

# Experiment NB-7: Crustacean Stretch Receptor

## Background

Animals use their senses to gather information about their environment. Most senses use specialized sensory organs (eyes, ears, nares) to receive stimuli. These organs vary widely between species. In turn, the information received by the sensory organ is coded in the impulses generated by sensory neurons and conducted to the animal's central nervous system for the generation of an appropriate response.

The stretch receptors in the tails of decapods (shrimp, lobsters, crayfish) are good examples of sensory organs that are integrated with sensory neurons which are connected to a central nerve chord. These receptors monitor the position and movement of the animal's tail, particularly important if the animal has to make a hasty retreat. The nerve tracts from these receptors are easily exposed, allowing recording electrodes to be attached to the nerves without damaging the sensory organs or the muscles that are being monitored.

Students will record responses from two different types of stretch receptors that are positioned next to the dorsal superficial extensor muscles in the tail of the crayfish. Each type of sensory organ responds to a different stimulus, generates impulses of a different amplitude and frequency, and adapts to prolonged stimuli at a different rate.

## The Stretch Receptor

To have the fine degree of neuromuscular control needed for coordinated movements, an animal's nervous system needs to monitor the positions of its body parts at all times. Position and tension in skeletal muscle is often monitored by a sensory organ known as a *muscle receptor organ* (MRO) that lays in parallel with the skeletal muscle. A muscle receptor organ is composed of a *receptor muscle* (RM) and a *sensory neuron* (SN). The dendrites of the sensory neuron are embedded in the receptor muscle and are stimulated when the receptor muscle is stretched. The receptor muscle stretches as the skeletal muscle does. The stretch-sensitive dendrites of the sensory neuron have ion channels that open and produce graded potentials in response to the stretching of the receptor muscle. When the graded potentials of the sensory neuron sum to reach its threshold, an action potential develops and propagates along the sensory neuron to its synapse with the central nervous system.

When a decapod curls its tail quickly and repeatedly to move backwards, a process known as scuttling, the ventral flexor muscles alternate contractions with the

dorsal extensor muscles in a fraction of a second. As the flexors contract, the extensors on the dorsal relax and stretch. In turn, the muscle receptor organs on the extensors stretch and the associated sensory neurons generate action potentials. These are the action potentials that will be recorded in this experiment as the tail of the crayfish is flexed.

As the dorsal extensor muscles in the crayfish tail contract, their receptor muscles must also contract. If a receptor muscle does not contract, it and the dendrites of its attached sensory neuron are too slack to create the action potentials needed to monitor the position and movement of the animal's tail. Receptor muscles have motor innervations and contractile elements just like other muscles. When tension is maintained in these receptor muscles, they and their sensory neurons are ready to create action potentials when stretched.

## The Crayfish

The tail of a crayfish is composed of interlocking exoskeletal plates known as *terga*. Each abdominal segment, or tergum, and the two most posterior thoracic segments of a crayfish contain two types of muscle receptor organs (MRO) along the dorsal superficial extensor muscles. One type of MRO is a slow adapting, *tonic*, receptor (MRO<sub>1</sub>); and, the other type is a quick adapting, *phasic*, receptor (MRO<sub>2</sub>).

On each side of the dorsal midline, one of each type of MRO originates at the anterior edge of a segment and inserts at the anterior edge of the following segment (Figure NB-7-1 on page NB-7-1). So, when the tail flexes (curls) and the tail segments rotate around the lateral hinge joints that connect the adjacent terga, the dorsal receptor muscles stretch and the attached sensory neurons generate action potentials that can be recorded from the lateral nerve tract that is caught in a suction electrode. When the tail extends (straightens out), the receptor muscles are relaxed and the sensory neurons cease firing.

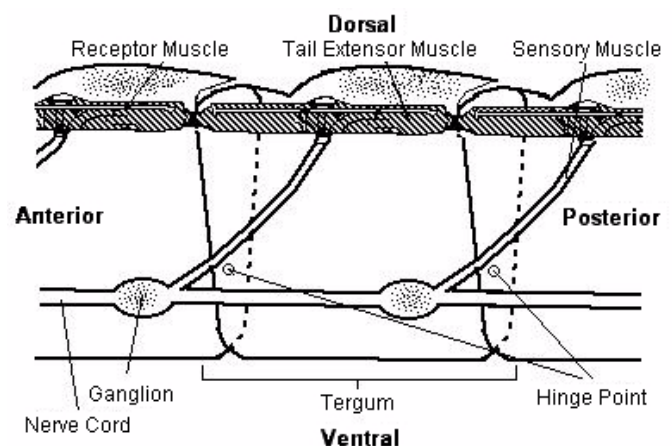


Figure NB-7-1: Cut-away diagram (side view) of a segment of crayfish tail.

