

Experiment 3: Nerve Conduction

Overview

The interior of a cell is negatively charged with respect to the outside, and the magnitude of the potential difference is usually between 50 and 80 mV. Some cells, like nerves and muscles, can transiently reverse their membrane potentials. This event is called an action potential and takes place in milliseconds. During this process, the membrane potential goes from negative to positive and back to negative, again. In the resting cell, the permeability of the membrane to potassium (PK) is greater than its permeability to sodium (PNa). Stimulation, like synaptic activity coming from other nerve cells, can depolarize (make less negative) the cell membrane. Sodium channels in the cell membrane are sensitive to membrane depolarization and they respond by opening, which increases membrane's permeability to sodium. If the depolarization reaches or exceeds a certain level (threshold), an action potential is produced. Action potentials develop because of a regenerative, positive feedback cycle. As the cell's permeability to sodium increases, sodium conductance increases, and increased sodium conductance leads to greater depolarization of the membrane. As depolarization increases, sodium permeability increases again, as more voltage-sensitive channels open. With more channels open, sodium conductance and membrane depolarization increase until the membrane potential reaches the equilibrium potential for sodium.

But, before the equilibrium potential for sodium is reached, two other events occur: the voltage-sensitive sodium channels close soon after they open, and the voltage-sensitive potassium channels open. With its channels open, potassium ions leave the cell and cause the membrane to repolarize (hyperpolarize) towards its resting level. This process of membrane hyperpolarization closes the voltage-sensitive potassium channels and reprimed the sodium channels so that they are ready to open once more.

Propagation of the action potential from the site of initiation to other locations along the nerve cell is caused by the positive charges in the cell leaking to an adjacent (unstimulated) region and depolarizing that region enough to create an action potential there. In this way, the signal moves from one region of the axon to adjacent one, and ultimately to the end of the axon. Some axons are myelinated; the axon is covered with a series of Schwann cells, a type of glial cell which electrically insulates the axon. The spaces between adjacent Schwann cells are called the nodes of Ranvier, and they are the only regions along the axon where the membrane is exposed to the extra cellular fluid. The myelin insulation prevents the currents associated with action potentials from leaking out of the membrane until they reach a node. So, action potentials take place only at the nodes in myelinated cells.

In this laboratory you will record action potentials from the ventral nerve cord of the crayfish (*Procambarus clarkii*). Each nerve contains hundreds of axons with different diameters, thresholds, and degrees of myelination. The large, myelinated axons with the fastest conduction velocities are known as Type A fibers. Type B fibers are also myelinated, but have smaller diameters and slower conduction velocities. Type C fibers are very small, unmyelinated axons. When a large stimulus is delivered to the nerve, many axons respond and the recorded potential is the summation of all the axons firing is recorded. This potential is known as the compound action potential (CAP).

You will examine certain principles associated with nerve conduction:

- The compound action potential—observing one or more populations of different fiber types, each type with similar conduction velocities.
- Stimulus-response/axon recruitment—how the response changes with increased stimulus voltage.
- The conduction velocity—you will measure how fast action potentials are conducted down the axons.
- The effects of temperature—how cooling the nerve changes the conduction velocity.
- Bidirectionality—whether crayfish ventral nerve cord conduct in both directions.

Equipment Required

PC computer

iWorx/214 and USB cable

Nerve Chamber

AAMI cable and nerve chamber leads (red and black)

Glass hooks

Stimulator cable

Grounding adapter or cable

Crayfish (*Procambarus clarkii*)

Van Harreveld's (or crayfish) saline (in g/L, NaCl 12; KCl, 0.4; CaCl₂·2H₂O, 1.2, MgCl₂·6H₂O, 0.5; NaHCO₃, 0.17) at two temperatures:

- 100ml per station chilled on ice
- 400 ml per station at room temperature

Equipment Setup

1 Connect the iWorx/214 unit to the computer (described in Chapter 1).

2 Attach the AAMI connector on the end of the gray patient cable to the isolated Channel 1 and 2 inputs on the iWorx/214 unit.

