Chapter 9

Two Simple Electrophysiological Preparations Using Grasshoppers

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181

181

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I. Introduction

Two long-lasting electrophysiological preparations utilizing grasshoppers are currently on display as live exhibits at the Exploratorium, a science museum in San Francisco. The techniques for preparing these demonstrations, as well as the maintenance of the two grasshopper species, *Schistocerca nitens* and *S. americana*, are described in the hopes that they will be of use in the college biology laboratory. These preparations were not developed with laboratory teaching in mind, but it is hoped that they may be successfully modified for use in that area.

"The Watchful Grasshopper"

The "Watchful Grasshopper" exhibit uses chronically implanted wire electrodes to record from the ventral nerve cord of a grasshopper. The neural activity which is detected by the electrodes and displayed via recording amplifiers and oscilloscope clearly shows the electrical activity of two paired neurons; the Descending Contra-lateral Motion Detector (DCMD) and the Descending Ipsi-lateral Motion Detector (DIMD). These cells usually generate nerve impulses in response to novel moving objects in the visual field and may show some activity in response to sounds. At the exhibit, visitors may explore and map the visual field of the grasshopper, determine what type of stimulus triggers impulses, and observe habituation to repeated stimulation in specific areas of the visual field.

The preparation is of interest because motion detector cells are found in the visual systems of many other animals, including most vertebrates. They frequently serve to alert the animal to possible prey or danger. The motion detection system of the grasshopper is one of the most completely understood of these systems.

"The Grasshopper Leg Twitch"

The "Grasshopper Leg Twitch" exhibit uses the isolated leg of a grasshopper to demonstrate that electrical stimulation can cause muscle contraction. In this exhibit, visitors may vary the voltage to determine that there is a "threshold voltage" which must be attained before the leg muscles will contract. They may also change the frequency of electrical pulses administered to the leg to show that the shock-stimulus is cumulative. The teaching value

of this preparation is limited by its use as a museum exhibit, whereas in a laboratory situation, there exists a much greater range for experimentation. For instance, measurements might be made of the strength of muscle contraction, or the biomechanics of the knee joint and muscle attachment can be studied. Also, muscle tetanus might be studied, the position of the stimulating electrode could be changed, or the voltage applied to the leg could be modified much more drastically than is possible in the museum demonstration. This preparation lasts up to 24 hours with minimal care and may be used to show most of the phenomena demonstrated in other muscle-nerve preparations.

Selection of Species

Schistocerca nitens and *S. americana* were selected because of their relatively large body size. Also these animals are conveniently available to us at the Exploratorium from research laboratories at Stanford University and the University of California at Berkeley. With the animals we have received from these institutions, we have established and maintained grasshopper colonies with reasonable success.

Most of the research on the grasshopper's visual system has been conducted on *Schistocerca nitens* but our studies indicate that the DCMD and DIMD cells of this species are identical to those of *S. americana*. Since it is difficult to both ship and maintain either of these species, we recommend that locally available hoppers be used in these preparations whenever possible. Use of locally available species may require some field work, cage-construction, and exploration of your hopper's neuroanatomy, but it does away with the bothersome need for numerous permits and the fabrication of exotic environments. For example, in experimentation on species of grasshoppers indigenous to the San Francisco Bay Area, motion detector cells very similar to those found in *S. nitens* have been observed. Therefore, one should consider the information presented here only as a model around which a technique for preparing and maintaining other species of grasshoppers might be developed.

II. The Solitary Desert Locust

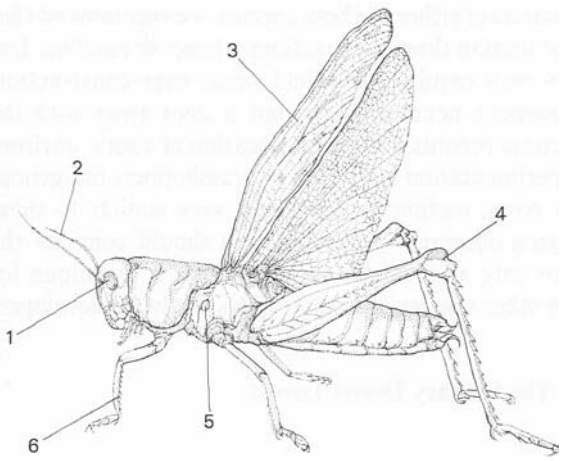
Natural History

The genus *Schistocerca* contains solitary and gregarious species in both the Old and New Worlds. The species *S. gregaria*, which lives in northern and eastern Africa and western Asia, is the most destructive species of locust in the world. From the descriptions available, we know that the locust has plagued and devoured people's food supplies since ancient times. *Schistocerca nitens*, the solitary desert locust with which we are most familiar in the United

