

# Experiment 13: Crustacean Stretch Receptor

## Overview

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 ( $MRO_1$ ); and, the other type is a quick adapting, *phasic*, receptor ( $MRO_2$ ).

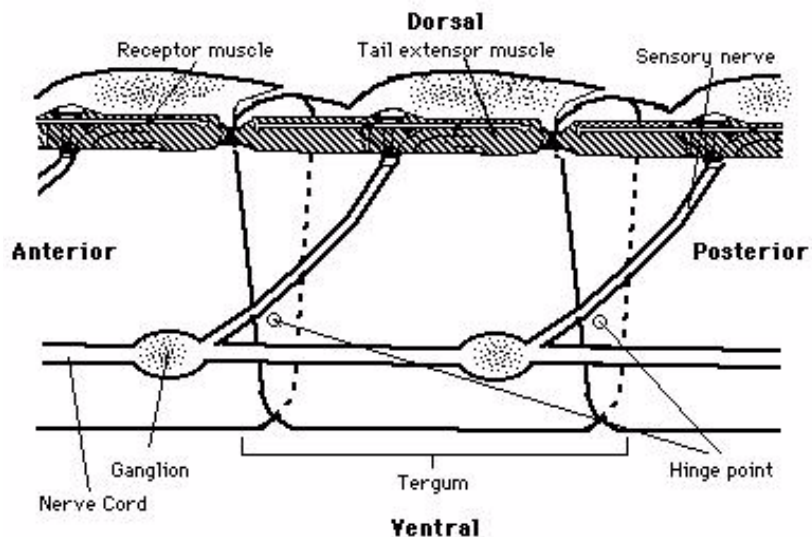


Figure 3-1: Cut-away diagram (side view) of a segment of crayfish tail.

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 3-1 on page 2). 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.

## Adaptation

Each receptor muscle ( $RM_1$  or  $RM_2$ ) is embedded with the dendrites of a its own sensory neuron (Figure 3-2 on page 3). 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 3-1 on page 2). 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.

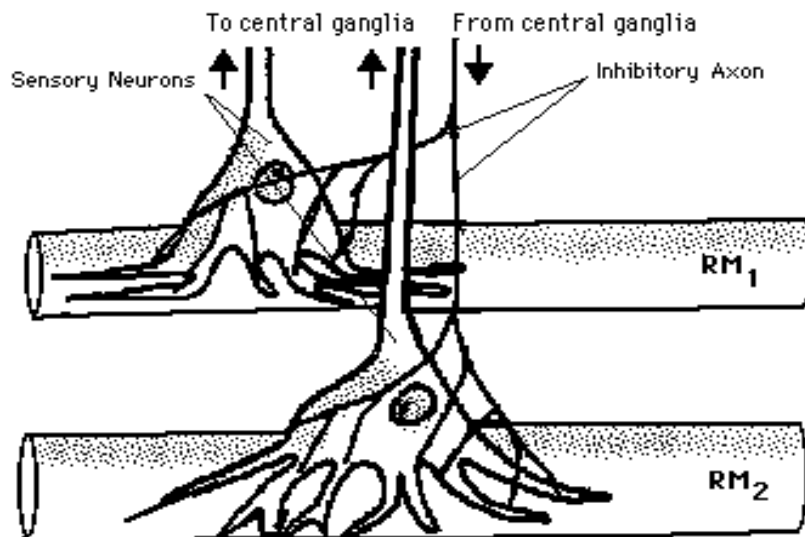


Figure 3-2: RM sensory and inhibitory innervation. Motor innervation is omitted.

## Experiment

The exercises in this experiment will demonstrate:

- the types of muscle receptor organs (MRO): MRO<sub>1</sub>, which is *slow-adapting* and discharges for long periods of time with a moderate, constant stretch; MRO<sub>2</sub>, 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<sub>1</sub> is directly proportional to the degree of tail flexion or stretching of RM<sub>1</sub>.

## Equipment Required

PC Computer  
iWorx unit, and USB or serial cable  
100X DIN8 gain input cable  
Suction electrode assembly and glass tips  
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)  
Assorted cables and alligator clips for grounding equipment  
Suture thread  
Crayfish and crayfish saline  
Dissection tools

## Equipment Setup

- 1 Connect the iWorx unit to the computer.
- 2 Attach a 100X DIN8 gain input cable to the DIN input of Channel 3 on the iWorx unit (114, 204, 214). With this type of input cable, the amplification of the input amplifier of the recording channel is automatically programmed to 100X. This cable should have connectors that are compatible with the connectors on the suction electrode assembly. The most common type of connectors used are banana jacks and plugs.
- 3 Attach the three color-coded connectors of the suction electrode assembly to the matching connectors of the 100X gain cable so that:
  - the recording electrode, that is inside the lumen of the suction tubing, is connected to the positive input of the recording channel.
  - the indifferent (reference) electrode, that is wrapped around the end of the glass micropipette, is connected to negative input.
  - the ground electrode, that is in the bath chamber, is connected to the ground input.

