Lab #10: Sensory Physiology of Crayfish Ventral Nerve Cord

In this lab we will investigate the sensitivity of chemosensory, mechanosensory and thermosensory receptors whose signals are propagated through the crustacean ventral nerve cord.

Background

Many animals have hairs on the exterior of their bodies that serve as **mechanosensors** – mechanical sensors that allow them to detect their physical environment via forces induced by fluid flow, breezes, or physical contact. For example, the hairs on our bodies are mechanosensory in that we can "feel" the movement of the hairs even if the skin does not move. In addition, mechanosensory hairs are involved in processes such as hearing, equilibrium, motion control, and tactile response. Other sensory receptors include **chemosensors** – detecting the chemical composition of the environment, and **thermosensors** – detecting the temperature of the environment.

Crayfish have hairs (called **setae**) involved in mechanical, chemical, and thermal sensory systems. Mechanosensors are diverse in function and located in various regions of the body. The major touch organs are in the antennae. **Statocysts** (an organ for orientation and equilibrium or balance) lie in the base of the antennules. They are composed of 400-500 hairs distributed in 4 rows that project into the statolith (sand grains cemented together). When the animal shifts its orientation, fluid in the statocyst moves the hairs in a particular direction relative to the statolith. This differential movement stimulates some hairs more than others and gives the animal a sense of the direction of the body's movement. **Proprioreceptors** are sensory hairs receive information from within the body about the position of an animal's extremities and body parts. Located at the joints and within muscles, proprioreceptors are stimulated upon movement and provide information used to coordinate limb movement, posture, and equilibrium. **Smell and taste** are highly developed in crayfish: they "sniff" their environment using chemosensory hairs on their prominent antennae and antennules, which they continuously wave and flick through the water. These hairs are especially sensitive to dissolved amino acids. The chemosensory hairs on the legs give the crayfish the ability to "taste" food items while using their legs to probe the sediment. Chemosensory hairs are also located on the mouthparts, where the final decision is made whether to ingest a potential food item.

Each setae that is involved in sensory function is anchored in a sensory cell that forms a **junction with a neuron**. Signals are transduced through the cell to the neuron and any resulting action potentials travel up the axon to the animal's central nervous system, where the signals are processed and the animal can make behavioral responses.

In today's lab, we will investigate the function of these sensory systems. We will examine the frequency and size distribution of action potentials that travel down the nerve chord during sensory stimulation. We will record from an exposed length of the ventral nerve chord, leaving these neurons in tact. We will then mechanically stimulate the animal or bathe the carapace in different solutions and record action potentials. If there is time, you are welcome to also investigate differences in sensory responses of different parts (such as legs, mouthparts, etc), or the neurons in the antennae and antennules (although getting at these neurons can be quite difficult).

Required Equipment

PowerLab, BioAmp, microhook electrodes, silver wire
Crayfishes, crayfish Ringer's solutions, dissection gear, Petri dishes, stands with micropositioners

—> REMEMBER: Invertebrate nerves are unmyelinated. They are very fragile!!!

Procedures

A. Make silver electrodes

1. Clean about 3 inches of silver wire with fine sand paper.
2. Cut two pieces of silver wire, about 1.5 inches long each.
3. Bend one end of one wire into a tiny hook using forceps.
4. Mount the two electrode wires to a pasteur pipette for easier mounting. Place the two electrodes on the open end of the pipette, one on each side, with the hook end pointing down. Tape around the middles of the wires, leaving the hook end exposed below the tape and a little bit above the tape bent away to clip the hook or alligator clip electrodes. Adjust the wire electrodes so that the straight ended wire is slightly longer (about 3mm) than the hook wire. It will be used as a ground in the animal's tissue while the hook lifts the nerve cord.
5. Mount the whole electrode assembly in the clamp of your micropositioner.