3 Attach two color-coded nerve chamber leads to the Channel 1 inputs on the lead pedestal. Connect the alligator clips or sockets on the other end of the leads to the electrodes on the nerve bath, so that:

- the red "+1" lead is attached to the distal (or positive) recording electrode.

This electrode is on the thread holding the nerve in place.

- the black "-1" lead is attached to the proximal (or negative) recording electrode. This electrode is on the nerve between the ground electrode and the positive recording electrode.

Note: *If the compound action potential (CAP) is recorded while the positive recording electrode is on the nerve, a biphasic wave is produced. As the propagating CAP passes the negative recording electrode, it is displayed as an upward deflection on the recording. This is followed by a downward deflection created as the CAP passes the positive recording electrode. If the CAP is recorded while the positive recording electrode is on the thread holding the nerve, a monophasic wave is produced.*

4 Plug the BNC-double banana adapter into the positive (red) and negative (black) sockets of the iWorx 214 stimulator. To insure the correct polarity of stimulation, check

the side of the double banana adapter for a tab, often embossed with the letters GND. This is the side of adapter that goes into the negative (black) socket of the stimulator.

5 Attach the BNC connector of the stimulator cable to the adapter already on the stimulator.

6 Attach the sockets or alligator clips of the stimulator cable to the closely - spaced electrodes at one end of the nerve bath chamber (Figure 3-6). The one closest to the end of the chamber is the positive stimulating electrode.

7 Attach the socket or alligator clip of the grounding lead/cable to the electrode that is closest to the negative stimulating electrode. The ground should always separate the stimulating electrodes from the recording electrodes. The other end of the grounding cable should be connected to the ground jack on the front or back of the iWorx 214 unit.

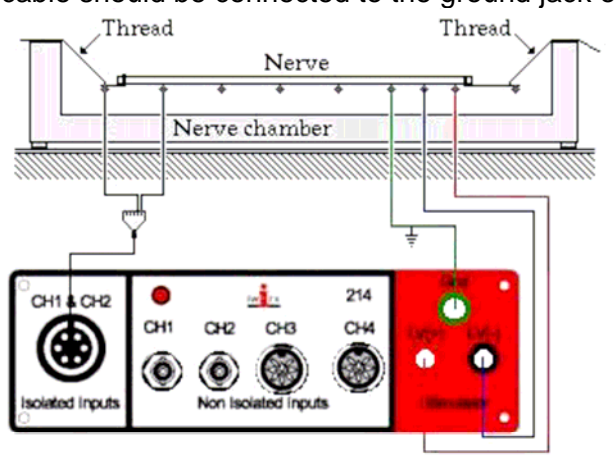


Figure 3-6: The equipment setup to record from the ventral nerve cord.

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 **ahk214.iws** and then click **Load**.

4 Click on the **Settings** menu again and select the **SciaticNerve-214** settings file.

5 After a short time, LabScribe will appear on the computer screen as configured by the **SciaticNerve-214** settings in Scope mode.

6 The **SciaticNerve-214** settings file adjusts:

- the stimulus amplitude to **0.25V**, with adjustable increments of **0.05V**.
- the stimulus duration to **0.1ms**, with adjustable increments of **0.1ms**.
- the sampling rate to **20,000** per second.
- the sweeps to be **Repetitive** and timed for a duration of **30 ms**.

These settings can be changed by selecting **Preferences** from the **Edit** menu.

The Dissection

1 Place a crayfish in the freezer for 5 minutes. Remove the crayfish and quickly cut off its head.

2 Remove the tail (abdomen) from the thorax by cutting around the joint (seam) connecting those two parts.

3 Pin the tail down with the ventral surface up in the preparation dish. Push one pin through the shell in the first abdominal segment and a second pin through the telson. Cover with saline. Remove the swimmerets.

4 Hold the tail and make a longitudinal cut along each side of the abdomen (below the hinge ridge) to loosen the ventral shell from the dorsal shell. Leave the tail fins attached to the dorsal exoskeleton.