## Start the Software

- 1 Click the Windows Start menu, move the cursor to **Programs** and then to the **iWorx** folder and select **LabScribe**; or click on the LabScribe icon on the Desktop
- 2 When the program opens, select **Load Group** from the **Settings** menu.
- 3 When the dialog box appears, select **AddedLabs.iws**. Click **Load**.
- 4 Click on the **Settings** menu again and select the **CrayfishStretchReceptor** settings file.
- 5 After a short time, LabScribe will appear on the computer screen as configured by the **CrayfishStretchReceptor** settings.
- 6 With these settings, Channel 3 (**MRO AP**) on an iWorx 114, 204, or 214 is programmed for recording action potentials from the MRO's. Channel 2 (**MRO Freq**) is programmed, using the **Frequency** function from the **Right-Click** menu, to display the frequency of firing of the MRO's.

## 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 3-3 on page 5).

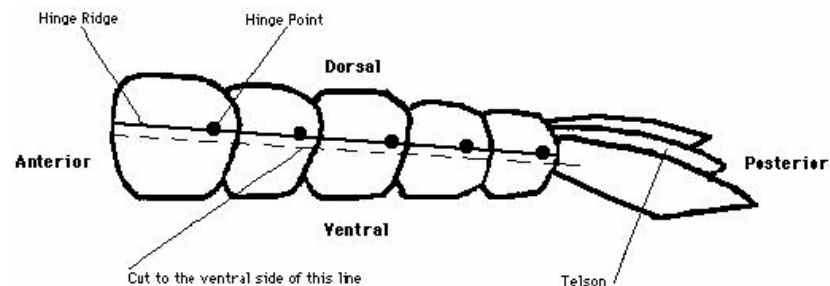


Figure 3-3: Diagram to show the dissection of the crayfish tail.

- 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 3-4 on page 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.

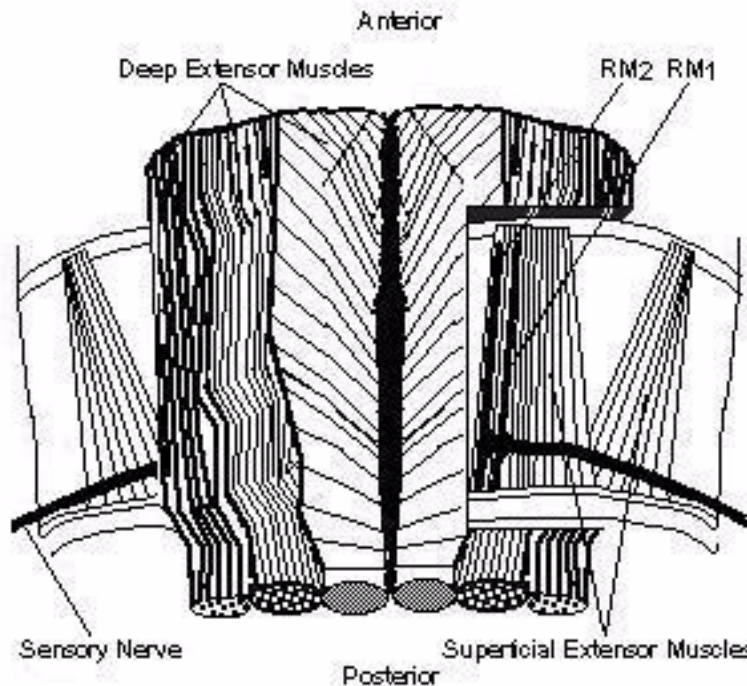


Figure 3-4: Ventral aspect of the second abdominal segment of crayfish. Dorsal musculature removed on the right side and intact on the left side.

- 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 (Figure 3-5 on page 8). 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 micro-electrode. 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.