States, inhabits the arid regions of the southwestern United States and northern Mexico. These animals are considered an agricultural pest but do not swarm and are not as destructive as their larger African and Asian relatives. See Figure 9.1

Adult *Schistocerca nitens* have an extreme sexual size dimorphism. Generally, females are about 6 cm from head to wing tip while the males are much smaller, being about 4 cm long. This difference is associated with the extra molt which females undergo.

In the wild, the desert locust usually has one generation a year. Occasionally, during an extremely mild winter, a second generation may be produced. The eggs are laid in loosely packed egg cases in the fall and stay in diapause through the winter. The young hatch with the spring greenery. The tiny (5 mm) hoppers immediately locate fleshy green plants and begin to feed. They undergo seven to eight molts, changing from a bright green wingless hopper to the intermediate unstriped beige morph, and finally to the winged brown adult with the characteristic leg striping and dark body pigmentation. Adults are capable of sustained flight, and it is only during the adult phase that mating and egg-laying occur.



1 eye
grasshoppers have a keen sense of vision. They can almost see 360°.

2 antenna
sensitive to touch and taste. You may see the insects cleaning their antennas to ensure good reception.

3 wings
are used for escape and migration.

4 hind legs
the powerful muscles enable the animal to jump long distances.

5 tympanic membrane
sounds are sensed by movements of the membrane.

6 receptor hairs
sensitive to touch and body position.

Figure 9.1. Gross external anatomy of the grasshopper. Female—*Schistocerca americana.*

The changing coloration of the growing locust provides an interesting study in adaptive coloration. All of the hoppers are green when they hatch and all of the adults the characteristic mottled brown or gray coloration. However, the intermediate instars are more variable. If the growing hoppers are crowded and reared in low humidity, they achieve dark coloration earlier in their lives than those raised in isolation and high humidity. It is thought that this coloration aids in camouflaging the animal from its natural predators. Dry, crowded conditions seem likely to dictate brown vegetation or exposed earth, either of which would favor a brown grasshopper. Conversely, humid, uncrowded conditions might favor a grasshopper which retains its green coloration.

In development, students should note the contrast between *Schistocerca nitens* and many familiar insects. The young hoppers are similar to the adults. They lack wings and genitalia and are a different color, but look and behave like the adults. This contrasts with the larval-pupal-adult life-cycle of the butterfly or moth. This difference in life-cycle strategies may be associated with the eating habits, food availability, and most propitious time of the plant-growing cycle for the larva or adult to exist.

Colony Maintenance

Maintaining a colony of *Schistocerca nitens* (or *S. americana*) can be a frustrating experience. The information presented here does not guarantee a successful grasshopper colony, but states some guidelines which appear to be important. The grasshoppers require a temperature around 35° C with high humidity for successful reproduction. Our grasshoppers are housed in 1.0 cubic-foot screened cages located inside of an environmental room, with about 20 to 30 hoppers per cage. The lighting is controlled to produce 14 hours of light and 10 hours of darkness each day. The animals are fed a mixture of wheat sprouts, wheat germ, and softy-dry dog or cat food (such as Gainesburger). When the colony is mature, 250 ml glass beakers full of wet, medium-coarse sand are placed in the cages for the females to lay their eggs in. The female grasshopper lays her eggs by drilling a hole several inches into the sand with her abdomen and depositing the eggs at the bottom. The eggs are covered with a protective foam which usually reaches to the top of the sand. See Figure 9.2.

The eggs may be left in the beakers through the incubation period if kept moist, or they may be collected. To collect the eggs, empty the contents of the beaker into a tray or bowl. Then pick the yellowish-brown egg clusters out of the sand and place them into a small sealable container of approximately 500 ml volume. We use refrigerator food storage containers. Next completely cover the egg clusters with moist sand.