5 Begin at the anterior end of the abdomen and separate the ventral and dorsal halves of the shell from each other. Hold and lift the anterior border of the ventral shell and scratch with pair of scissors (or the tip of a scalper) as close as possible to the ventral shell. **Use caution and do not cut any tissue if necessary.** Try to scratch any muscle tissue attached to the ventral shell exclusively.

6 Discard the ventral portion of the shell after reaching the telson (Figure 3-2).

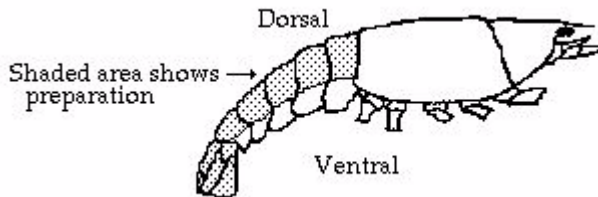


Figure 3-2: Diagram to show the dissection of the crayfish tail.

7 You should notice the ventral nerve cord (similar to the spinal cord in humans but with fewer nerves!) running along the middle of the tail. Carefully hold and lift the anterior portion of the nerve. To separate the nerve from surrounding connective and muscle tissue, scratch with pair of scissors (or the tip of a scalper) as far as possible from the nerve. **Use caution and do not cut any tissue if necessary.**

8 Once the nerve has been completely free, place a piece of thread under the anterior end of the nerve (close to the thorax). Ligate the nerve; leave a small piece of thread attached to the nerve. This procedure is needed to align the nerve in the nerve chamber.

10 Fill the nerve chamber with saline. Lift the nerve out of the tail and place it on top of the nerve chamber.

Important Notes

1 The proximal end of the nerve (with the piece of thread) should be over the stimulating electrodes, and the distal end (close to the telson) should be over the recording electrodes.

2 The thread can be secured on the edge of the bath with tacky wax or clay to prevent the nerve from moving when the bath is drained.

IMPORTANT: Remember to keep the chamber filled with saline during experiments to prevent nerve drying out.

Remember to drain the chamber before starting an experiment. If the fluid touches the electrodes they will short-circuit.

Exercise 1: The Compound Action Potential

Aim: To apply a brief stimulus at the proximal end of the nerve and record a compound action potential from the distal end.

Procedure

1 Check values listed the stimulator panel, which is below the LabScribe toolbar (Figure 3-8 on page 41). The stimulus amplitude should be **0.25 V** and the pulse width should be **0.1ms**.

2 Remove enough crayfish saline from the nerve chamber to insure that the nerve is no longer contacting the solution. If necessary, carefully blot any large drops of saline from the recording electrodes and the nerve with the corner of a wipe.

3 Click **Start** to stimulate and record from the nerve. LabScribe is set to use **Scope** mode and to display **Repetitive** sweeps. This means that the nerve will be stimulated again, after the preceding sweep is completed, A new recording of the nerve response replaces the previous sweep on the **Main** window. **Scope** will continue to stimulate the nerve and display new compound action potentials until the **Stop** button is clicked. Click the **Stop** button to preserve the latest sweep displayed on the window.

4 A mark line appears on the left side of the screen to indicate the point in time when the stimulus was delivered to the nerve. There may be a stimulus artifact at the mark. The compound action potential usually reaches a peak a few milliseconds after the artifact (Figure 3-7).

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 **iWorx** or class folder). Click the **Save** button to save the file (as an ***.iwd** file).

