Chapter 13

Recording Action Potentials From Cockroach Mechanoreceptors

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INTRODUCTION

Action potentials can be recorded with both intracellular and extracellular electrodes. With intracellular electrodes the tiny tip of a micropipette pierces the plasma membrane, allowing the actual electrical potential difference across the membrane to be recorded. At rest, a steady membrane potential of about -70 mv is recorded. As an action potential passes by the point of the recording, the membrane depolarizes to about +50 mv and then about one millisecond later returns to the resting level.

Often, however, a neurophysiologist does not need to know the actual changes in the membrane potential, but only when an action potential occurs. In this case, an extracellular recording is usually adequate. Electrodes are placed outside a neuron to record the electrical potential (voltage) changes occurring in the extracellular fluid. The technique works because ionic current flowing across membranes during depolarizations simultaneously causes ionic current to flow in the extracellular fluid, producing electrical potential changes.

The electrical potential changes detected by extracellular electrodes are much smaller than those recorded with an intracellular electrode. Also, the time course of the potential changes is not the same, but distorted.

Although analyzing extracellular potential changes in detail is complex, a few simple principles help understand the recording. Electrical potentials are always recorded between two locations, since it is the difference in electrical potential that is significant. Usually the electrode connected to the positive input of the voltage recording device (the "positive electrode") is placed just outside the neuron. The negative electrode is then placed either outside the same neuron at a distance or else anywhere in the fluid surrounding the neuron. Often recordings pick up less interference if the negative electrode is connected to ground. At rest, no electrical potential is recorded -- both electrodes are in the extracellular fluid. If only the positive electrode is close to the neuron, the voltage measuring device detects a positive electrical potential as an action potential moves towards the positive electrode; conversely, an action potential travelling away from the positive electrode causes a negative electrical potential change. Thus, if an action potential conducts towards and then past the electrode, the electrical potential will first be positive and then negative. If both the positive and negative electrodes are near the neuron, the recording is more complex, consisting of a summation of the potential changes detected at the two electrodes.

Sensory (afferent) axons in the leg of a cockroach offer an excellent opportunity for observing action potentials and for studying important concepts in sensory physiology. Most of the largest sensory neurons detect movements of the spines ("bristles") on the leg. The long portion of the leg closest to the body is termed the femur. The next portion is the tibia.

As Figure 1 shows, each spine on the femur or tibia is suspended on a flexible membrane within a stiff socket. The sensory innervation is not in the spine itself, but at the flexible membrane at the base of the spine. The sensory structure is called a campanifom sensillum. It consists mainly of a tiny dome of cuticle with the dendrite of a single sensory neuron attached to its inner surface. The dendrite passes through a canal in the wall of the spine to the cell body at the base of the spine. The axon projects all the way to the central nervous system via a leg nerve that contains the axons of many sensory receptors, as well as axons of motor neurons innervating the muscles.

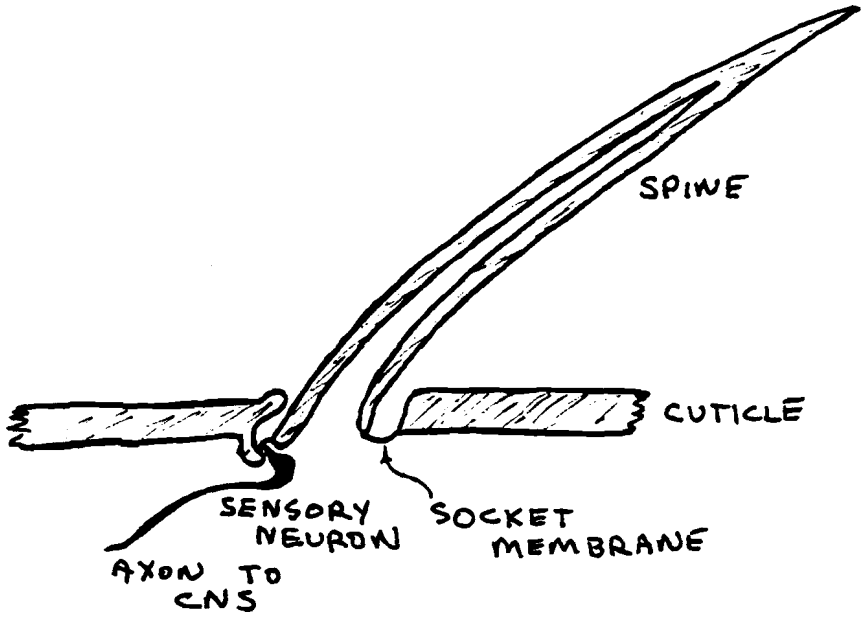

Figure 1. Sensory innervation of femur or tibia spine.