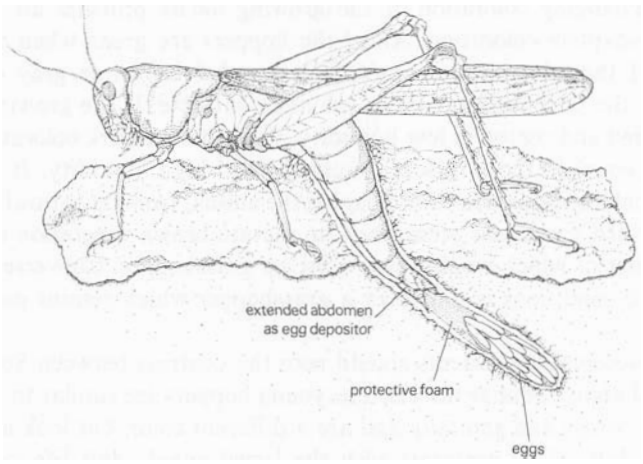


Figure 9.2. Female grasshopper laying eggs.

It is important to collect the eggs every three days so that approximately the same age groups of baby grasshoppers are raised together. The incubation period is variable and is dependent upon the temperature, but usually takes about 21 days. It is important to keep the egg-containing sand moist but not so wet as to drown the developing embryos. Also, do not allow the temperature to rise above 37° C, as this will kill the embryos. When the baby grasshoppers hatch, they wriggle to the surface and immediately begin to hop, so it is important that they either hatch into already prepared cages or are in closed containers which can be moved to prepared cages. Window screen-sized mesh is sufficient to contain them in their cages whereas larger mesh may be used for the adults. It takes approximately six weeks for juvenile grasshoppers to grow to maturity at 35° C, and under the conditions just described, the animals will reproduce year-round.

Neurobiology

Research on *Schistocerca nitens* and its near relatives initially began in England under the auspices of the Anti-Locust Research Center and World Health Organization, the object of which was to control them as pests. In recent times, however, investigators have been more interested in examining the neural mechanisms underlying some of the hopper's behavior. One of the first central nervous system oscillators to be discovered was shown to coordinate the rhythmic contractions of the locust's flight muscles. The inherent frequency of the wing beat is modified by sensory inputs from the periphery. Likewise, the escape jump of the locust has been shown to have a centrally

sequenced pattern which may also be modified by sensory feedback. The tremendous acceleration in the initial phase of the jump comes from the energy stored in the deformed exoskeleton of the leg. This requires coordination in the contraction of the flexor and extensor muscles of the femur.

First the flexor muscle contracts the lower leg into the pre-jump position, then the extensor muscle contracts a spring-like compression of the femur. To jump the flexor muscle is relaxed. Relaxation releases the compressed exoskeleton and highly tensioned extensor muscle causing the animal to jump.

A. *The Watchful Grasshopper—Background Information*

The visual system of the locust is an important sensory system and has been shown to alert the animal to possible dangers in the environment. It contains a group of extensively studied cells, known as the Descending Movement Detector (DMD) cells. These cells, as a group, generally receive input from cells in the optic lobe and have outputs to the thoracic ganglia. DMD cells may be divided into three types. The first type makes no connection with the compound eyes but gives a tonic off response to light falling on the ocellar field. A second group contains units which receive inputs which are only visual or are visual and tactile. These cells produce excitatory or inhibitory responses to light. The third group contains the Descending Contra-lateral Motion Detector (DCMD) and Descending Ipsi-lateral Motion Detector (DIMD) cells. These paired cells are driven by connections from the contralateral and ipsilateral compound eyes and show a similar response to movement. For this reason the DCMD and DIMD are treated as a group. Recording extracellularly from *S. nitens*, action potentials from the DCMD are produced which are approximately 30% larger than those of the DIMD. This extra-cellularly recorded size difference is accounted for by the position of the DCMD within the ventral nerve cord and its cross-sectional diameter. The DCMD is situated on the periphery of the ventral nerve cord and has a relatively large diameter of about 17 um. The exact anatomical description of the diameter and position of the DIMD is incomplete, but it is suspected to be a large axon buried deeper within the ventral nerve cord. Interestingly, in crickets the DCMD and DIMD are also present, but the size of the action potential is reversed.

