials of the fibers?

- 2 Do some of the values fall outside the range of the mean  $\pm$  2 Standard Deviations (S.D.)?
- 3 Why do resting membrane potentials recorded from different fibers vary?
- 4 Can lower values for the membrane potentials be attributed to the state of health of the muscles or the ease at which the fibers were penetrated?

**Exercise 2: Action Potentials in Motor Neurons**

Aim: To record motor nerve action potentials from the SBIII nerve.

**Procedure**

- 1 Turn on the Model 3000 amplifier. Make sure it has warmed up for five minutes before it is used for the recording.
- 2 Use the second micromanipulator to position the tip of the suction electrode near the SBIII nerve of the slow flexor muscle fibers from which the membrane potentials were recorded.
- 3 Check the size of the tip of the suction electrode. It should be the same size or only slightly larger than the diameter of the nerve.
- 4 Without moving the tubing, pull back on the plunger of the syringe, that is connected to the tubing of the suction electrode, to pull the end, or a loop, of the nerve into the suction electrode.
- 5 Turn on the audio monitor connected to the output of the Model 3000 amplifier. The action potentials in the motor neurons should be heard as a series of clicks.
- 6 Type **Motor Neuron** in the **Mark box** to the right of the **Mark button**.
- 7 Click **Record** to begin recording.
- 8 To improve the display of the action potentials in the SBIII nerve on the **Main window**:
  - Increase the gain of the Model 3000 amplifier.
  - Minimize the 60 Hz noise by using the filters of the amplifier and the digital filters of the LabScribe2 software.
- 9 If the spontaneous rate of firing from the motor neurons is too slow, brush the sensory hairs on the lateral edge of the same or adjacent tail segments to increase the firing frequency.
- 10 Continue to record for two minutes.
- 11 If only two or three different types of action potentials are detected, use a suction electrode with a smaller tip or record from a different SBIII nerve.
- 12 Click **Stop** to halt recording.
- 13 Select **Save** in the **File** menu.

## Analysis

- 1 Measure and record the parameters of the action potentials coming from the neurons of the SBIII nerve using the techniques described in Exercise 2.
- 2 Inspect the recording made from the SBIII nerve to determine the number of different types of action potentials present. Use these parameters to distinguish the different types of action potentials recorded from the SBIII nerve:
  - **Amplitude (Amp):** Measure the amplitude of an action potential by placing one cursor on the baseline before the action potential, and the second cursor on the peak of the potential. The value for the **V2-V1** function on the **Nerve Action Potential channel** is the amplitude.
  - **Duration (Dur):** Measure the duration of an action potential by placing one cursor at the onset of the action potential, and the second cursor on the point where the potential returns to the baseline. The value for the **T2-T1** function on the **Nerve Action Potential channel** is the duration.
  - **Rise Time (Rise):** Measure the rise time of an action potential by placing one cursor at the onset of the action potential, and the second cursor on the peak of the potential. The value for the **T2-T1** function on the **Nerve Action Potential channel** is the rise time.
  - **Fall Time (Fall):** Measure the fall time of an action potential by placing one cursor on the peak of the potential, and the second cursor on the point where the potential returns to the baseline. The value for the **T2-T1** function on the **Nerve Action Potential channel** is the fall time.
  - **Frequency (Freq.):** Measure the frequency of a type of action potential by placing one cursor on the peak of an action potential, and the second cursor on the peak of the successive potential. The value for the **T2-T1** function on the **Nerve Action Potential channel** is the period of the wave type. The inverse of the period is the frequency.
  - **Polarity (Pol.):** note whether the waveform of the action potentials goes up (positive) or down (negative) on the screen.
- 3 Enter information about the types of action potentials recorded in the **Journal** and Figure NB-4-5 on page NB-4-9.

**Table NB-4-5: Properties of Action Potentials Recorded from SBIII Nerve.**

AP Type	Amp. (mV)	Dur. (msec)	Rise (msec)	Fall (msec)	Freq. (Hz)	Pol. +, -
1						
2						
3						
4						
5						
6						
7						

## Question

- 1 How many different types of action potentials did you detect?

- 2 What membrane properties affect the shape and size of action potentials?

## Exercise 3: Action and Synaptic Potentials

**Aim:** To correlate the activity of action potentials in the SBIII nerve with the activity of synaptic potentials in the muscle fibers.

### Procedure

- 1 With the suction electrode attached to the SBIII nerve and a microelectrode placed in the membrane of a slow flexor muscle fiber, record the presynaptic action potentials and the postsynaptic membrane potentials at the same time.
- 2 Record from several muscle fibers since most muscle fibers are not innervated by every motor neuron. The synaptic potentials from a particular muscle fiber are usually the same size and shape every time an action potential from a particular motor neuron occurs.