## Adaptation

Each receptor muscle ( $RM_1$  or  $RM_2$ ) is embedded with the dendrites of a its own sensory neuron (Figure NB-7-2 on page NB-7-2). The axons from the sensory neurons of the MRO's on the same side of a segment join together in a nerve that circles the major dorsal and ventral interior muscles of the tail on its track to the ventral nerve cord (Figure NB-7-1 on page NB-7-1). MRO's lie on top of the medial superficial extensor muscles that line the inside dorsal surface of the crayfish tail. In turn, the deep medial flexor muscles of the tail lie over these receptor muscles.

Each MRO also has an accessory nerve fiber that originates in the animal's central nervous system, and its activity creates inhibitory post-synaptic potentials (IPSP's) in the sensory neuron as the tail flexion ends and extension begins in preparation for another flexion. IPSP's effectively raise the threshold of the sensory neuron, requiring a greater degree of dendritic stretching to create an action potential.

The exercises in this experiment will demonstrate:

- the types of muscle receptor organs (MRO):  $MRO_1$ , which is *slow-adapting* and discharges for long periods of time with a moderate, constant stretch;  $MRO_2$ , which is *fast-adapting* and responds only briefly to sharp, vigorous stretching.
- *adaptation*, or a decrease in the frequency of firing, in the slow-adapting MRO as the time after the initiation of a constant stretch increases.
- the frequency of firing of  $MRO_1$  is directly proportional to the degree of tail flexion or stretching of  $RM_1$ .

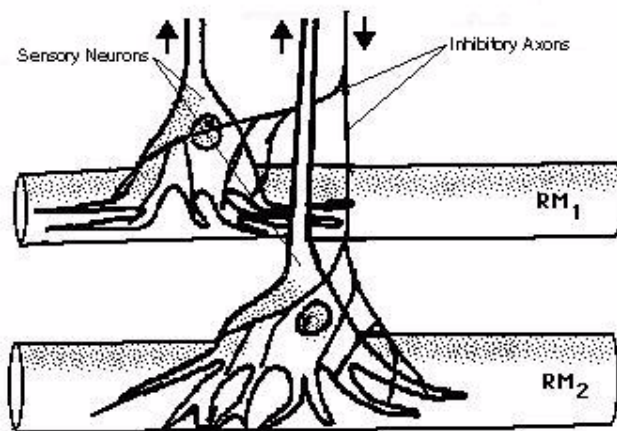


Figure NB-7-2: RM sensory and inhibitory innervation. Motor innervation is omitted.

## Equipment Required

- PC Computer
- IWX/214 data acquisition unit
- USB cable
- IWX/214 power supply
- Model 3000 AC/DC extracellular differential amplifier
- C-DB15-P3 Nerve bath recording cable
- Suction electrode assembly and glass tips
- Audio monitor
- BNC T-connector
- Stand and clamp, or micromanipulator, for electrode.
- Plastic crayfish bath chamber
- Dissection microscope and light source
- Tissue tensioner, or micromanipulator, for flexing tail.
- Faraday cage and steel base plate (if noise in room)
- Cables and alligator clips for grounding equipment
- Suture thread
- Crayfish
- Crayfish saline (See Appendix)
- Pasteur pipettes and bulbs
- Dissection tools

## 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**.

- Pull down the **Settings** menu again. Select the **Crustacean SR-NBK** settings file.
- After a short time, **LabScribe** will appear on the computer screen as configured by the **CrustaceanSR-NBK** settings.
- For your information, the settings used to configure the **LabScribe** software and the **IWX/214** unit for this experiment are listed in Table NB-7-1 on page NB-7-3. 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**.

**Table NB-7-1: Settings on the Channel Window of the Preferences Dialog that Configure the iWorx System for Experiment AN-2.**

Parameter	Units/Title	Setting	Mode/Function
Acquisition Mode		Chart	
Start		User	
Stop		User	
Display Time	Sec	5	
Speed	Samples/Sec	10000	
Channel A1	MRO Potentials	✓	BNC
Channel C1	MRO Firing Freq.	✓	P.Freq.

### Model 3000 Amplifier and Audio Monitor Setup

- Place the Model 3000 AC/DC extracellular amplifier (Figure NB-7-3 on page NB-7-3) and its AC power supply on the bench near the **IWX/214**.



Figure NB-7-3: The front panel of the Model 3000.

- Place a T-connector (BNC) on the Channel 1 BNC Input of the **IWX/214**.
- 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**.
- 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**.

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

- Locate the C-DB15-P3 recording cable (Figure NB-7-4 on page NB-7-3) in the **NBK/214** kit. Attach the DB15 connector on the end of the cable to the DB-15 probe socket of the Model 3000 amplifier.

