Teaching insect retinal physiology with newly designed, inexpensive micromanipulators

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Krans, Jacob, Cole Gilbert, and Ron Hoy. Teaching insect retinal physiology with newly designed, inexpensive micromanipulators. Adv Physiol Educ 30: 254–261, 2006; doi:10.1152/advan.00029.2006.—In this article, we detail how to produce two inexpensive micromanipulators that offer high precision (~25 μm) along a single axis of movement. The more expensive of the designs provides improved versatility along multiple axes. Both manipulators offer substantial savings over commercially available micromanipulators with comparable capabilities. Plans and instructions are given such that a novice can produce the manipulators with simple tools. The manipulators are designed to serve undergraduate teaching exercises in physiology. An electroretinogram exercise is suggested in adult house flies (Musca) or flesh flies (Neobellieria). Measuring the intensity-response function and temporal characteristics of visual transduction are discussed. A brief introduction to the field of visual transduction and the physiology of the laboratory exercises is provided as well.

-electroretinogram; vision

TO MAKE CUTTING-EDGE AND CONTEMPORARY SCIENCE ACCESSIBLE TO teaching institutions on limited budgets, the Cornell University neuroscience development team has developed inexpensive alternatives to equipment used in neuroscience, genetics, and physiology. These include an electrical stimulator (8), amplifier and suction electrode (9), and temperature control device (5), all of which are suitable for teaching laboratories and can be produced for under $50. With the advent of computer-based oscilloscopes and inexpensive IO boards, one of the few remaining essential components is a micromanipulator. The manipulators we describe here can be used in place of expensive (i.e., >$400) commercially available micromanipulators in many teaching exercises that require precise positioning. These might include crayfish abdominal recordings, both intracellularly from superficial musculature and extracellularly from nerves (17); intracellular recordings of voltage changes in plants (4); calibrated displacement of cockroach tibial mechanoreceptors (19); and force recordings of various tissues (with a force transducer), including skeletal, cardiac, and smooth intestinal muscle. These manipulators are excellent for extracellular recording and stimulation applications; however, they can also be used to make intracellular recordings from the body wall musculature of many arthropods. The muscles of the crayfish and larval fly are particularly accessible with these manipulators and have been utilized in established teaching exercises (6, 16). A new application of teaching interest, on fly vision, is described in the latter portion of the article.

Commercial micromanipulators with precise movement in three dimensions (3-D) cost between $400 and several thousand dollars, whereas single dimension (1-D) manipulators are available for $125–500 (Table 1). If more than one manipulator is needed per rig or station in a teaching setting, the cost can reach several thousand dollars. Moreover, the favorite models of physiologists, those that have lasted generations and continue to require minimal service, are not the inexpensive models but cost in excess of $1,000. Here, we introduce alternatives that meet the basic requirements and can be produced at relatively low cost.

Making an inexpensive manipulator is difficult in part because of the accuracy and stability required of many classic experiments in neuroscience and physiology. Additionally, even basic commercial micromanipulators offer movement in two dimensions (2-D) or 3-D, which is difficult to replicate for those without technical expertise or access to a machine shop. The least expensive manipulator described here consists of a flat base with an extendible sliding section that is advanced via a micrometer head unit. This type of manipulator, referred to as the “sliding base” design (Fig. 1), advances with high precision (~25 μm) along a single axis and can be produced from readily available materials for under $15.

A second, slightly more expensive design offers improved versatility and compactness. Because this manipulator does not rely on a base, it is referred to as “floating.” The positioning of this manipulator is more flexible because of its mounting system, which makes use of swivel clamps and bars found in most undergraduate science laboratories (see Table 1, Aluminum stock and miscellaneous materials). The floating manipulator can be assembled for around $80, about half that of the least-expensive commercial single-axis alternatives. Our design offers an advantage that most 1-D commercial units do not: its low profile is valuable in the space around a biological system, which makes use of swivel clamps and bars found in most undergraduate science laboratories (see Table 1, Aluminum stock and miscellaneous materials). The floating manipulator can be assembled for around $80, about half that of the least-expensive commercial single-axis alternatives. 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1 All prices are estimates, given in United States dollars, at the time of publication.