### Questions

- 1 Is one particular type of nerve action potential associated more closely with postsynaptic potentials more than any other type of action potential?
- 2 Are particular types of action potentials associated with particular sizes of postsynaptic potentials? For example, are the largest action potentials always followed by largest excitatory post synaptic potentials (EPSP) or by the smallest EPSPs?
- 3 At least one of the motor neurons in the SBIII nerve is inhibitory. Can you tell which type of action potential from the SBIII nerve produces inhibitory postsynaptic potentials (IPSP)? Does this type of nerve action potential produce IPSPs in all muscle fibers?

## Exercise 4: Evoked Synaptic Potentials

**Aim:** To evoke and record postsynaptic potentials in slow flexor muscle fibers while stimulating the SBIII nerve.

### Procedure

- 1 If it has not already been done, cut the SBIII nerve at the point where this nerve enters the ventral nerve cord. Pull the cut end of the nerve into the tip of the suction electrode by pulling back on the syringe attached to the end of the suction tubing.
- 2 Locate the C-STIM-BNC-P2 BNC stimulator cable and double male banana-female BNC adapter in the NBK-214 kit.
- 3 Plug the male double banana-female BNC adapter into the positive (red) and negative (black) banana jacks of the IW/214 stimulator. The banana plug that goes into the negative (black) stimulator output is identified by a tab, embossed with the letters **GND** (ground), on that side of the adapter.
- 4 Attach the BNC connector of the C-STIM-BNC-P2 stimulator cable to the adapter on the stimulator outputs.

- Connect the lead wire for the electrode inside the lumen of the suction electrode to the red pin connector of the C-STIM-BNC-P2 stimulator cable. Connect the lead wire for the electrode wrapped the tip of the suction electrode to the black pin connector of the C-STIM-BNC-P2 stimulator cable.
- Pull down the **Settings** menu. Select the **SynapticPotentials-B-NBK** settings file. The LabScribe Main window appears on the computer screen as configured by settings in the **Channels** and **Stimulator Preferences Dialog** windows. The settings, which can be viewed by selecting **Preferences** from the **Edit** menu on the LabScribe Main window, are listed in Table NB-4-6 on page NB-4-10 and Table NB-4-7 on page NB-4-10.

**Table NB-4-6: Settings on the Channel Window of the Preferences Dialog Used to Configure the iWorx Recording System for Exercise 4.**

Parameter	Units/Title	Setting	Mode/Function
Acquisition Mode		Scope	Multiple
Sweeps	Number	1	
Delay between Sweeps	Sec	0.000	
Start		User	
Stop	Sec	Timed - 0.020	
Display Time	Sec	0.020	
Speed	Samples/Sec	10000	
Channel A2	Synaptic Potentials	✓	BNC
Channel S1	Stimulus	✓	Record

- Click the **Stimulator Preferences** icon on the LabScribe toolbar (Figure NB-4-5 on page NB-4-4) to open the **stimulator control panel** on the **Main window**.
- Check the settings on the **stimulator control panel** against the settings for this portion of the exercise that are listed in Table NB-4-7 on page NB-4-10. To make changes to the stimulus parameters:
  - Click on the **arrow buttons** to the right of the window that displays the value of the parameter to increase or decrease the value; or,
  - Type the value of the parameter in the window next to the label of the parameter.
  - Click the **Apply** button to effect the change to the stimulator.
- Click the **Record** button to stimulate the SBIII nerve and record from the postsynaptic slow flexor muscle fiber.
- If a response did not occur in the muscle fiber as recorded on the **Membrane Potential channel**, increase the stimulus amplitude. Remember to click the **Apply** button to finalize changes.
- Continue to increase the stimulus amplitude until a synaptic potential occurs. Note the amplitude at which this potential

occurred.

- Continue to increase the stimulus amplitude and record the response of the fiber. As the stimulus amplitude is increased, the amplitude of the synaptic potential will increase in a stepwise manner. These steps correspond to activation of different axons in the nerve.

**Table NB-4-7: Settings on the Stimulator Window of the Preferences Dialog that Configure the iWorx System for Exercise 4.**

Parameter	Units/Title	Setting
Stimulus Mode		Pulse
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	0.002
Amplitude (Amp)	Volt	0.010
Pulses (#pulses)	Number	1
Pulse Width (W)	msec	0.1
Time Off (T Off)	msec	0.9
Time Off Amplitude	Volts	0
Holding Potential (HP)	Volts	0

### Questions

- At what stimulus amplitude did the muscle fiber first generate a synaptic potential?
- At what stimulus amplitude did the amplitude of the synaptic potential reach a maximum level?
- How many times larger is the stimulus that delivered the maximum response than the stimulus that delivered the first measurable synaptic potential?

### Appendix

**Table NB-3-8: Recipe for Crayfish Saline Solution.**

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		