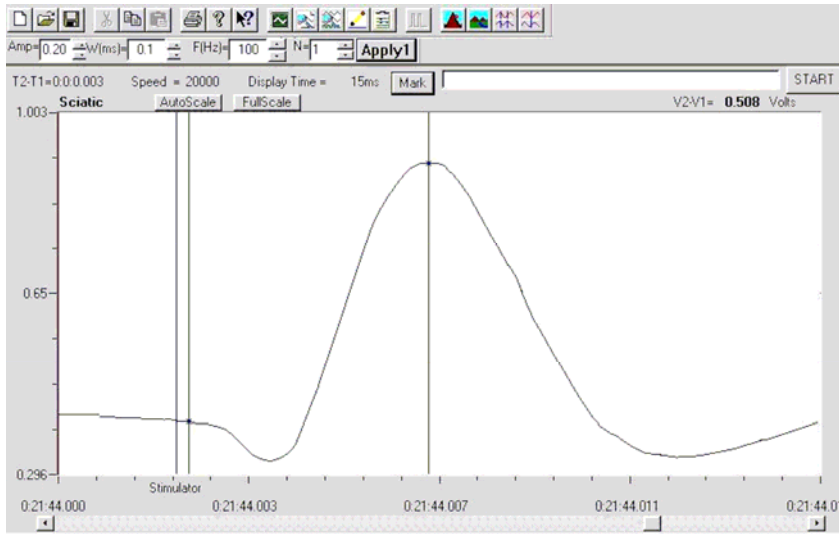


Figure 3-7: The compound action potential.

Exercise 2: Stimulus and Response

Aim: To quantify the relationship between amplitude of the stimulus and amplitude of the compound action potential.

Procedure

- 1 Use the arrow buttons in the stimulator panel to change the stimulus amplitude to **0.00 V** (zero). Click the **Apply1** button on the right of the stimulator panel to effect the change in the stimulus.
- 2 If necessary, drain the crayfish saline from the nerve chamber, and carefully blot any large drops of saline from the recording electrodes and the nerve with the corner of a wipe.
- 3 Click **Start** to stimulate the nerve with **0.00V**. A flat line should be observed. Click **Stop** to display the last sweep on the **Main** window.
- 4 Click the **1-Cursor** icon in the LabScribe toolbar (Figure 3-8), type "0.00V" on the comment line to the right of the **Mark** button. Press the **Enter** key on the keyboard to attach the comment to the sweep.

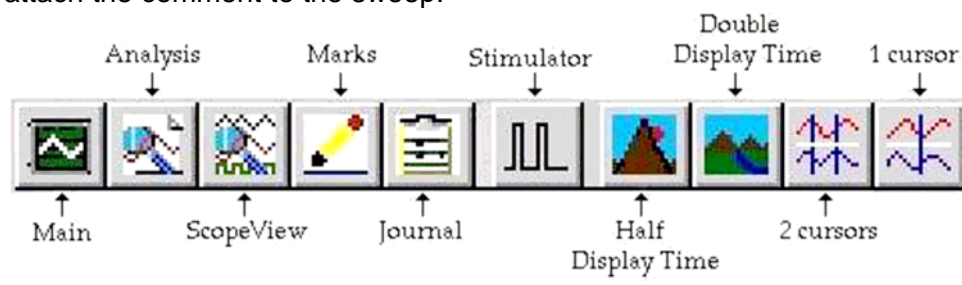


Figure 3-8: The LabScribe toolbar

- 5 Use the arrow buttons in the stimulator panel to change the stimulus amplitude to **0.05 Volts**. Click the **Apply1** button on the right side of the stimulator panel to effect the

change in the stimulus. Click **Start**. After the trace appears, click **Stop**. On the recording window, there may be a small peak at the stimulus mark; this is the stimulus artifact. Type "0.05V" on the comment line, and press the **Enter** key to attach the comment to the sweep.

6 Continue to increase the stimulus amplitude in **0.05V** increments until a small compound action potential is observed. Remember: click the **Apply1** button each time you want to effect a change in the stimulus; and, click the **Stop** button to record the sweep. This small compound action potential is the summation of action potentials from axons in the nerve with the lowest thresholds. Record comments in the same manner as before.

7 Continue to increase the stimulus amplitude in **0.05V** increments until the maximum compound action potential is observed. Change voltages, record sweeps, and record comments in the same manner as before. C fibers have thresholds and latencies up to 30 times those of A fibers, so higher stimulus amplitudes and longer display times are required to see C fibers.