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supply, Burlington, NC), but several interesting mutants must be requested from the investigator or laboratory responsible for

The laboratory exercises described here are designed to achieve two educational goals: one technical and the other biological. The technical goal is to efficiently introduce students to an electrophysiological setup with automated stimulation and data gathering. Many electrophysiological experiments are complex, requiring students to first master a dissection and then carefully arrange and monitor the electrode interface between the wet preparation and the electronics. Much of the time during the laboratory period is spent on such activities, and less time is available for developing an understanding of the operational characteristics of electrophysiological equipment. The exercise described below has minimal dissection, and the electrode placement is trivial. Within 15 min, students are ready to collect data and can thus focus much more time on developing a deep understanding of how the amplifiers and data-collecting software operate that will be required for this and subsequent electrophysiological exercises. In the Physiology course regularly taught by one of us (C. Gilbert), this has always been the first exercise of the semester,

Table 1. Sources for commercially available products and components referenced in the text

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Prices micrometer are given in United States dollars at the time of publication. Suppliers were located using on-line search engines at the time of publication. To facilitate micrometer searches, key words of interest may include the following: blade micrometer AND non-rotating spindle OR micrometer head. PN, part number; SKU, stock-keeping unit number. *Items may not be in stock at the time of purchase, which we found was particularly pertinent to purchasing a nonrotating spindle micrometer. On-line auction sites (e.g., eBay) intermittently list these at a substantial savings.
and students have enjoyed the ease with which they were able to collect data.

The second goal is biological, namely, to investigate several important properties of sensory systems that are ubiquitous in animals. Thus, there is broad generality to investigating such sensory properties as intensity-response functions, effects of adaptation, and temporal dynamics. Students are introduced to these concepts as well as the fact that neural information in the first stage of sensory processing is carried by graded electrical potentials rather than the action potentials that they will see in subsequent exercises. Thus, the study of the sensory system lays the groundwork for examining nervous signals in the central nervous system and then finally at the neuromuscular interface. These sensory properties are easily recorded in the insect visual system as an extracellular electroretinogram (ERG): the summed electrical potentials of photoreceptor cells and first-order interneurons. The biological interface between the fly and recording equipment is readily achieved, the physiological phenomena are robust, and the preparation can last for hours, which allows students to focus on building competence with the data collection equipment and on exploring the biology of sensory systems.

MATERIALS AND ASSEMBLY

**Sliding base design.** The first manipulator design relies on a simple aluminum base and sliding tab (Fig. 1) and can be assembled from widely available parts for less than $15. The manipulator functions by extending and retracting a thin tab through an aluminum base. Plans for cutting the aluminum pieces necessary to assemble the sliding base manipulators are given in Fig. 2. These plans can be enlarged or reduced and transferred to raw materials as cutting patterns, which reduces the number of measurements required for assembly.

The sliding base manipulator shown in Fig. 1A is assembled from precut aluminum, which offers very straight edges and thus reduces unwanted lateral movements as the slide extends within the base. Appropriately sized aluminum is available at many home improvement and hardware stores and can also be purchased online at low cost. For example, the website www.doityourself.com sells extruded aluminum in the desired size that is small enough to avoid heavy freight shipping costs. To produce three slide-based manipulators, the following aluminum flat bar stock is necessary: one piece of 1/16 in. × 1/2 in. × 3 ft (thickness × width × length) and one piece of 1/16 in. × 1–1/2 in. × 4 ft (less than $12 for both pieces, shipped; Table 1). Purchasing presized pieces of stock aluminum minimizes the number of cuts required to assemble the sliding base. We used a Dremel rotary tool (www.dremel.com/en-us/tools) to make the necessary cuts. Rotary tools are available at many home improvement stores for between $30 and $50, but any cutting tool (e.g., a “hacksaw”) will suffice. Finally, there is little price difference between a larger piece of sheet stock and the flat bar stock specified above.

