A student apparatus for recording action potentials in cockroach legs

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Linder, Thomas M., and John Palka. A student apparatus for recording action potentials in cockroach legs. Am. J. Physiol. 262 (Adv. Physiol. Educ. 7): S18–S22, 1992.—A comparatively simple apparatus allows even beginning students to observe action potentials in the cockroach leg. The recordings are made extracellularly by impaling the leg on two insect pins. Deflection of large spines on the leg, which are each innervated by one sensory neuron, initiates the action potentials. Using this technique, students observe the all-or-nothing nature of action potentials, their coding of information by frequency, and sensory adaptation.

ACTION POTENTIALS are among the most striking of biological phenomena. Moreover, the all-or-nothing character of action potentials and their coding of information by frequency are key concepts for students learning the basics of neurophysiology. Ideally, students should be able to observe real action potentials and explore directly some of their most important properties. However, much of the value of a neurophysiology experiment is lost if the students spend most of their time trouble shooting. To be most effective, the equipment and procedures should be reliable and streamlined so that attention is focused on the biology.

Intracellular recordings of action potentials are most informative but present difficulties. The experiments require a relatively complex apparatus and a delicate touch that most students have not developed. Also the dissection and impalement of neurons tend to be difficult in the readily available preparations. Although muscle cells are easier, they contract and thus often dislodge the electrode. Moreover, in many invertebrate preparations, muscle cells do not produce action potentials but only postsynaptic potentials.

Extracellular recordings are often more appropriate for an initial experiment, requiring less skill and equipment. Although they are not especially suitable for analyzing the ionic basis of electrical excitability, they do focus attention on the information contained in a sequence of action potentials and thus on the overall functioning of the nervous system.

One especially favorable preparation for extracellular recording, we have found, is the cockroach leg. The experiment is based on the long-known fact that action potentials can be recorded simply by inserting pins into an isolated leg. This technique has been used in research (1, 2, 6, 7), and versions of a student experiment have appeared in an out-of-print invertebrate physiology laboratory manual (8), a neurophysiology laboratory manual (5), and a workshop volume of limited distribution (4). It is our purpose here to describe a simplification of the technique, one which brings the experiment into the realm of even beginning students. We also point out how students can use their simple measurements for a semi-quantitative analysis of the structure and function of the system.

EXPERIMENTAL SYSTEM

Sensory spines in the cockroach leg. A cockroach leg is studied with long stiff spines, which are each connected to the surface of the leg by a flexible membrane (Fig. 1). At the base of each spine is a sensor consisting of a single afferent neuron and a specialized cuticular structure. The cell body of the neuron lies just under the cuticle of the spine, with the unbranched dendrite of the neuron projecting up through a narrow canal in the overlying cuticle (1, 3). As the spine is moved, the flexible membrane and thus the dendrite of the neuron is distorted. On the basis of the evidence collected in other systems, one can assume that the distortion opens mechanically gated ion channels in the dendrite, allowing positive ionic current to flow into the neuron. This creates a receptor potential, which in turn triggers action potentials.

As an action potential moves down an axon, ionic current flows in the extracellular spaces, causing electrical potential differences. In the cockroach leg, these potential differences may be >1 mV. The unusually large amplitude of the potentials is probably a consequence of the comparatively narrow extracellular spaces and thus high extracellular electrical resistances.

Other types of sensors are found in the leg, although in extracellular recordings their action potentials are smaller than those triggered by movement of the spines. Some sensors, for example, respond to deformation of the cuticle around the joints. The structure of these sensors is closely related to that of the sensors associated with the spines. Also, most of the small hairs projecting from the surface of the cuticle are sensory, responding to air puffs and other gentle stimuli.

Animals used. We usually use the cockroach Periplaneta, because in our experience it produces the largest signals. However, other large insects, such as crickets, grasshoppers, and locusts, can be used in the same way and provide a nice comparison. Cockroaches are available from biological supply companies and can be kept in the laboratory for long periods. Rat chow or pet food is convenient for feeding, and clean water, of course, is essential.