B. Setup of equipment

1. Connect two micrograbber hook electrodes to the BioAmp cable, one as "positive", and one as "ground" and connect this cable to the BioAmp.
2. Attach the BioAmp cable to the stand with tape to stabilize the wires. You will use the micropositioner to adjust the height of the tips of the silver wires at the top of the Petri dish.
3. Grab the silver electrodes with the micrograbbers. It doesn't matter which wire you choose

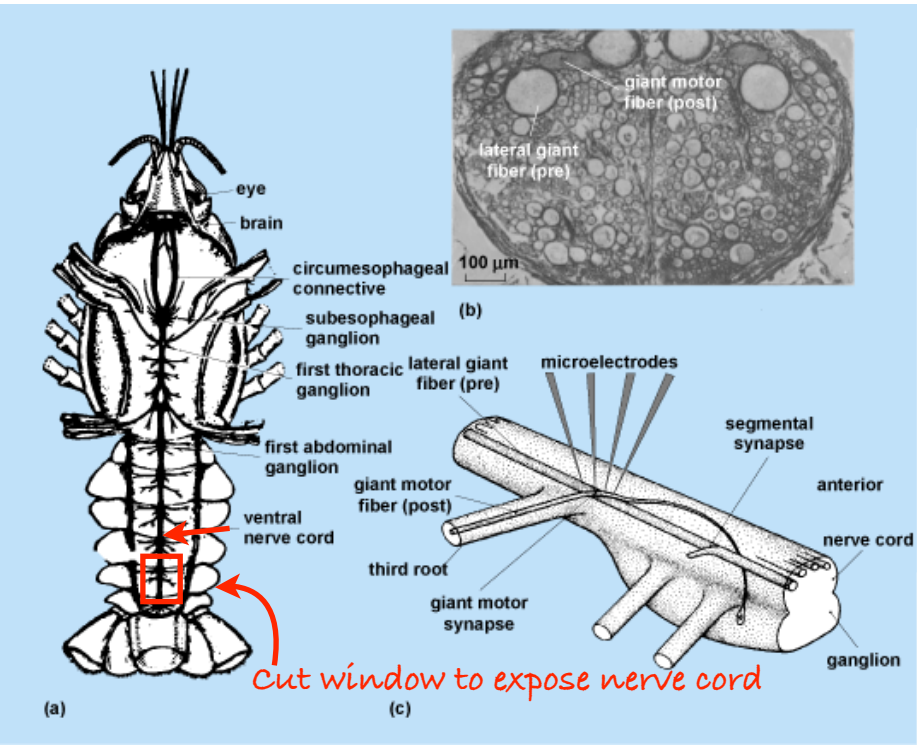
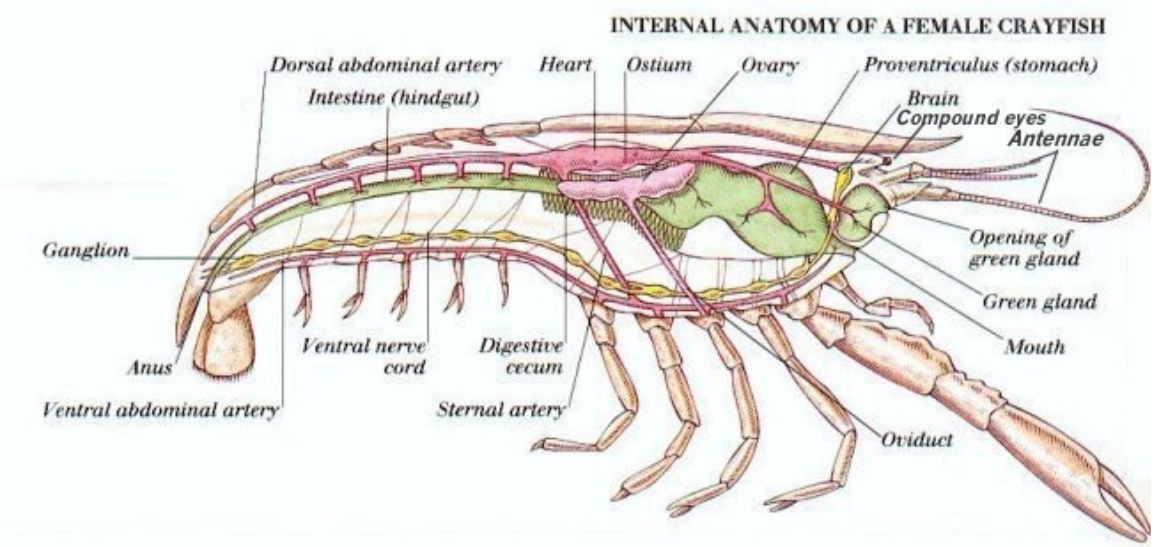