Parts of the design that influence the efficacy of the sliding mechanism, which is vital to the precision of the manipulator, are labeled on the design plans shown in Fig. 2. Part a (gray; Fig. 2) is the hind structure, or “base,” in the photograph shown in Fig. 1A. Parts b and c (black; Fig. 2) are glued to the bottom of part a; only their forward-most sections are visible in the photograph shown in Fig. 1. These two pieces define the edges of the channel through which part d (Fig. 2), the “sliding tab,” moves. The distance between parts b and c should be precisely the width of the sliding tab, part d. Using part d as a place-holder—tightly sandwiching it between parts b and c—is an effective way to size the channel made by parts b and c. This technique provided a favorable compromise between snugness and the ability to slide freely. Care should be taken to keep glue away from the sliding tab when attaching parts b and c to the underside of part a. Superglue (LocTite, Henkel, Rocky Hill, CT) was used for this application and to hold the micrometer head unit to the base. Various other glues, including those with longer curing times and epoxies, could also be used.

This design utilizes a low-cost (less than $10; Table 1) but high-precision micrometer head unit. The least-expensive micrometer head units come within a frame (Fig. 3A, the C-clamp-like structure). The frame was removed by making a single, shallow cut. Removal of the plastic housing and locking apparatus (Fig. 3B) permits clamping of the frame in a vise or to a secure surface, which simplifies cutting off its sleeve portion (Fig. 3C). Mounting the micrometer head to the manipulator’s base is accomplished in two steps. First, a hole is drilled in the vertical portion of the base (Fig. 1). The micrometer’s spindle is inserted through this for support. The diameter of the hole must accommodate tilting the micrometer head and thus should narrowly exceed the diameter of the spindle being.
placed through it (spindle = 8 mm; hole = 10 mm). Second, a strut is glued to both the base and micrometer head to support its weight. This bracing is positioned about 1/2 in. behind the vertical portion of the base (Fig. 2). The exact size and shape of the brace should be determined empirically by placing the micrometer head unit through the hole in the base’s vertical support and tilting it upward. Tilt is incorporated to facilitate turning the micrometer by hand without bumping the base, but should be minimized so that when the micrometer head is fully extended its spindle remains above the bottom of the slide. It is important that the micrometer’s spindle not push vertically upon the slide; this could lift the device during use.

Electrodes can be mounted directly to the vertical portion of the sliding tab (Fig. 1B). A rounded cutout can be made using a rotary cutting tool with a grinding attachment. This increases the rigidity of the contact between the electrode and tab (e.g., using a banana plug as in Fig. 1B). This assumes that the electrode has a round, or tubular, portion, which is the case with common electrodes, such as the electrode holders and headstage units from AxoClamp (www.axon.com) or A-M Systems (www.a-msystems.com). The sliding tab can be modified to accommodate any electrode type. A small spring provides tension on the slide and facilitates its retraction into the base (Table 1, Aluminum stock and miscellaneous materials). Each end of the spring is connected by insertion through a small hole drilled in the aluminum and then secured using an epoxy. Recoil is further facilitated by a thin layer of Vaseline or oil between the slide and base. Blue periphery wax (Surgident, Heraeus Kulzer, NY) was applied over the edges of the completed manipulator (parts b and c; Fig. 2) to secure it to the work surface.