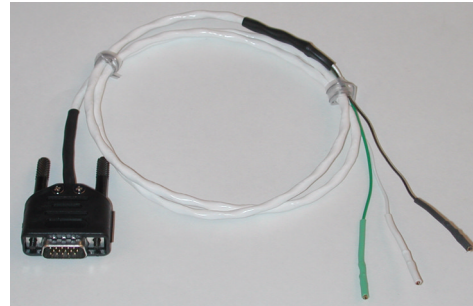


Figure NB-7-4: The C-DB15-P3 recording cable.

- Attach the three connectors of the suction electrode assembly to the matching connectors of the C-DB15-P3 recording 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. Place the barrel of the suction electrode on a micromanipulator placed close the crayfish preparation dish.

**Table NB-7-2: Settings on the Model 3000 Amplifier for Recording MRO Potentials.**

Function	Setting
HIGH PASS FILTER (Hz)	1
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

- Place the barrel of the suction electrode on a micromanipulator placed close the crayfish preparation dish.

- 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-7-2 on page NB-7-3.

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**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.*

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

## 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 Model 3000 amplifier has two different connections for grounding:

- One method uses the driven shield on the input cable as described earlier.
- The other method uses the ground (**GND**) input of the Model 3000.

- 1 Try recording when the ground electrode in the prep dish is connected to the green wire lead on the input cable.
- 2 If the recordings have a lot of 60Hz noise while using the driven shield of the input cable, connect the ground electrode to the ground input of the Model 3000 amplifier.

**Warning: Never connect the driven shield to the ground (GND) input of the Model 3000, either directly or indirectly.**

- 3 If the recordings still have a lot of 60Hz noise, connect the ground electrode to one of the green ground inputs of the iWorx 214 unit.

## Signal Improvement

- 1 To improve recordings of the stretch receptor 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 Noise can be decreased by changing the settings of the **High** and **Low Pass Filters**, or using the **Notch Filter**, on the Model 3000. If the new settings alter the shape of the action potential, they cannot be used.
- 3 Noise can also be decreased by applying the digital filter function in the LabScribe 2 software 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.
- 4 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.
- 5 Set the **Low Cutoff filter value** to **65** and the **High Cutoff filter value** to **8000**. 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 icewater for 10 minutes. Remove the crayfish from the icewater and quickly cut off its head.
- 2 Remove the tail 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 tail; 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 tail (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 tail and separate the ventral and dorsal halves of the shell from each other. It may be necessary to cut (use small forceps) the connections of that the segmental flexor muscles make to the dorsal shell.
- 6 Discard the ventral portion of the shell (Figure NB-7-5 on page NB-7-5).

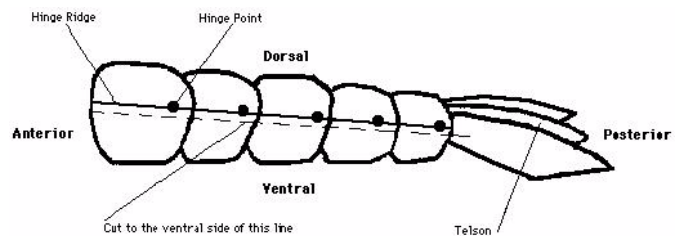


Figure NB-7-5: Diagram to show the dissection of the crayfish tail.

- 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 prep.
- 10 Examine the preparation, compare with Figure NB-7-6 on page NB-7-5 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.

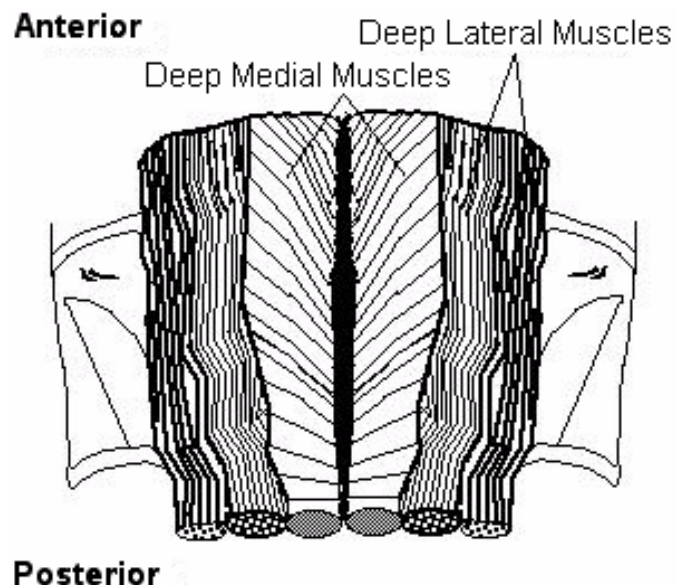


Figure NB-7-6: Ventral aspect of the second abdominal segment of crayfish. Dorsal musculature removed on the right side and intact on the left side.