When the spine is pushed from its resting position, the dendrite becomes depolarized due to mechanical forces which are not fully understood. The depolarization produces action potentials at a frequency reflecting the degree of depolarization.

PROCEDURE

Cut off the hind leg of an adult cockroach. Cockroaches are easier to handle if cooled in a refrigerator first. The wound will heal, so place the animal back in the container. Make sure the two insect pins on the holder are clean. Use fine sandpaper to clean if necessary. Gently impale the femur on one of the insect pins on the holder and the tibia on the other. Position the holder under a dissecting microscope so that you can see the leg with its spines.

Connect the cable from the holder to the preamplifier and oscilloscope. Adjust the gain to give a deflection of the oscilloscope beam of one centimeter for each 0.2 mv. The preamplifier should be recording frequencies between roughly 50 and 5000 Hz. The oscilloscope should be recording AC. Observe the action potentials. What is their amplitude and form?

Make a careful drawing of the leg and the position of the large spines that you can see on the femur and tibia.

Use a small probe to touch various parts of the leg and observe the response. Why doesn't touching each spine give an action potential of the same height?

What types of movements of a spine give the largest response? Test as many spines on the leg as you can and mark the direction of their maximal sensitivity with an arrow on the diagram you drew.

Can you verify that only one sensory receptor appears to be associated with each spine?

Do receptors differ in their response to a steady stimulus? A receptor that continues to respond is called tonic; a receptor that responds only briefly is called phasic. Indicate any observations of this type on your diagram.

Test some fine hairs, as opposed to stiff spines. Any response? Tap the holder. What receptors might produce this response? Blow on the preparation. What receptors are responsible? The terminal segments on the leg are called the tarsi, including the one at the tip with claws. Test responses to moving the tarsi.

CONSTRUCTION OF THE HOLDER

To make the holder you will need a number 15 cork, two 00 insect pins, some fine sandpaper, needle-nose pliers, solder and a soldering iron, and a shielded cable suitable for connecting the holder to your voltage measuring device. Usually this would be a preamplifier, which in turn would be connected to an oscilloscope. The electronic apparatus used for the traditional frog sciatic nerve experiment is ideal. However, just about any voltage measuring device capable of recording brief voltage changes of about 0.1 mv should work. The negative input to the voltage recording device can be grounded.

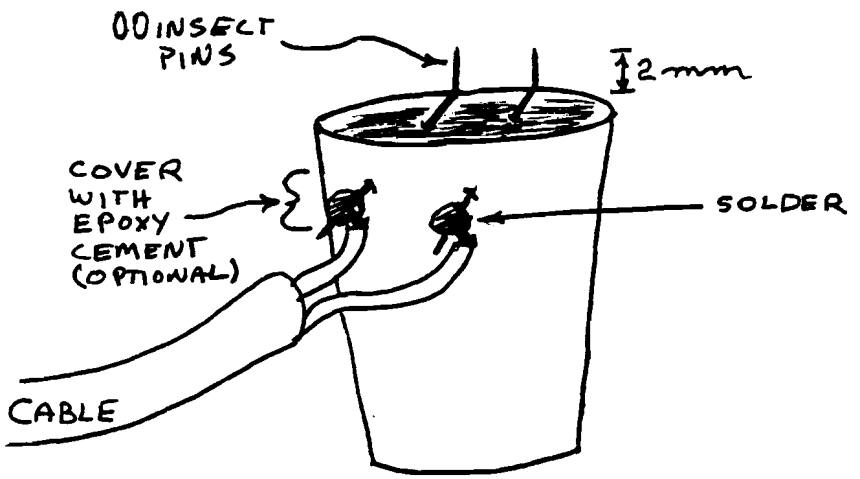

Figure 2. Holder.

Sand any paint or varnish off the insect pins. Grasp one pin about one centimeter from its tip with the needle-nosed pliers and force the pin obliquely into the side of the cork until about 5 mm protrudes from the top of the cork (see Figure 2). Insert the second pin in the same fashion approximately one centimeter away. Use the needle-nose pliers to bend the final 2 mm of the tips of the needles so that they point vertically. Finally, solder the wires in the cable to the pins. One pin can be ground if your apparatus is of this type. If your cable is relatively stiff, it would be a good idea to solder several inches of a more flexible cable to the end and attach the more flexible cable to the pins. The holder will be sturdier if the junction of the pin and the cable is covered by a thick layer of epoxy glue. Also, a few drops of any type of black wax applied to the top of the cork will make the spines more visible under the dissecting microscope.

REFERENCES

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Note: This experiment was developed in collaboration with John Palka