Floating design. The floating design manipulator is shown in Fig. 4. Micrometer head units with nonrotating spindles are the basis of this more versatile and expensive design. The spindle of these micrometers does not turn as it extends, which distinguishes it from the less-expensive micrometers utilized above. We found nonrotating spindle micrometers priced at $70 or more (Table 1). Various electrode types can be mounted to the micrometer’s spindle by way of a simple adaptor. The nonrotating nature of the spindle is thus essential to this manipulator’s design. By mounting the micrometer head unit to standard laboratory bars and clamps (Fig. 4A and Table 1, Aluminum stock and miscellaneous materials), the degrees of freedom increase significantly. This permits the use of the manipulators in tasks that require particularly steep mounting angles to reach nerves, muscles, or tissue that is deep within a preparation. This versatility is instrumental in approaching a tissue normal to its surface, as in puncturing the cornea of adult flies (described below).

The adaptor necessary for mounting electrodes to the spindle can be customized to meet the requirements of a particular electrode type. An adaptor that is both simple to produce and is compatible with a large assortment of electrode types is shown in Fig. 4B. A 1-ft length of 3/8 3/4-in. aluminum bar stock (Table 1) would suffice to produce 10 adaptors. The spindle diameter of Mitutoyo micrometer heads (Table 1) is precisely 8 mm. Drilling an 8-mm hole through a 1-in. length of this aluminum stock allows for pressure, or friction, mounting of the aluminum block to the spindle. A channel in the top of the aluminum block provides an area for mounting elec-

![Fig. 3. A–C: steps in the process of removing a micrometer unit’s frame and sleeve so that just the “head unit” can be used in assembly. A: plastic housing of the frame portion of the micrometer has been removed. B: an arrow shows the cut through the area where the locking apparatus once was, which is the thinnest section for cutting. C: the head unit removed from the frame portion.](image)

![Fig. 4. Floating design manipulator. A: the nonrotating spindle micrometer head is attached to a standard rod clamp, rods, and magnetic stand. Scale bar = 1 in. B: the adaptor from the spindle to electrode is shown holding a tungsten electrode housed within a syringe tip and connected via a banana plug. The sharpened tungsten rod is inserted into a 26.5-gauge syringe tip such that it extends out each end. The banana plug is then inserted into the opening of the syringe tip, allowing for future changes of the electrode. The junction between the electrode holder and manipulator is shown by an arrow, which also indicates the use of periphery wax. Scale bar = 0.5 in.](image)
telescopes (Fig. 4B). This channel can be made by drilling a second hole and opening it by cutting along its diameter (Fig. 4B). Blue wax was used to affix electrodes to the extending portion of the manipulator in both designs.

**STUDENT EXERCISES AND RESULTS**

We tested the manipulators in the context of teaching the electrophysiology of sensory systems, by investigating the visual system of adult flies. Each manipulator design provided suitable precision and stability in these experiments. The exercises outlined below demonstrated that the manipulators function particularly well in confined space, supporting their utility with flies, other insects, and generally small preparations. Data were gathered from the house fly (*M. domestica*). In our experience, flesh flies (*N. bullata*) work as well.

**ERG exercise.** The means by which sensory information from the periphery is communicated to the central nervous system varies widely with sensory modality, species, and evolutionary and environmental factors. Nevertheless, most sensory systems share certain organizational principles. Three examples of broadly applicable sensory principles are intensity-response functions, which describe the increase from threshold to saturation with increased stimulus intensity; adaptation, which can influence the response to equivalent stimuli; and temporal dynamics, which can affect the encoding of changing environmental conditions and the rate of information transfer to the central nervous system. The visual system of adult flies is a salient and accessible preparation with which to investigate these principles ofafferent transduction. Further background information on the visual system of flies can be found in Refs. 2, 10, and 16.

The simplest method of acquiring information about the physiology of the visual system is to record extracellular ERGs (for reviews, see Refs. 7 and 18). The ERG is easily recorded by measuring the voltage difference between an active electrode placed in the eye and a ground electrode placed in the dorsal region of the head, medially to avoid recording the activation of photoreceptors (Fig. 5). ERGs represent the summed electrical activity of many photoreceptors, and in some organisms, including flies, the response may include that of visual interneurons. In flies, the typical ERG waveform in response to a square-wave illumination is a negative plateau of ~2–8 mV. The plateau portion of the response is contributed by the photoreceptors; positive ON and negative OFF transients may appear (Fig. 6A). These are generated by first-order visual interneurons, so-called lamina monopolar cells (1). In our hands, a superficial penetration of the eye minimizes transient potentials and optimizes the plateau portion of the ERG.