Cockroaches can be narcotized by cooling in a refrigerator. Also, students find it easier to touch and pick up a cockroach if they have first observed another person confidently handling one.

Instruments required. An oscilloscope and a preamplifier are the primary instruments required, with an audio monitor highly recommended. The preamplifier may be either the single-ended type, in which one lead is ground, or the differential type. A stimulator is required for the second part of the experiment. It is likely that a course that has used the frog sciatic nerve preparation has the basic equipment for the cockroach leg. Newer computer-based systems would be even better.

Construction of the apparatus. Action potentials are recorded in this experiment by impaling an excised leg on a special holder. The essential portion of the holder is constructed from a no. 15 cork and two 00 insect pins. Sand the insect pins with fine sandpaper to remove any paint, varnish, or oil. With the use of needle-nosed pliers, grasp one of the insect pins ∼1 cm from its point. Now force the pin obliquely through the side of the cork until ∼5 mm protrude from the surface of the cork, as shown in Fig. 2. Insert the second pin in the same fashion ∼1 cm away. Then use the pliers to bend the final 2 mm of the pins
Fig. 1. Large spine from leg of a cockroach. Deflection of spine triggers action potentials in afferent neuron.

Fig. 2. Simple holder for recording action potentials from cockroach leg. Leg is impaled on 2 insect pins, which serve as electrodes for recording action potentials extracellularly.

so they point vertically. Next solder ~5 cm of flexible shielded cable to the bases of the insect pins, as shown in Fig. 2. If the preamplifier is single ended, the center wire connects to one pin and the grounded shield connects to the other pin. If the preamplifier is differential, the shielded cable should have two center wires. Connect one center wire to each of the two pins and leave the grounded shield free, unconnected to either pin. Because the insect pins are stainless steel, the proper solder to use is silver solder along with the appropriate flux. In practice, however, ordinary resin-core solder usually suffices. The other end of the flexible cable is soldered to ordinary shielded cable leading to the preamplifier. Cut off the excess portions of the insect pins and, for durability, apply a thick coat of 5 min epoxy glue to the region around the solder joints. Also, a tiny epoxy bead may be placed ~2 mm below the point of each pin to prevent the leg from being forced too far down the pin. A few drops of melted black wax applied to the top of the cork will help stabilize the pins and provide a more uniform background against which to observe the spines under the dissecting microscope. At this point the holder is ready for simply observing action potentials.

Some additional apparatus, however, allows for more precise stimulation and quantitative analysis. First, it is helpful if the cork is mounted in a manner that allows it to be easily moved and rotated. A ¾ in. polyethylene T, such as used for tubing, offers one solution (Fig. 3). Hollow out a ¾ in. hole from the bottom of the cork, using a cork borer. Trim the limb of the T that is perpendicular to the other two limbs so that it is ~2 cm long. Insert this limb into the hole in the cork. The fit should be snug but still allow the cork to be rotated. To one of the other two limbs connect a short length of a stiff rod or pipe. The rod or pipe is then used to mount the holder on a small ring stand or a micromanipulator. The final apparatus looks a bit like a smoking pipe and allows the cockroach leg to be moved easily and precisely into an orientation suitable for controlled stimulation.

A spine can be deflected accurately by using an ordinary 5-in. acoustic suspension speaker, such as may be purchased at a retail electronics store. Also obtain a no. 1 cork, and drill a hole through it that will just allow a hematocrit capillary tube to be easily inserted. Glue the large end of this cork to the center of the cone of the speaker. Next insert the point of a no. 1 insect pin (or any similar pin) a short distance into a hematocrit tube, and glue it in place. Insert the other end of the hematocrit tube into the hole in the cork in the speaker. Position the speaker so that it faces the cockroach leg. Now move the cockroach leg until the head of the pin just touches one of the spines. For this last step, it is much better for the holder to be mounted on a micromanipulator than on a ring stand.