The DCMD is an interneuron which extends from the optic lobe to the metathoracic ganglia. See Figure 9.3. It is a high-order sensory interneuron responding to changing light intensities over a small portion of the visual field. The cell becomes habituated to a repetitive stimulus in the same area of the visual field, but will be dishabituated to the repetitive stimulus if it is presented in a new area of the visual field. Changes in illumination of the whole visual field usually ellicit a response from the DCMD, and the cell is incapable of discriminating direction. It is an ON/OFF detector which is strongly stimulated by small contrasting objects moving across the visual field. Such a stimulus to the cell produces local rapid changes in light intensities at the individual visual units of the compound eye.

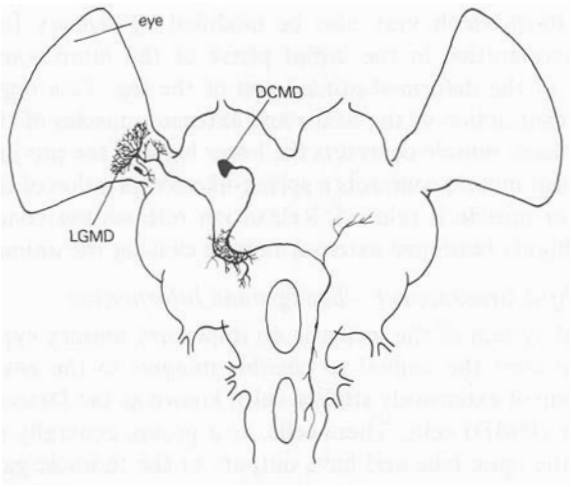


Figure 9.3. Relative positions of the LGMD and DCMD in the eye, brain and ventral nerve cord of *Schistocerca niten.*

The specificity of the DCMD response is not completely understood, but it is related to a synaptic input and dendritic connections which are made by the Lobular Giant Motion Detector (LGMD). The LGMD is a sensory interneuron which has a fan-shaped dendritic field receiving retinotopic projections. The DCMD has an electrically transmitting synapse with the LGMD and follows the output of the LGMD one to one. Intra-cellular recordings from the LGMD show that there is a compound excitatory potential generated in the dendritic field in response to an appropriate visual stimulus. Habituation of the LGMD to a repeated stimulus has been shown to be the result of a decrease in the size of the compound excitatory post-synaptic potentials. The failure of the LGMD to respond to changes in illumination across the entire visual field probably results from a lateral inhibition network at the retina and post-synaptic inhibition at the LGMD. It is thought that the neuroanatomy of the LGMD and the integrating effect of its fan account for the selective responsiveness of this MD system.

The behavioral function of the LGMD and DCMD seems to be directly connected with the escape jump of the hopper. All of the DCMD's afferent connections in the metathoracic ganglia are made with jump motorneurons. But the DCMD is not solely responsible for the locust jump. As you will see from the preparation, there appears to be little relationship between the activity of the cell and the behavior of a freely moving animal.

See the bibliography section for references dealing with the neurobiology of locusts.

B. *The Watchful Grasshopper—Method of Preparation*

Those unfamiliar with the gross neuroanatomy of the locust should sacrifice one or two animals and study the position of the ventral nerve cord by removing the thoracic plates. See Figure 9.4. The ganglia are yellow and located just below the trachea. The ventral nerve cord is seen as two semitransparent tubes interconnecting the ganglia.

The materials needed to make an extracellular recording from the DCMD include standard neurophysiology equipment: a Faraday cage, an oscilloscope with sensitivity to lmv/division, a Grass P-15 pre-amplifier, an audio amplifier, and a dissecting microscope. The special equipment needed for this preparation include the following: .003 to .005-inch teflon-coated stainless steel wire (available from Cooner Sales, Chatsworth, CA, #AS765–40), hemostats, a 26-gauge needle, #5 jewelers forceps, dental wax or clay, a beeswax rosin mixture (which will have to be prepared), an alcohol lamp, wooden applicator sticks, two test lead clips (Pomona, Pomona, CA, #3925), and shielded hookup wire.