8 Select **Save** in the **File** menu.

9 Fill the nerve chamber with fresh crayfish saline to prevent dessication of the nerve.

Data Analysis

1 Click the **ScopeView** icon in the LabScribe toolbar (Figure 3-8 on page 41) to view all the recorded sweeps. A single sweep or group of sweeps can also be selected for display from the **Display Sweeps** list. Sweeps can also be superimposed on each other, for comparison, by checking the **Stacked** box

2 Select **Title**, **V2-V1**, and **T2-T1** from the **Table Functions** list. Start making measurements on the last recorded sweep, the one created by the highest stimulus amplitude.

3 Click the **2-Cursor** icon in the LabScribe toolbar. Drag the cursors left and right to place one on the baseline preceding the compound action potential (CAP) and the second on its peak (Figure 3-7 on page 41). The value for **V2-V1** in the table at the top of the **ScopeView** window is the absolute amplitude of the CAP.

4 Data can be entered into the **Journal** by either typing the titles and values directly or by using the right-click menu. Place the cursors to take measurements; then, select **Add Title to Journal** or **Add Data to Journal** from the right click menu to add the measurements to the **Journal**.

5 Record the stimulus amplitude used to generate the nerve response along with the other data for the sweep in the **Journal**.

6 Select the preceding sweeps from the **Display Sweeps**, and measure the absolute amplitude of each sweep. Record its value and the value of the stimulus amplitude used to generate the CAP in the **Journal**.

7 Graph or tabulate the absolute amplitude of the CAPs as a function of the stimulus amplitude.

Questions

- 1 Does the action potential in a single axon increase in amplitude when the stimulus amplitude is increased?
- 2 Does the amplitude of the compound action potential increase because more fibers are firing, or the amplitudes of the action potentials from single fibers are increasing, or a combination of both?
- 3 How many fiber types did you observe in your monophasic recording of compound action potentials?
- 4 The first peak of the compound action potential is composed of the responses of the fibers. How does the threshold and diameter of these fibers compare to other fibers?

Exercise 3: Conduction Velocity

Aim: To measure the velocity of action potential conduction

Procedure

- 1 Use the arrow buttons in the stimulator panel to change the stimulus amplitude to **0.25 V** or a voltage that produces a CAP with a maximum amplitude. Click the **Apply1** button on the right of the stimulator panel to effect the change in the stimulus.
- 2 If necessary, drain the crayfish saline from the nerve chamber, and carefully blot any large drops of saline from the recording electrodes and the nerve with the corner of a wipe.
- 3 Click **Start** to stimulate the nerve. Click **Stop** to display the sweep on the **Main** window.
- 4 Click the **1-Cursor** icon in the LabScribe toolbar (Figure 3-8), Type "Long path" on the comment line to the right of the **Mark** button. Press the **Enter** key on the keyboard to attach the comment to the sweep.
- 5 Move the lead cable for the negative recording electrode one or two electrodes closer to the ground electrode. Measure the distance (in mm) from the old position of the negative recording electrode to its new position.
- 6 Click **Start** to stimulate the nerve. Click **Stop** to display the sweep on the **Main** window.
- 7 Click the **1-Cursor** icon in the LabScribe toolbar (Figure 3-8), Type "Short path" on the comment line to the right of the **Mark** button. Press the **Enter** key on the keyboard to attach the comment to the sweep.