The cone of the speaker and hence the cockroach spine are moved by applying pulses from a stimulator to the speaker. To protect the stimulator from backinductance from the speaker, connect a diode across the output terminals of the stimulator. The end of the diode that is marked should be connected to the positive output terminal. If the diode is connected backwards, it

Fig. 3. Ordinary speaker is used to reproducibly deflect a spine on cockroach leg. Pulse from stimulator causes cone of speaker to move. Movement is transferred to spine by head of a pin glued in capillary tube, which in turn is attached to speaker with a cork. Inset: holder mounted on a polyethylene T, an arrangement that allows holder to be rotated.

Fig. 4. Results such as a student might observe using apparatus. Top trace: extracellularly recorded action potentials. Each action potential is 150 μV high. Bottom trace: duration of 0.75-s pulse from stimulator. This pulse moved cone of speaker and hence spine. Sweep of oscilloscope was triggered by onset of pulse from stimulator. Calibration marks were added afterwards: 100 ms, 0.1 mV.
will short the output of the stimulator. With our specific stimulators, we have found we can dispense with the diode without damaging the stimulators.

**Student experiments.** The student first sets up the equipment, impales the leg on the holder, and observes action potentials (Fig. 4). The all-or-nothing principle and the coding of information by frequency are observed at this time. The student then stimulates the spines in several ways, leading to the concept that a sensor tends to respond best to a stimulus of a specific modality and orientation. Next a spine is stimulated in a more precise fashion using the speaker, allowing quantitative measurements of a stimulus-response curve and the rate of sensory adaptation.

To make it easier for instructors to assemble directions for their students, we present step-by-step protocols for the specific experiments in the APPENDIX.

**DISCUSSION**

**Technical advantages of the cockroach leg preparation.** Compared with other neurophysiology experiments, the cockroach leg preparation is comparatively simple to organize. Cockroaches are readily available, easy to maintain, and, as invertebrates, avoid the objections that may arise with other animals. In addition, many institutions have most of the required equipment on hand. Newer computer-based equipment would also be appropriate and would afford an opportunity for more extensive quantitative analysis. The holder is simple, inexpensive, and has been thoroughly tested with students. Also the dissection and set-up are comparatively easy, do not require special experience, and provide a preparation that is usually stable for several hours. Using our simplified apparatus, we have found that even beginning students rarely fail to record discrete all-or-nothing action potentials; the experiment proves to be well within the manual capabilities of most students.

**Usefulness of the preparation for illustrating concepts.** The most important feature of the cockroach leg, however, is that it is a ready vehicle for the students to explore key physiological principles. With this preparation it is comparatively easy to stimulate a single sensory neuron and to observe each axon potential produced. In this way, the all-or-nothing characteristic of action potentials and the coding of information by frequency are both obvious. Moreover, an actual functioning sensory structure is still present during the recording of the action potentials, allowing students to explore several basic topics by direct observation.

Various further questions can be posed, depending on the level of the students. For example, one might approach discussions of current flow during an action potential by asking why the extracellular recordings are so much smaller than the intracellular potential changes shown in textbooks. Similar discussions also can begin with the observation that action potentials recorded from different axons vary in size. Likewise, the biphasic shape of the recorded action potential can be pursued.

Questions can also be asked that link the biophysical properties of the sensors with the biology of the animal. For example, different neurons adapt at different rates. What might be the value of this variation to the animal? Indeed, what are the naturally occurring stimuli that normally stimulate these spines? Are properties of the sensors, such as the rates of adaptation and the stimulus-response curves, well suited for monitoring the pertinent environmental phenomena? Reciprocally, do the observed properties of the sensors enable one to make reasonable predictions about their role in behavior? How could these predictions be tested? Can correlations be made between the effect of temperature on the sensors in the leg and on the whole animal? Are there other conditions or substances, such as neuromodulators or hormones, that alter the behavior of these sensory axons? What effect would these alternations have on the animal?