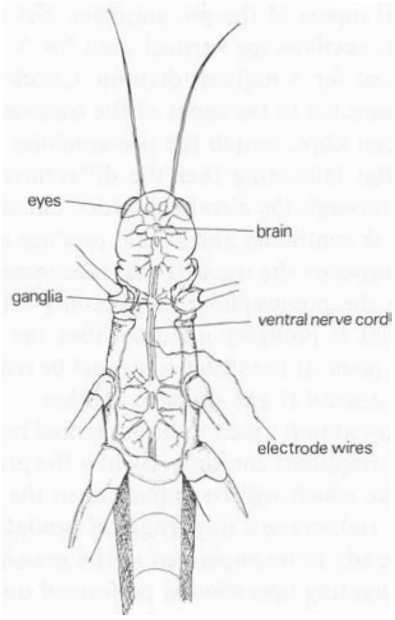


Figure 9.4. Cutaway diagram of the ventral side of the grasshopper showing the ventral nerve cord and hypothetical position of the electrode wires.

The beeswax rosin mixture is made by mixing together equal volumes of beeswax and rosin, and should be prepared ahead of time and allowed to cool until solid. Heat the wax until it is molten, then dissolve the rosin into it. The mixture can then be poured into small suitable containers to cool (for example, paper Dixie cups which may be torn off after the mixture has set).

The stainless steel electrodes are prepared for implantation as follows: Cut a 38 to 46 cm length of stainless steel wire. Clamp the two ends together with the hemostat. This will form a large loop. Hang the loop from a pencil or other small-diameter dowel and spin the hemostats to twist the wire together. It should be twisted fairly tightly but not so tight that it breaks or kinks. Gently pull on the now twisted loop. Unclamp the hemostats, cut and untwist the previously clamped end so that two suitable lengths of wire remain, approximately 4 cm long. Remove the teflon coating from the last 1.6 cm of the two ends by gently scraping the wire between the tips of the jeweler's forceps.

Check the electrode with the P–15 pre-amplifier as follows: Attach the uncoated ends of the electrode to the test clips which are connected via shielded wire to the differential inputs of the pre-amplifier. Set the pre-amplifier for a gain of 100. Set the oscilloscope vertical gain for 5 mv division. Set the oscilloscope sweep speed for 5 millisec/division. Check to see that the pre-amplifier output is connected to the input of the oscilloscope. With the electrode in place in the test clips, switch the pre-amplifier on. The oscilloscope trace should remain flat indicating that the differential inputs of the pre-amplifier are shorted through the electrode. Next, cut the looped end of the electrode, this will break continuity and should produce a large amount of 60 cycle electrical interference on the trace. If the trace remains flat make certain that it is not because the pre-amplifier has become saturated. If the trace continues to remain flat it probably indicates that the electrode is shorted together at some other point. If the situation cannot be remedied by shortening the electrode 2–3 cm, discard it and produce another.

The electrode is now almost ready to be implanted in the grasshopper and is also attached to the amplifiers correctly. Switch the pre-amplifier off. Trim the end of the electrode which will be implanted in the grasshopper so that two even stubs remain and scrape a tiny length of insulation off of these ends. The electrode is now ready to be implanted in the grasshopper.

The electrode implanting operation is performed under a dissecting microscope. The grasshopper should be attached, dorsal side down, to a small movable platform using dental wax, making sure that its legs are secured but leaving its visual field unobstructed. The hopper may be anesthetized by placing it in a refrigerator for about ten minutes.

Place the hopper on the stage of the dissecting microscope. Using the 26-gauge needle, make two superficial punctures approximately 1 to 1½ mm apart, through the cuticle of the prothorax along the midline of the animal. A small amount of green blood should appear. Carefully insert an electrode tip into each hole. This may take some practice. Figure 9.5 shows this preparation.