8 Fill the nerve chamber with chilled crayfish saline.

9 Select **Save** in the **File** menu.

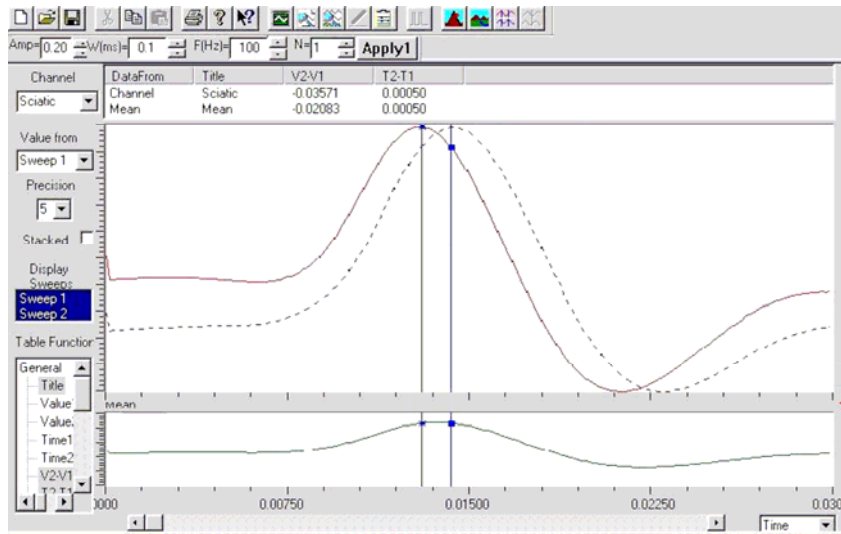


Figure 3-9: Conduction velocity—two stacked traces.

Data Analysis

1 Click the **ScopeView** icon in the LabScribe toolbar (Figure 3-8 on page 41) to view the recorded sweeps. Select the last two sweeps recorded from the **Display Sweeps** list. Superimpose these sweeps on each other by checking the **Stacked** box.

2 Select **Title** and **T2-T1** from the **Table Functions** list.

3 Click the **2-Cursor** icon in the LabScribe toolbar. Drag the cursors to the peaks of the two compound action potentials (Figure 3-9 on page 41). The value for **T2-T1** in the table at the top of the **ScopeView** window is the time it took the action potential to travel the distance between the two positions of the negative recording electrodes.

4 Data can be entered into the **Journal** by either typing the titles and values directly or by using the right-click menu. Place the cursors to take measurements; then, select **Add Title to Journal** or **Add Data to Journal** from the right click menu to add the measurements to the **Journal**.

5 Read off the **T2-T1** value (0.0005s or 0.50ms in Figure 3-9 on page 44).

6 Calculate the conduction velocity (in m/s). For example: 10mm distance between electrodes/ 0.5 ms = 20mm/ms = 20m/s

Exercise 4: Conduction Velocity and Temperature

Aim: To examine the effects of cooling on the velocity of action potential conduction

Procedure

1 Measure the conduction velocity of the nerve after draining the chilled crayfish saline from the chamber, as done in the previous exercise.

Note: *This part of the experiment must be done quickly since the nerve will begin to warm as soon as the bath is drained.*

2 Fill the bath with room temperature crayfish saline after data is recorded for this exercise. Allow the nerve to warm as you determine the conduction velocity of a chilled nerve.

Questions

1 Does the conduction velocity change when the nerve is cooled?

2 What properties of the ion channels may change with temperature?

Exercise 5: Bidirectionality

Aim: To examine whether an action potential travels in the wrong direction and if so, at what velocity.

Procedure

1 Reverse the positions of the leads attached to the electrodes on the nerve bath. Put the stimulating electrodes on the distal end of the nerve where the recording electrodes used to be, and vice versa

2 If necessary, drain the crayfish saline from the nerve chamber, and carefully blot any large drops of saline from the recording electrodes and the nerve with the corner of a wipe.

3 Stimulate the nerve with the same amplitude used to record the last sweep

4 Measure the conduction velocity of the nerve after draining the chilled crayfish saline from the chamber, as done in the previous exercise.

Questions

1 Do you record an action potential from the proximal end of the nerve?

2 What is the conduction velocity for the nerve when stimulated in reverse direction? Is this similar to the value recorded when the nerve was stimulated from the proximal end to the distal end?

3 How can an axon conduct as action potentials in both directions? **Hint:** *Where are the cell bodies and synapses in this preparation?*

1. The crayfish nerve cord.

The crayfish abdominal nerve cord has been widely investigated because of its relative simplicity. The cord consists of six **ganglia** (AB1 to AB6 in the figure) joined by **connectives**. From each ganglion, three bilateral (left & right) pairs of nerves ("**roots**") innervate the muscles and sensory receptors in that abdominal segment. (The sixth ganglion, nearest the uropods and telson, is a fusion of two embryonic ganglia.) Each of the three roots has a different destination in its segment of the abdomen, as described below. A supplement on the [Anatomy of the Crayfish Nervous System](#) has figures of stained neurons and spectacular videos showing serial sections through a ganglion.