- 11 Poke a hole through the most posterior segment (telson) and then thread a suture through this hole. Tie the suture to the tail using a loose loop. The exoskeleton in this area is very thin and tears very easily if knots are tied too tightly.
- 12 Gently pulling on the string should flex or curl the tail. If the hinge ridge has been damaged, the tail will not flex correctly.
- 13 Examine the cut edges of the exoskeleton with a dissecting microscope. Locate any cut ends of nerves containing the axons of sensory neurons, extensor motor fibers, and other sensory fibers. Usually the nerve ends float free of other tissue near the posterior and lateral wall of each tergum.
- 14 With fine forceps, carefully remove any large segments of damaged flexor muscles that obscure the viewing of nerves or interfere with the placement and operation of the suction electrode.

### Placement of the Electrode

- 1 Place the chamber containing the crayfish on the table (or steel grounding plate inside a Faraday cage, if necessary).
- 2 Position the dissecting microscope over the preparation chamber and focus on the nerve trunks along the edge of the exoskeleton.
- 3 Place the coiled ground electrode over the side of the specimen chamber, using wax or clay to hold it in place. The copper wire in the lead and the solder joint holding the silver electrodes to the leads must not touch the saline bath.
- 4 Place two micromanipulators at the end of the chamber nearest the most anterior segment of the tail. Attach the extracellular suction electrode to one of the micromanipulators.
- 5 Locate a nerve from which to record and grossly position the electrode near the nerve. Pin down the segment in which the nerve is located. Avoid injuring the MRO's or the nerves by pinning along the midline or well to the sides.
- 6 Align the electrode and its manipulator (or stand and clamp) at an angle that still permits the posterior segments of the tail to be flexed without touching the electrode. View the electrode tip through the microscope and move it until it is near or touching the cut end of the nerve. The opening in the tip of the electrode should be the same size or only slightly larger than the diameter of the nerve. Carefully pull back on the plunger of the 3cc syringe (without moving the tubing) and pull the end (or a loop) of the nerve into the electrode.
- 7 Check the height of the saline in the chamber and inside the glass microelectrode. Both the positive and negative electrodes should be in contact with the saline since the amplifier of the recording channel is used to record differentially between the inner and outer wires.
- 8 Position another micromanipulator (or tissue tensioner) on the end of the bath with the anterior tail segments. Attach the suture on the telson to the horizontal axis of this device, so the suture does not interfere with the recording electrode when the tail is flexed by movement of the horizontal axis of

the micromanipulator or tensioner.

### Exercise 1: MRO<sub>1</sub>

Aim: To record action potentials from the slow-adapting MRO<sub>1</sub>.

- 1 Click **Record** on the **LabScribe Main window** to begin recording.
- 2 Type **MRO1** in the **Mark box** to the right of the **Mark button**. Press the **Enter key** on the keyboard to attach this notation regarding the type of receptor potentials to be recorded.
- 3 Move the horizontal axis of the micromanipulator holding the suture on the telson and flex the crayfish tail to a position where the MRO<sub>1</sub> begins firing slowly.
- 4 Click the **AutoScale button** on the **MRO Potential** and **MRO Firing Frequency** channels to adjust the size of the traces displayed on the **Main window**.
- 5 Note the readings on the scale of the horizontal axis of the manipulator or the number of turns of the tensioner required to create this MRO<sub>1</sub> firing frequency. A slow-adapting MRO<sub>1</sub> should generate action potentials, like the ones with a low amplitude and high frequency in Figure NB-7-7 on page NB-7-6.

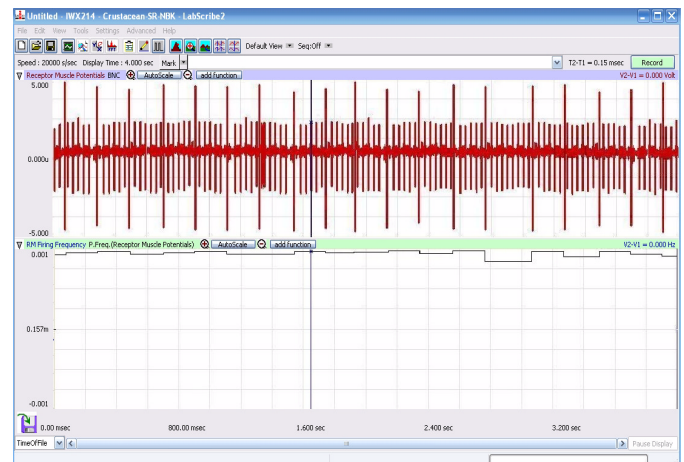


Figure NB-7-7: The action potentials from MRO<sub>1</sub> (with their lower amplitude and higher frequency) and MRO<sub>2</sub> (with their higher amplitude and lower frequency) are displayed on the upper channel. The frequency of action potentials from MRO<sub>2</sub> are displayed on the lower channel.