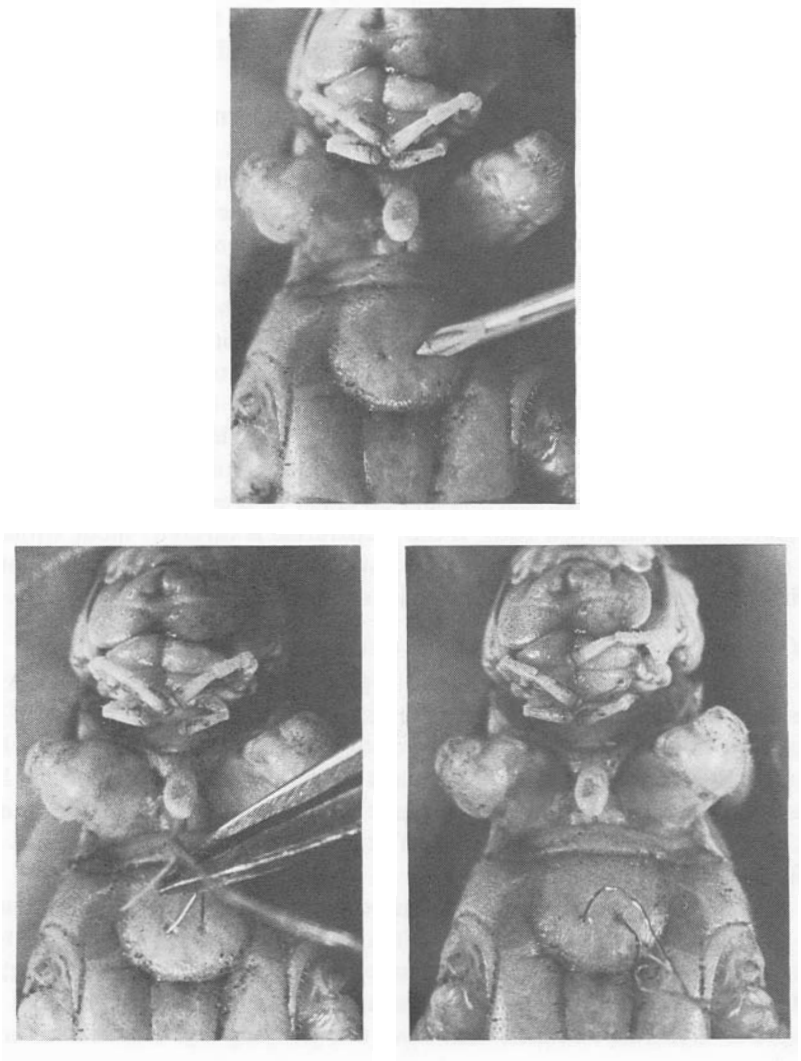


Figure 9.5. Procedure for placement of electrodes.

Set the P–15 amplifier to 300 Hz on the low pass filter and 10 kHz on the high pass filter. Switch the pre-amplifier on. If the electrodes are near the ventral nerve cord, its activity will be recorded. Try stimulating the DCMD by moving your hand in the visual field of the locust. A sharp train of pulses will occur with each novel hand movement. The electrodes may be carefully moved deeper or shallower to improve the signal. DCMD signal strength of 1 division at 5 mv/division on the oscilloscope is good. If the electrodes are too deep, a regular large deflection will occur. This is the contraction of the heart muscle. Shallow electrodes are usually indicated by high impedances, microphonic leads, and 60-cycle interference.

Once implanted, the electrodes are sealed and cemented to the thoracic plates of the locust using the beeswax rosin mixture. Heat an edge of the beeswax rosin mixture over the alcohol lamp until it is slightly molten. Scrape off a small quantity of the wax rosin mixture onto the end of an applicator stick. With the electrodes in place and pre-amplifier on, re-heat the mixture on the end of the applicator stick until it just flows. Carefully drip a small amount of the mixture on the area where the electrodes are implanted and let it harden. Check to see that the electrodes are functional. Fold the wires so that they lie flat across the thorax and place another drop of wax on the wires for added strength. If the legs are accidentally waxed into a permanent position, they may be freed by carefully chipping away the wax.

Once the wax rosin mixture has hardened, the animal may be removed from the restraining wax. Care should be taken to prevent the hopper from jumping against electrode leads. If the preparation is carefully made without too much trauma to the animal, it will last one week or more, up to a month, without noticeably affecting the behavior or health of the animal.

C. *Grasshopper Leg Twitch—Method of Preparation*

The jumping legs of grasshoppers and locusts contain a powerful set of muscles which, in conjunction with the animal's skeletal system, are used to propel the animal away from danger or to jab would-be attackers with leg spines. Through the use of paired stimulating electrodes implanted in the leg muscles, contractions are initiated by electrical stimulation. Careful placement of the electrodes allows stimulation of both flexor and extensor muscles at different voltage settings.