The student also can be pointed in a more quantitative direction. For example, viewing the spine as a simple lever, it is possible to estimate the mechanical stimulus that is applied to the sensory dendrite. When the spine is deflected toward the proximal end of the limb, the rim of the socket acts as a fulcrum (3). The dendrite is ~50 μm below this fulcrum (1). Knowing the distance above the fulcrum at which the stimulating probe moves the spine, one can estimate the movement at the invisible sensory dendrite. The student, in other words, derives a number than cannot be obtained by direct measurement. Also a "reasonable estimate" is being made here, something scientists do all the time but that is not necessarily an obvious procedure for everyone. Another area for quantitative work would be to introduce the technique of curve fitting to determine the rate of adaptation. In addition, challenged with the task of measuring the minimum displacement of a spine required to elicit an electrophysiological response, one might discuss the meaning of "threshold," the general importance of operational definitions, and the use of statistical procedures in an inherently noisy situation.

A closing note. Today there is a great need for physiological experiments that allow students themselves to work with physiological reality, to be initiated into abstract analysis, and to think of the whole organism as well as of cellular function. After all, direct observation and measurement of physiological processes are the essence of physiology. It is not easy to achieve all these pedagogical goals in a single experimental system, but we believe the cockroach spine preparation does unusually well.

**APPENDIX: SAMPLE PROCEDURE FOR THE STUDENT**

**Procedure for observing action potentials.** Mount the holder for the cockroach leg under a dissecting microscope. Take a small piece of fine sandpaper and lightly sand each of the two pins protruding from the top of the holder. The sanding cleans the pins so that they make good electrical contact with the leg. Next connect the cable to the input of the preamplifier. The wire leading from one of the pins should be connected to ground; the other wire goes to the input of the preamplifier. (If the bandwidth of the preamplifier can be adjusted, select ~500-5,000 Hz. The lower frequency is not critical, except for reducing the amplitude of interference from the power lines. If the upper frequency is much lower than 5,000 Hz, the extracellular action potentials will appear smaller and more rounded; if the upper frequency is significantly higher, high-frequency noise will be unnecessarily included in the signal.)

Next connect the output of the preamplifier to the positive input of an oscilloscope. The input should be set to AC, because steady signals are not being recorded. The gain on the oscilloscope should be set so that a signal of 0.1 mV will cause a 1 cm
deflection on the oscilloscope screen. (Be sure to take the gain of the preamplifier into account.) A sweep speed of 100 ms/cm is convenient initially. If an audio monitor is available, connect the output of the preamplifier to this as well. Also use wires with alligator clips to ground all pieces of metal near the holder to reduce interference from the power lines.

Select a cockroach from the cage. It is usually easier to pick up a cockroach if you have seen another person do it first. The cockroach may be narcotized by placing it in a refrigerator until its movements have slowed. With a small sharp pair of scissors, cut one of the hind legs from the cockroach at the coxa, which is the short leg segment adjacent to the body. The cockroach should be returned to the cage.

Using forceps with fine points, gently impale the femur of the leg on one of the pins of the holder (Fig. 2). Next impale the tibia on the other pin. The leg should be suspended completely above the surface of the cork to avoid stimulation of the spines or other sensors by contract with the cork surface.

Check to be sure the preamplifier and oscilloscope are on and properly adjusted. Now blow on the preparation or tap the table. You should see action potentials. If you wish the deflection of the action potentials to be in the opposite direction, move the output of the preamplifier to the negative input of the oscilloscope.

Exploring the effects of various stimuli. As you have just seen, tapping the table top and blowing gently on the leg both elicit action potentials in the leg nerves. Look through the dissecting microscope and observe what happens when you blow. Can you identify structures that might be involved in detecting air movement? Can you guess what sort of structure might detect vibration? You could also try a warming stimulus, produced by holding a heated, but unplugged, soldering iron a few inches from the leg tapping the table top and blowing gently on the leg both elicit action potentials in the leg nerves. Look through the dissecting microscope and observe what happens when you blow. Can you identify structures that might be involved in detecting air movement? Can you guess what sort of structure might detect vibration? You could also try a warming stimulus, produced by holding a heated, but unplugged, soldering iron a few inches from the leg.