- 6 Move the horizontal axis of the micromanipulator or tensioner to flex the tail to a greater degree. The frequency of firing from MRO<sub>1</sub> should increase.
- 7 Move the horizontal axis of the micromanipulator or tensioner to flex the tail to an even greater degree. The frequency of firing from MRO<sub>1</sub> should be increase, again.
- 8 Click **Stop** to halt the recording. Note the readings on the scale of the horizontal axis of the manipulator rack. The tail may then be returned to the same degree of flexion in order to verify the measurements. Relax the tail from its flexed state between recordings to conserve neural activity.

- 9 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.

## Exercise 2: MRO<sub>2</sub>

Aim: To record action potentials from the fast-adapting MRO<sub>2</sub>.

- 1 Click **Record**. Move the horizontal axis of the micromanipulator holding the suture on the telson and flex the crayfish tail to a position where the MRO<sub>1</sub> is firing slowly.
- 2 Type **MRO2** in the **Mark box** to the right of the **Mark** button. Press the **Enter** key on the keyboard to attach this notation regarding the type of receptor potentials to be recorded.
- 3 Click the **AutoScale** button on the **MRO Potential** (CH3) and **MRO Firing Frequency** channels to adjust the size of the traces displayed on the **Main window**.
- 4 While recording action potentials from MRO<sub>1</sub>, use a pencil to tap the suture holding the tail in the flexed position. This tap should be quick, as if the pencil is bouncing off the suture. If the movement of the pencil depresses and holds the suture too deeply, the electrode could be pulled off the nerve or the nerve could be damaged. With this technique, MRO<sub>2</sub> should generate a burst of larger action potentials in the midst of action potentials from MRO<sub>1</sub>, as in Figure NB-7-7 on page NB-7-6.
- 5 Click **Stop** to halt the recording. Relax the tail from its flexed state between recordings to conserve neural activity.
- 6 Select **Save** in the **File** menu to add this data to the current data file.

## Questions

- 1 Were you able to elicit a response from the MRO<sub>2</sub>? if you did, what technique did you use?
- 2 Did you observe any differences between the responses of MRO<sub>1</sub> and MRO<sub>2</sub>? Explain these differences.

## Exercise 3: Adaptation of MRO<sub>1</sub>

Aim: Subject MRO<sub>1</sub> to constant stretch and measure the decline in its rate of firing or adaptation.

## Procedure

- 1 Click **Record**. Move the horizontal axis of the micromanipulator holding the suture and flex the crayfish tail to a position where the MRO<sub>1</sub> is firing rapidly.
- 2 Type **MRO1 Adaptation** in the **Mark box** to the right of the **Mark** button. Press the **Enter** key on the keyboard to attach this notation regarding adaptation of the MRO<sub>1</sub> firing rate
- 3 Click the **AutoScale** button on the **MRO Potential** and **MRO Firing Frequency** channels to adjust the size of the traces displayed on the **Main window**.

- 4 As you continue to record, press the **Enter** key on the keyboard every five seconds to place a mark on the recording. Continue recording for 2 minutes or until the MRO<sub>1</sub> has stopped firing, whichever is shortest.
- 5 Click **Stop** to halt the recording. Relax the tail from its flexed state between recordings to conserve neural activity.
- 6 Select **Save** in the **File** menu.
- 7 Repeat Steps 1 through 6 for a position where the MRO<sub>1</sub> is firing less rapidly.
- 8 Repeat Steps 1 through 6 for a position where the MRO<sub>1</sub> is firing even less rapidly.

## Analysis

- 1 Scroll through the data file and locate the first recording of constant stretch on the muscle receptor organ.
- 2 Use the **Display Time** icons to adjust the **Display Time** of the **Main window** to display a fifteen-second section of recording 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-7-8 on page NB-7-7) to expand or contract the fifteen-second recording to the width of the **Main window**.

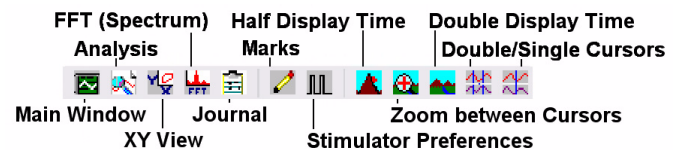


Figure NB-7-8: The **LabScribe** toolbar.