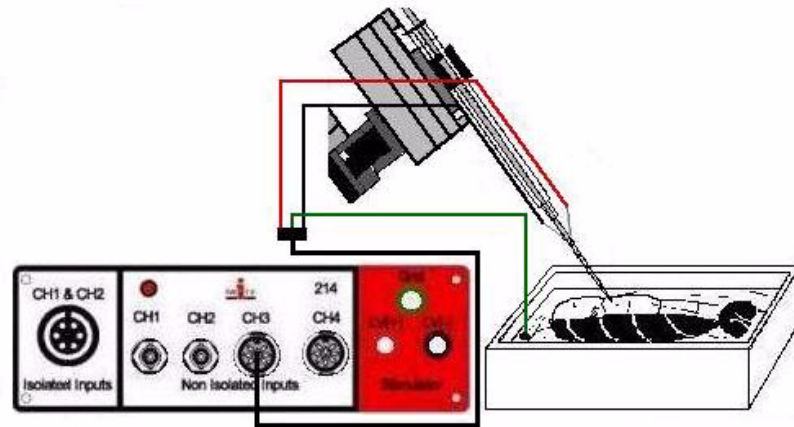


Figure 3-5: Recording electrode positioned to record from sensory nerve in the second abdominal segment.

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

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

- 1 Click **Start** on the LabScribe **Main** window to begin recording. 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. Click the **AutoScale** button on the **MRO AP** (CH3) and **MRO Freq** (CH 2) channels to adjust the size of the traces displayed on the **Main** window. 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 3-6 on page 9.
- 2 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.
- 3 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.
- 4 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.
- 5 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 (e.g. the **My Documents** folder). Click the **Save** button to save the file (as an \*.iwd file).

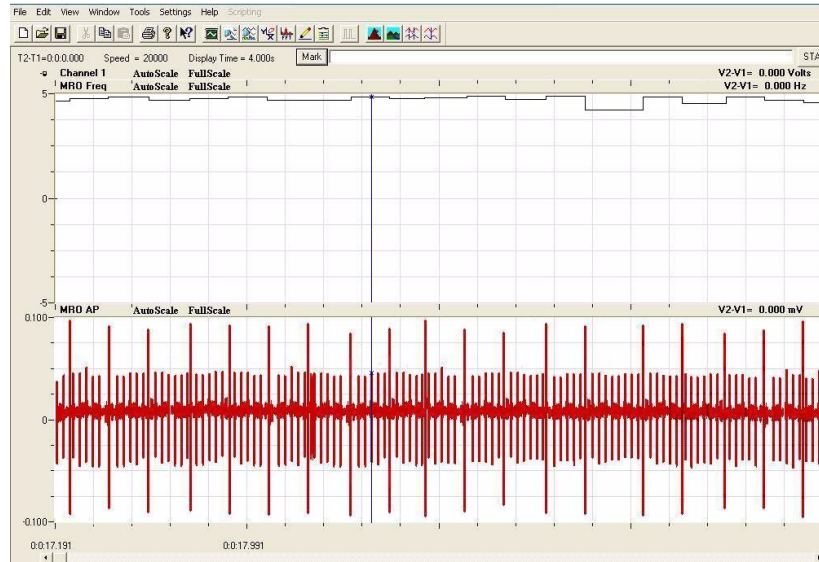


Figure 3-6: Frequency of action potentials from MRO<sub>2</sub> displayed on upper channel. The action potentials from MRO<sub>1</sub> (with their lower amplitude and higher frequency) and MRO<sub>2</sub> (with their higher amplitude and lower frequency) are displayed on the lower channel.

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

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

- 1 Click **Start**. 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. Click the **AutoScale** button on the **MRO AP** (CH3) and **MRO Freq** (CH 2) channels to adjust the size of the traces displayed on the **Main** window.
- 2 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 3-6 on page 9.
- 3 Click **Stop** to halt the recording. Relax the tail from its flexed state between recordings to conserve neural activity.
- 4 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 **Start**. 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. Click the **AutoScale** button on the **MRO AP** (CH3) and **MRO Freq** (CH 2) channels to adjust the size of the traces displayed on the **Main** window.
- 2 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.
- 3 Click **Stop** to halt the recording. Relax the tail from its flexed state between recordings to conserve neural activity.
- 4 Select **Save** in the **File** menu to add this data to the current data file.
- 5 Repeat Steps 1 through 4 for a position where the MRO<sub>1</sub> is firing less rapidly.
- 6 Repeat Steps 1 through 4 for a position where the MRO<sub>1</sub> is firing slowly.