Notice that the action potentials arising from movement of any one spine are all similar in size, but action potentials from different spines have different amplitudes. Refering to a textbook, however, you will find that the actual change in the membrane potential of all these cells is nearly the same. How can you reconcile this apparent conflict?

Quantitative analysis of the stimulus and response. Now we will use a small loudspeaker carrying an insect pin to deliver controlled stimuli to single spines. This will enable us to construct a stimulus-response curve and thus analyze the coding of stimulus intensity by the frequency of the action potentials. Insert the capillary tube with the insect pin into the cork at the center of the speaker and then connect the wires from the speaker to the stimulator. (Be sure the pulse amplitude is first set to zero.) Choose a spine that produces large extracellular action potentials and decide whether you wish to move the end of the spine toward the distal or the proximal end of the leg. The sensory neuron is stimulated in either direction, although not equally. Position the speaker near the leg holder and move the holder until the head of the insect pin just touches the spine. (It is always easier to move the holder than the speaker.) A minute amount of a sticky wax applied to the head of the pin helps to stabilize its contact with the spine. Alternately, the pin may be bent at a right angle ~1 mm from the end. This allows the spine to be either pushed or pulled.

Select a pulse duration of 100 ms and a sweep speed for the oscilloscope of 10 ms/cm. Also connect the "sync out" terminal of the stimulator to the "external trigger" terminal of the oscilloscope, and set the oscilloscope for external triggering. In this way the beam will sweep across the oscilloscope face during the same time the stimulus is being applied.

Now begin increasing the pulse intensity. The moving insect pin should cause visible movement of the spine. If the spine is not moving optimally, reposition the holder or reverse the polarity of the stimulator. Make a rough estimate of the voltage (and thus movement) required to elicit a minimal response and also the voltage required for a vigorous response. Choose five to eight voltages that include these two extremes and obtain at least five responses at each voltage. Calculate the mean of the responses at each voltage and plot a stimulus-response curve, that is, the number of action potentials produced as a function of stimulus intensity.

At the conclusion of the experiment you can calibrate the actual movement of the insect pin by using an ocular micrometer. Begin by measuring the movement when applying a large pulse from the stimulator. Because the relationship between voltage and movement of the speaker is linear, you can safely extrapolate down to low stimulus values where the movement cannot be directly measured. Now you can plot your stimulus-response curves as the number of action potentials versus the magnitude of the movement measured in micrometers.

It is also possible to estimate the distance the dendrite is moved during a stimulus. When the spine is moved toward the proximal end of the leg, the rim of the socket acts as a fulcrum around which the spine pivots. The distance from the fulcrum down to the dendrite is ~50 μm. You can determine the length of the other arm of the lever by measuring the distance from the fulcrum to the point at which your moving probe contacted the spine. You now have three known distances: dendrite to fulcrum, fulcrum to probe, and movement of probe. Express all these distances in micrometers and calculate the estimated distance the dendrite moves for each deflection of the spine. About how much would you estimate that the dendrite moves at threshold?

Sensory adaptation. Select a stimulus duration long enough to show the phenomenon of sensory adaptation clearly. The duration will probably be in the range of 200 ms to 1 s. Choose a stimulus strength that elicits a vigorous response, as determined earlier. Elicit a response and count the number of action potentials in each centimeter division of the screen. Now plot the frequency (not number) of the action potentials for each time interval, in this way showing the time course of sensory adaptation.

Compare the rates of adaptation of several different spines. How much do the rates vary? If you make a map of the leg surface and mark the location of the different spines, does a pattern emerge? Do spines in different locations behave similarly or differently? What advantage might sensors with different rates of adaptation provide to the animal?

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