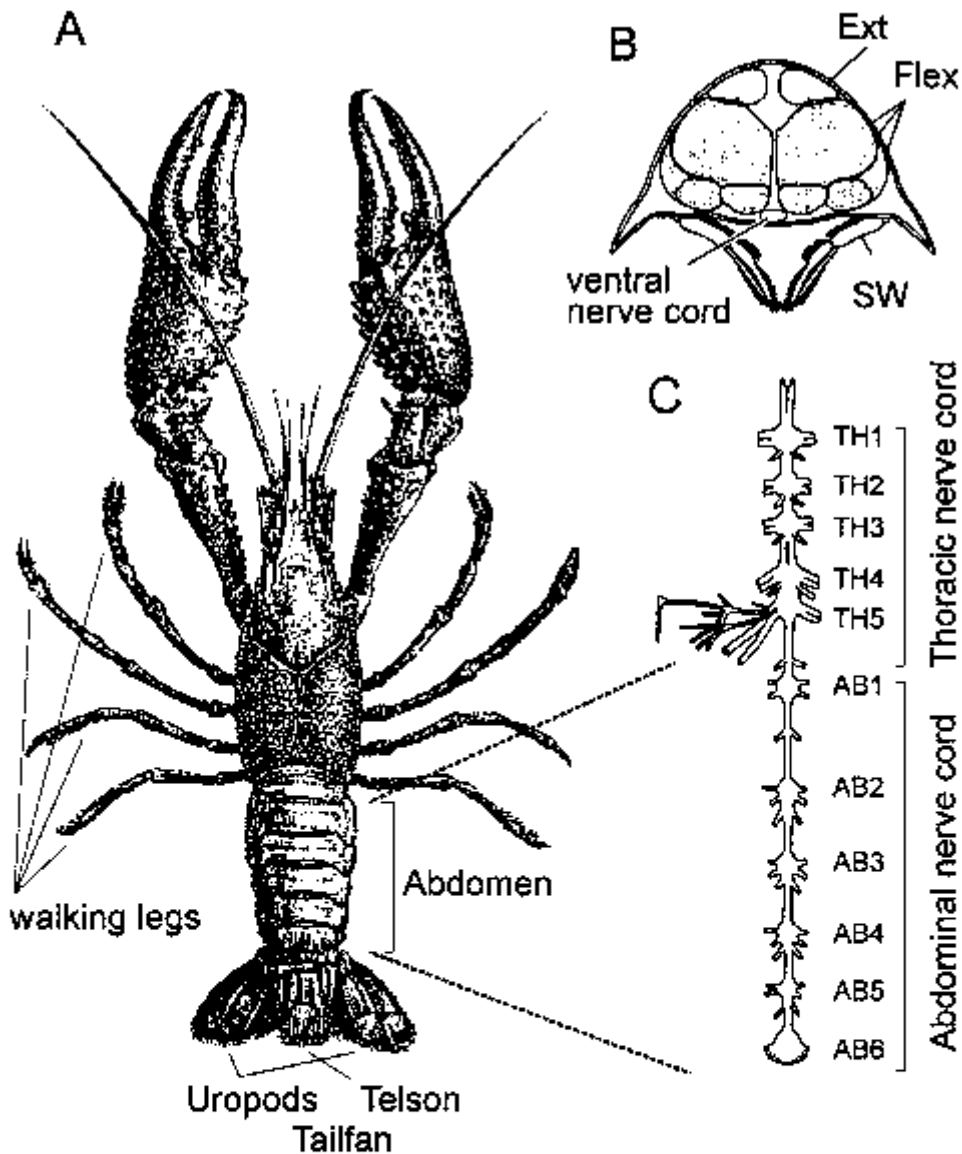
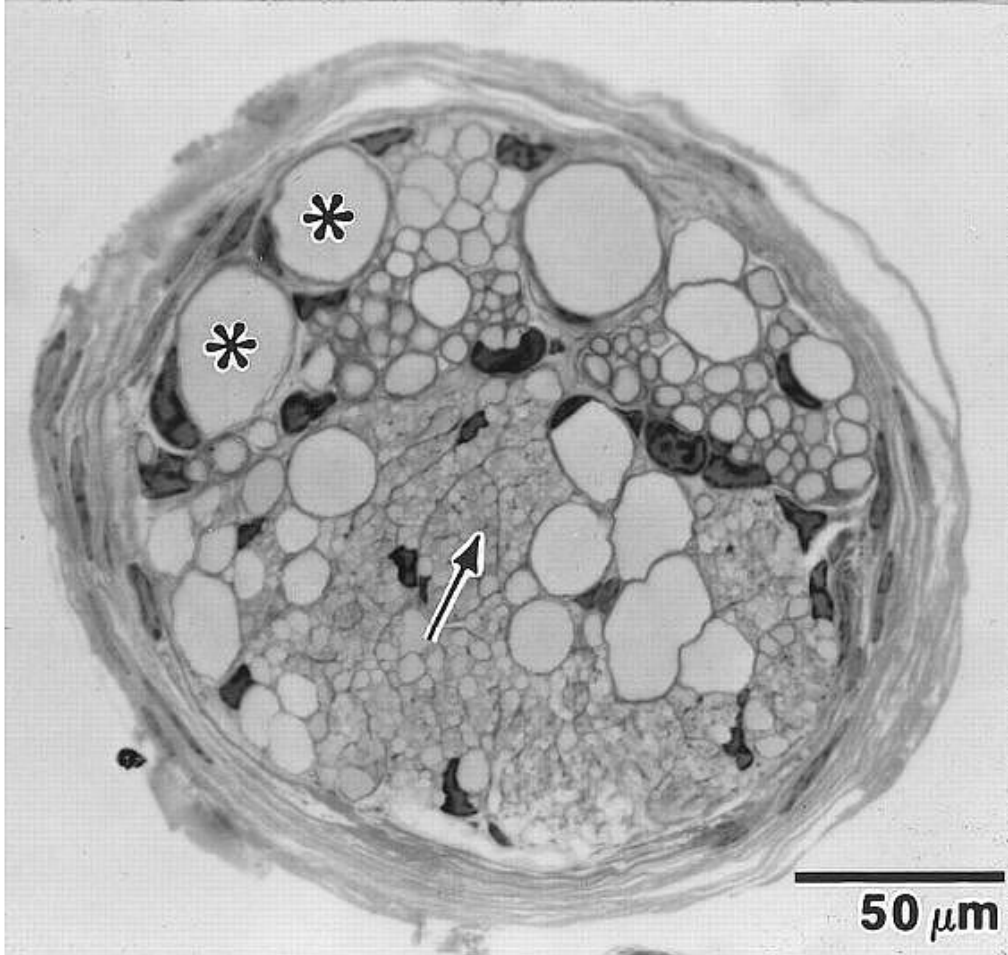


Fig. 1. Motor systems in crayfish. (A) Organization of the thoracic and abdominal motor systems. The legs (for walking), the swimmerets disposed by pair under each of the abdominal segments (for swimming), and the abdomen itself with its last segment differentiated in a telson and two uropods (for escape reaction) represent the three main locomotor and equilibrium systems of the crayfish. (B) Transversal view of the abdomen showing both its flexor (Flex) and extensor (Ext) musculature, as well as a pair of swimmerets (SW) and the ventral localization of the nerve cord. (C) Isolated ventral nerve cord composed of five thoracic (TH1 to TH5) and six abdominal (AB1 to AB6) ganglia. On TH5, the proximal innervation of the left fifth leg is also shown.



Cross section of a first root. Note the axons of many different sizes. The two large axons (*) are those of nonspiking stretch receptors. Smallest axons (arrow) are probably sensory afferents. From Sherff & Mulloney 1997, Fig 1B.