Figure 1. Crayfish ventral nerve cord and ganglia. The nerve cord is shallow and located ventrally. It will appear white. The nerve cord has 13 ganglia and many different fibers including four giant motor neurons which are involved in the escape reflex. The sensory neurons are much smaller. Image from http://people.usd.edu/~cliff/Courses/Behavioral%20Neuroscience/Crayfish/CrayEscfigs/Crayfishcirc.html

C. Dissection of crayfish

1. Put the crayfish on ice to anesthetize.
2. Using fine scissors, you will very carefully cut a small window (about 1cm tall and the width of the abdomen) in the carapace to expose the ventral nerve cord as shown in figure 1. The cuts should be very shallow and near the terminal fins (uropods and telson). Gently lift the carapace as you cut to avoid damaging the tissue below. Careful not to tear. Tease the carapace away with your scalpel as you go.
3. Find the ventral nerve cord. It should be a white cord, superficially located and in the center of the abdomen. You will also see the gut, which is often a darker color.
4. Pin the animal down in the tray on its dorsal side with the ventral nerve cord facing up.
5. Keep the ventral nerve cord moist with Ringer's solution at all times.



D. Using the hook electrodes

1. Adjust the position of the mounted wire electrode to be just over the center of the window, at a height just above the nerve cord.
2. Lower and gently pick up the nerve cord with the silver hook using the micropositioner.
3. With the forceps, move the straight silver electrode into the ventral tissue of the animal to serve as the ground.
4. Using the micropositioner, lift the hook with the nerve cord high enough so that it is just above the surface of the Ringer's.
5. Without touching or shaking the electrodes, place a drop or two of mineral oil down the hook electrodes to insulate the nerves that are out of the Petri dish. Alternatively, if you cannot do this without hitting the electrode you can just place a drop directly on the exposed nerve cord.
6. Use the input amplifier function on chart to examine the action potentials your crayfish neurons are transducing. You should see a great diversity of spikes, some larger than others.
7. If there is excessive electrical noise, try shielding the prep with aluminum foil.

Start the "Lobster sensory settings". To get really excellent resolution of individual action potentials, normal recording rates might be 100 kHz or faster. However, these recording rates will generate data files that are unmanageably large. Try 10kHz for a sampling rate, and the 5:1 time display. If that doesn't work well, sample different recording speed and pick the one that you feel is the slowest where you do not lose any information about the heights of the action potentials.

Each type of receptor should produce spikes of different height, so you will want to be sure that you can record this. You should see a lot of spikes. Determine which size spikes correspond to the sensory nerves.

D. Mechanoreceptors

The easiest sensory receptor cells to measure are the mechanoreceptors. In fact, it is hard not to because they are so exquisitely sensitive. Your animal's mechanoreceptor neurons will fire spontaneously because of vibrations in the room or even elsewhere in the building. Normally, these types of measurements are made on fancy anti-vibration tables that insulate all mechanical stimuli. We do not have these, however, so we'll have to measure increases in action potential generation above background. You should be able to elicit an increase in action potential activity simply by tapping the table with a finger, by blowing air on the surface of the liquid in the Petri dish, or by adding a drop of lobster saline into the Petri dish. Try to determine the sensitivity to each of these stimuli by tapping at greater distances, and dripping liquid from greater heights.

E. Thermoreceptors

Warm and cold crayfish Ringer's is available for you to determine how changes in temperature are sensed. Make sure to have control and experimental treatments so that you can distinguish between effects of temperature and mechanical stimulation.

F. Chemoreceptors

Crayfish Ringer's with dissolved amino acids (meat extract) and Ringer's with dissolved sugars are available so that you can examine the responses of chemoreceptors to these two categories of molecules. You should try dilute solutions before concentrated solutions (you dilute these yourselves) and be sure to include appropriate controls, as above.

G. Variation in sensory systems of different body parts

Various appendages and parts may be specialized for different sensory functions. Repeat steps D-F on each walking leg, mouth parts, or whatever you wish to try to examine variation in function.

Feel Free to do other experiments as well, time and equipment (and your creativity) permitting.