Catch a lively grasshopper and remove a jumping leg from it by grasping the leg between the index finger and thumb while allowing the rest of the animal to move freely. This usually causes the animal to automize the leg; if it does not release the leg, gentle twisting and pulling may be required to remove it. Generally, animals that have two jumping legs will release one,

while those with only one jumping leg will require more persuasion. It is not advisable to remove the leg with scissors, since this will cause the animal to bleed profusely at the leg stump and possibly damage the jumping leg at the anterior end of the femur.

Once the leg has been removed, the second step of the preparation may begin. The materials needed for this may be as sophisticated as a standard electrophysiological stimulation set-up or as simple as a contact-switch, potentiometer, and nine-volt battery. These components are hooked up in series to pass a variable current through the stimulating electrodes. Special equipment includes fine wire or insect pins (these will serve as stimulating electrodes), #5 jewelers forceps, dental wax, some light-gauge hookup wire, and attachment clips. These materials are the same as those specified in the methodology section of the "Watchful Grasshopper".

Implanting the electrodes is a relatively easy procedure. Anchor the femur in a small piece of dental wax so that the tibia is free to move from a fully contracted position to a fully extended position. This is usually accomplished by placing the femur ventral side up, or laid sideways, so that the tibia moves in a horizontal plane. Using the tip of the #5 forceps or a dissecting pin, poke two small holes through the exoskeleton, one hole located several millimeters from the anterior end and the other several millimeters from the posterior end of the femur on either the dorsal or ventral surface. See Figure 9.6. Connect the electrodes to the stimulating device and insert them 2–3 mm into the holes in the femur. Set the voltage at zero level on the stimulator. If a standard electrical stimuliator is being used, adjust the device for delivery of a pulse of 1 millisec duration several times a second. If a contact-switch and battery device is being used, pulse the switch with your finger in such a manner as to produce short electrical pulses of approximately one millisec in length. Gradually increase the voltage of the stimulating pulse until the leg muscles begin to contract. This usually happens at two to three volts. If the muscles do not begin contracting with each pulse, increase the voltage or poke the wires in a tiny bit further. If the leg muscles contract but only partially, the electrodes may be in too deep and thus interfering with the muscle activity. To correct this, pull them out a tiny amount. If stimulation still does not occur by this point, check the electrodes to make sure they are clear of insulating material which can interfere with electrical conduction.

At lower voltage settings, only the flexor muscles contract, causing the leg to bend inwards. At higher settings, the extensor muscles contract, causing the leg to extend. It is probable that the leg extension which occurs at higher voltage levels happens because both the flexor and extensor muscles contract, but the larger extensor muscles are able to overpower the flexor muscles. It is these powerful extensor muscles which are actually used in jumping.

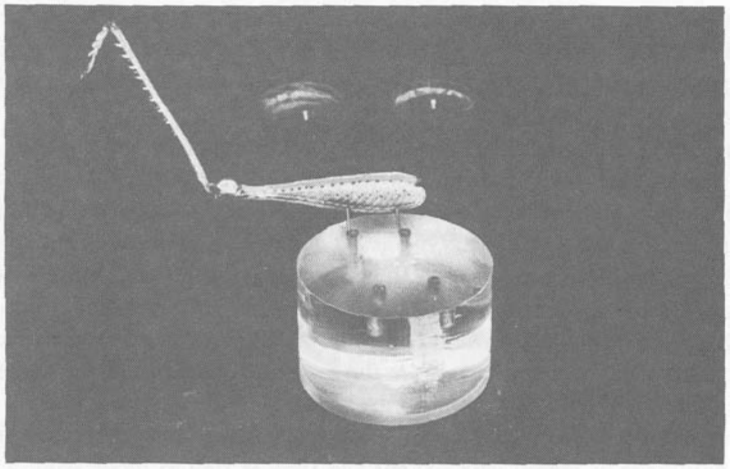


Figure 9.6. Position of leg on electrodes.

Further experimentation may be conducted on the leg once initial stimulation observations have been made. One can move the electrode to new positions, measure the amount of force that the leg muscle exerts on the tibia, observe muscle tetanus, and determine threshold voltages. With minimal care, the leg will continue to function for many hours after removal from the animal.

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