- 3 Click on the **Analysis window** icon in the toolbar (Figure NB-7-8 on page NB-7-7) 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 **MRO Potential** channel in the **Analysis window**. The mathematical function, **Mean**, should appear in this table. The value for **Mean** firing frequency is displayed in the table across the top margin of the **MRO Firing Frequency** channel.
- 5 Once the cursors are placed in the correct positions for determining the mean frequency of MRO firing in this section of the recording, the value for **Mean** 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 location used to measure the mean firing frequency.

- Transfer the names of the parameters to the **Journal** using the **Add Title to Journal** function in the **MRO Firing Frequency channel menu**.
  - Transfer the values for the parameters to the **Journal** using the **Add Ch. Data to Journal** function in the **MRO Firing Frequency channel menu**.
- 7 On the **MRO Firing Frequency channel** displayed in the **Analysis window**, use the mouse to click on and drag the cursors so they are about five seconds apart.
  - 8 Record the value for the mean firing frequency (**Mean**) in the **Journal** using the one of the techniques described in Steps 5 or 6.
  - 9 Move the left (first) cursor to a point on the recording that is five seconds to the right of the position of the second cursor. Determine the frequency of firing in this five second block of time. Enter that value in the **Journal**.
  - 10 Continue to move the cursor to successive five-second marks, determining the frequency of firing at each mark, and entering those values in the **Journal**. Continue until the end of the recording is reached.
  - 11 Repeat Steps 1 through 10 for the other two constant stretch recordings that started with lower initial firing frequencies.

### Questions

- 1 Plot the frequency of firing of MRO<sub>1</sub> in the first segment as a function of the time after the initiation of the constant stretch. Is the graph linear?
- 2 What can be concluded about the rate of adaptation of MRO<sub>1</sub>? How does the duration of the constant stretch affect the response of the MRO<sub>1</sub>?
- 3 Plot the frequency of firing of MRO<sub>1</sub> in the third (slowest) segment as a function of the time after the initiation of the constant stretch. Is the relationship linear?
- 4 Can you make any conclusions about the degree of constant flexion and the rate of adaptation?
- 5 How might an animal benefit from adaptation?

### Exercise 4: Flexion & Firing Frequency

Aim: Find the rate of MRO<sub>1</sub> firing for different degrees of flexion in the crayfish tail.

#### Procedure

- 1 Make sure the pinned segment of the crayfish tail remains secure as this exercise is performed.
- 2 Click **Record**. Relax the crayfish tail to a position where the MRO<sub>1</sub> is scarcely firing. Note the position of the index mark on the scale of the micromanipulator or the position of the tensioner. Click the **AutoScale** button on the **MRO Potential** channel to adjust the size of the trace.
- 3 As the recording continues, quickly rack the manipulator, or tensioner, to increase the flexion of the crayfish tail by one

scale unit, or a fraction of a turn. Press the **Enter** button on the keyboard to mark the recording as the final angle of flexion is reached.

- 4 Record the firing of the MRO<sub>1</sub> with the micromanipulator or tensioner in this position for a period of 5 seconds. Then, relax the crayfish tail to the starting position where the MRO<sub>1</sub> was scarcely firing.
- 5 If you are ready to flex the crayfish tail to a greater degree, continue recording.
- 6 Quickly rack the micromanipulator or the tensioner with a greater degree of tail flexion (two scale units, or fractions of a turn), Mark the recording, and record the firing of the MRO<sub>1</sub> in this position for 5 seconds. Note the position of the index on the scale of the micromanipulator or the turns of the tensioner. Relax the crayfish tail to the starting position as done in Step 4.
- 7 Repeat Steps 3 and 4 three more times with flexions equal to 3, 4, and 5 scale units, or fractions of a turn.
- 8 Click **Stop** to halt the recording. Relax the tail from its flexed state to conserve neural activity.
- 9 Select **Save** in the **File** menu.

### Data Analysis

Use the same techniques used in Exercise 3 to measure the frequency of MRO<sub>1</sub> firing at different degrees of tail flexion.

### Questions

- 1 Plot the initial firing frequency of MRO<sub>1</sub> at each degree of tail flexion as a function of the degree of tail flexion (expressed in units of movement for the micromanipulator or tensioner).
- 2 What is the relationship between the degree of flexion and the initial frequency of firing? Is the relationship linear?
- 3 Does MRO<sub>1</sub> respond to any and all degrees of flexion? Why or why not?