#### Data Analysis

- 1 Scroll to the beginning of the first segment of the constant stretch recording on the LabScribe **Main** window. **AutoScale** both the **MRO Freq** and **MRO AP** channels (CH2 and CH3). Click on the **1-cursor** icon in the LabScribe toolbar.
- 2 Place the cursor on one of the plateaus of the **MRO Freq** trace near the beginning of the recording. The **Value** in the upper right margin of the **MRO Freq** channel display is the frequency of firing of MRO<sub>1</sub>. Record this value in the **Journal**.
- 3 Move the single cursor to the plateau at the first five-second mark. Determine the frequency of firing at that time. Enter that value in the **Journal**.
- 4 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.
- 5 Repeat Steps 1 through 4 for the other two constant stretch segments 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  $MRO_1$  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  $MRO_1$  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. Click **Start**. Relax the crayfish tail to a position where the  $MRO_1$  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 AP (CH3)** channel to adjust the size of the trace.
- 2 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. Record the firing of the  $MRO_1$  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  $MRO_1$  was scarcely firing.
- 3 If you are ready to flex the crayfish tail to a greater degree, continue recording. 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  $MRO_1$  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 2.
- 4 Repeat Step 3 a few more times with flexions equal to 3, 4, and 5 scale units, or fractions of a turn.
- 5 Click **Stop** to halt the recording. Relax the tail from its flexed state to conserve neural activity.
- 6 Select **Save** in the **File** menu to add this data to the previously saved data file.

#### Data Analysis

As performed in Exercise 3, measure the frequency of firing of  $MRO_1$  at the marks (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?

## Literature

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Kuffler, S.W.: Mechanisms of activation and motor control of stretch receptors in lobster and crayfish. *J. Neurophysiol.*, 17: 558-574, 1954.

Wiersma, C.A.G., Furshpan, E. and Florey, E.: Physiological and pharmacological observations on muscle receptor organs of the crayfish, *Cambarus clarkii* (Girard.). *J. Exp. Biol.*, 30: 136-150, 1953.

## Appendices

### *Extracellular Suction Electrode*

- 1 Obtain the following items to make your own suction electrode (Figure 3-7 on page 13): 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.

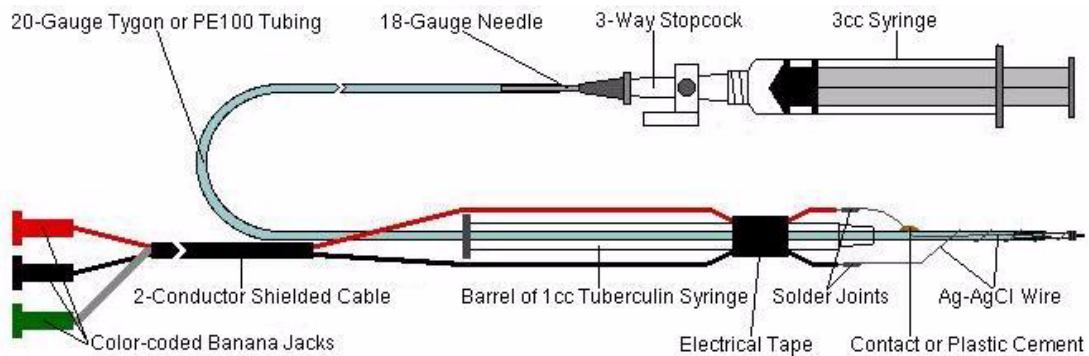


Figure 3-7: Typical suction electrode assembly.

- 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.
- 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 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 3-8 on page 15). **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!**

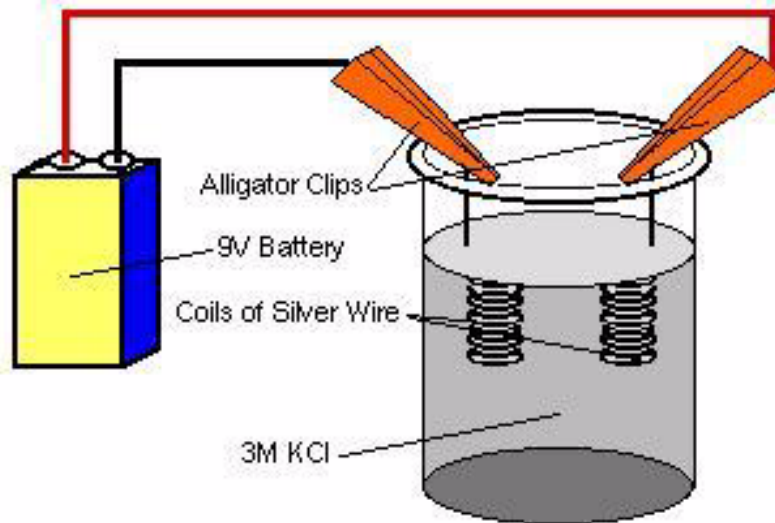


Figure 3-8: Setup used to chloride silver wire.

- 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.