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## Appendices

### Extracellular Suction Electrode

- 1 Obtain the following items to make your own suction electrode (Figure NB-7-9 on page NB-7-9): a soldering iron; solder; a wire stripper; a wooden-handled dissecting pin; an alcohol burner; a can or tube of contact or plastic cement; a fine flat file; emery cloth; electrical tape; a popsicle stick; two pieces of chlorided silver wire (0.005" dia, 5" long); three feet of shielded, two-conductor, insulated cable; three color-coded connectors that will mate to the connectors on the input cable for the amplifier; three feet of flexible plastic tubing (20 gauge Tygon or PE 100); an 18-gauge needle, a 3-way stop cock, a 3cc syringe, a 1cc tuberculin syringe; glass micropipette tip.
- 2 Connectors and electrodes need to be attached to the ends of the shielded, two-conductor cable. Take one end of the cable and carefully strip 5 inches of insulation off the end. Minimize the number of strands of braided shielding cut during the removal of the insulation. Avoid cutting the conductor wires under the braided shielding.
- 3 Unbraid the 5 inches of exposed shielding. Pull the conductor wires aside. Gather all the strands of the shielding in a bundle and twist them together. Solder a small alligator clip to the end of the twisted shielding.
- 4 Strip a quarter of an inch of insulation off the end of each conductor wire. Solder a piece of the chlorided silver wire to the end of each wire.
- 5 Take the other end of the cable and carefully strip 3 inches of insulation off the end. Again, minimize the number of strands of braided shielding cut during the removal of the insulation. Avoid cutting the conductor wires under the braided shielding.
- 6 Unbraid the 3 inches of exposed shielding. Pull the conductor wires aside. Gather all the strands of the shielding in a bundle and twist them together. Solder a color-coded connector, that mates with the connector on the input cable of the amplifier, to the end of the twisted shielding. Since the shielding is the ground wire, the connector is usually color-coded green.
- 7 Strip a quarter of an inch of insulation off the end of each conductor wire at this end of the cable. Solder a color-coded connector to the end of the each conductor wire. A red connector is usually put on the wire conducting the signal from the recording electrode. On this suction electrode assembly, the recording electrode will be the silver wire that is inside the lumen of the suction tubing. A black connector is usually put on the wire connected to the indifferent or reference electrode, which is a silver wire wrapped around the outside of the electrode tip.

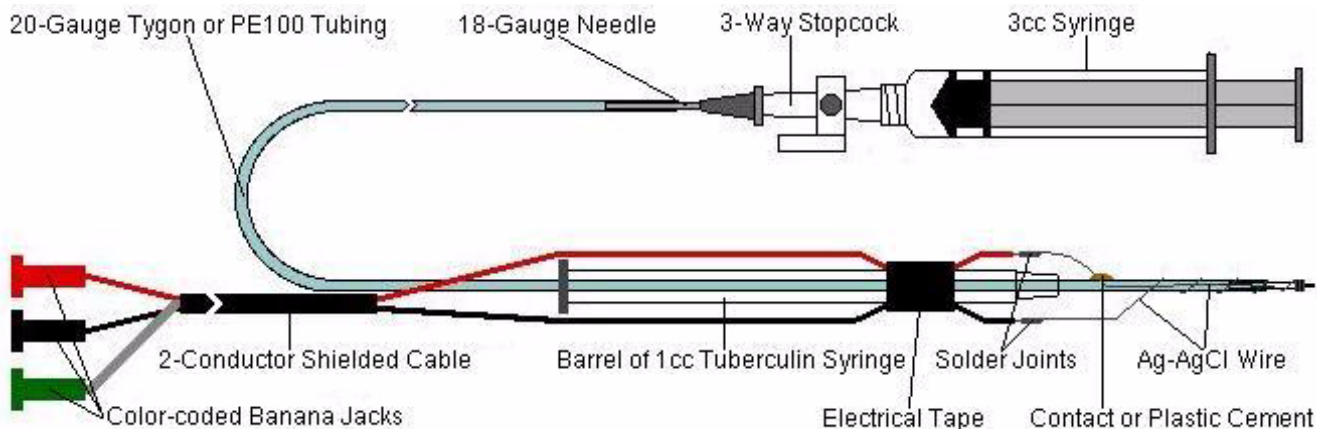


Figure NB-7-9: Typical suction electrode assembly.

- 8 Heat the tip of the dissecting pin in the flame of the alcohol burner. Use the heated tip to poke a small hole in the side of the plastic tubing that is about 3 inches from the end of the tubing and angled toward the end at 45 degrees.
- 9 Remove the plunger from the 1cc tuberculin syringe. Find the end of the plastic tubing without the hole. Push this end of the tubing through the hole in the front end of the 1cc syringe barrel until the tube exits the rear of the syringe barrel. Push and pull the tubing through the syringe barrel until the small angled hole in the side of the tube is about half of an inch from the front of the syringe barrel.
- 10 Find the silver electrode that is designated as the recording electrode by being color-coded to the red conductor wire or connector. Push the tip of this electrode wire through the hole in the side of the plastic tubing. Keep the solder joint of the electrode to the conductor wire about a quarter of an inch away from the hole in the tubing.
- 11 Mix up the contact or plastic cement. Use the popsicle stick to place a drop of cement over the hole in the tubing to create an air-tight seal around the silver electrode wire going through the hole. Make sure the cement does not drip into the lumen of the tubing and block it. Contact and plastic cement usually dries to the touch in 10 to 15 minutes.
- 12 Once the hole is sealed, cut the end of the silver recording electrode so that a quarter of an inch of the wire is sticking out of the end of the tubing.
- 13 Use the flat file and emery cloth to remove the point and smooth the tip of the 18-gauge needle. Once smoothed, push the tip of the needle into the other end of the plastic tubing.

- 14 Attach the 3-way stopcock to the 18 gauge needle, and the 3cc syringe to the 3-way stopcock.
- 15 When the nerve has been isolated, determine the diameter of the tip of the glass micropipette needed to fit the nerve. Snap the tip of the micropipette at the correct diameter, and fire-polish the tip to remove and jagged edges.
- 16 Place the glass micropipette over the wire sticking out the end of the plastic tubing. Push the micropipette into the plastic tubing to make a tight seal. The silver wire should be sticking a short distance into the glass micropipette.
- 17 The silver wire that is the indifferent or reference electrode is wrapped around the outside of glass micropipette and part of the plastic tubing supporting the micropipette. The tip of the indifferent electrode should be as close as possible to the tip of the glass micropipette, but, the positive and negative electrodes and their connectors must not touch each other!

### Chloriding Silver Wire

- 1 Obtain the following items to chloride your own silver wire: a 9V transistor battery; a 9V transistor battery connector with color coded lead wires; two small alligator clips; a 200 ml beaker; 175 ml of 3M KCl; 1 roll of silver wire (0.005" dia); a #2 pencil; dental wax or clay; forceps
- 2 Attach an alligator clip to the each lead of the 9V transistor battery connector.
- 3 Pour the 3MKCl solution into the beaker.
- 4 Wrap a length of the silver wire around the pencil to form a coil of ten turns. At one end of the coil, there should be a straight segment about 1.5" long. Make two coils for each chloriding session.
- 5 Put two 1" long beads of dental wax or clay on opposite sides of the rim of the beaker.
- 6 Attach a coil of silver wire to each lead of the battery connector by clamping the straight segment of the wire in the jaws of the alligator clip.
- 7 Position each alligator clip in the wax or clay on opposite sides of the rim of the beaker, so that the two wire coils are in solution (Figure NB-7-10 on page NB-7-10).

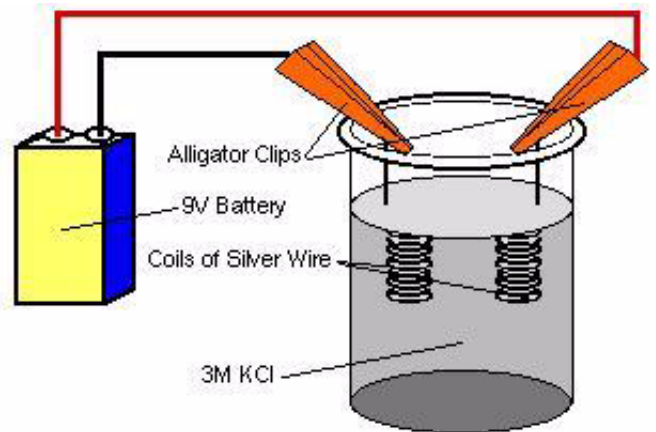


Figure NB-7-10: Setup used to chloride silver wire.

**Warning: It is important that one wire coil does not touch the other, and that the alligator clips or the lead wires of the battery connector are not in solution!**

- 8 Attach the 9V transistor battery to the battery connector. The solution near the coils will bubble and the coils will change color during the chloriding procedure. Chloride the coils for 8 minutes.
- 9 Disconnect the 9V battery from the connector. Reverse the chloriding process by putting each coil of silver wire on the other alligator clip. Use the forceps to hold a coil as it is removed from an alligator clip and moved to the other.
- 10 Re-position the alligator clips on the wax and the wire coils in the solution. Attach the 9V battery to the connector and chloride the wire coils in this polarity for another 8 minutes.
- 11 At the end of the second 8-minute period of chloriding. Put the wire coils back on the alligator clips to which they were initially attached and chloride the coils in this configuration for 5 minutes.
- 12 Finally, reverse the chloriding of the coils, as performed in Steps 9 and 10, for 5 minutes. At the end of the 5 minutes, disconnect the battery, remove the wire coils from the clips, and rinse the coils with deionized water.
- 13 The coils of chlorided silver wire are now ready to be used as electrodes.