The preparation is simple and the interface with the equipment is straightforward (Fig. 5). Flies can be immobilized by chilling them (~0°C) for a few minutes. The bases of the wings and legs can be immobilized and the head fixed to the thorax using a low-melting-point wax, such as dental wax (Ted Pella, Reading, CA), which can be melted and applied with a low-temperature fine-tip cautery (Acuderm, Ft. Lauderdale, FL). Flies can be mounted on any sort of holder, such as a toothpick, wooden applicator stick, or small rod, using a dollop of wax. Placing the fly on warmed wax alleviates the necessity for leg removal by restricting movement upon cooling and solidification of the wax. In mounting the fly, care should be taken to avoid covering the compound eye with adhesive. The active electrode should be placed just beneath the cornea (Fig. 5). Sharp electrodes, such as 0.2-μm tungsten rods (A-M Systems) electrolytically etched to a fine tip (12), work best, but we have also had success with narrow-gauge silver wire that has been chloridored. Silver wire is easier to manipulate and solder, whereas tungsten, which defies soldering, remains sharp and more easily penetrates the cornea. When using blunter electrodes, it is desirable first to make a hole in the cornea with a stiff, sharp pin, such as a “00” insect pin, and then insert the electrode into the hole. If the cornea dries a bit, simply let it come back to convexity; the preparation is very robust. The electrode penetrates more efficiently if it is more perpendicular to the corneal surface. In principle, the ground electrode can be placed anywhere in the fly, but in practice it is best to avoid areas of muscle that the fly may contract in synchrony with the light stimulus. The back of the head is free of muscle and is a good location for the ground (Fig. 5). Finally, most direct current (DC) amplifiers should suffice to amplify ERG potentials for display on an oscilloscope or monitor. Two amplifiers that we have used successfully are the Warner DP-301 (Warner Instruments, Hamden, CT) and model 1600 Neuroprobe (A-M Systems). Alternatively, basic DC amplifiers can be constructed simply and assembled for very little cost (e.g., using an operational amplifier (http://en.wikipedia.org/wiki/Operational_amplifier]). The ERG signal could also be taken directly into an analog-to-digital converter and processed by associated software with adequate gain, such as iWorx (Dover, NH).

A variety of physiological investigations are possible with this preparation, and we outline only three exercises here. Intensity-response functions can be determined from a series of stimuli with a light source at various intensities or distances from the eye. This experiment can be done with the room lights on, but allow at least 15 s between stimuli. The typically sigmoid function is below threshold at low stimulus intensities, proceeds through threshold, and rises steeply up to saturation at brighter intensities (Fig. 6B). Depending on the light source, saturation may be difficult to achieve, but we achieved satura-
tion with a 10-mm-diameter green LED, available from any large supplier of electronic components (Table 1, Aluminum stock and miscellaneous materials). They can be driven with a 9-V battery through a dropping resistor and switch or from the digital-to-analog output port of a software program such as iWorx. Maximum intensity was achieved by setting the LED as close as possible to the cornea, near the electrode. Submaximal intensities can be achieved by reducing intensity directly or by moving the light source progressively farther away from the eye. One caveat is to be wary of changing the spectral properties of the light source. Simply dimming an incandescent bulb induces a red spectral shift that confounds stimulus intensity with wavelength. The fly visual system has photopigments with absorption maxima centered on UV, blue, and green wavelengths; thus, using white light or a green LED are the most efficient stimuli (2).

A variant of this basic exercise is to investigate the effect of adaptation. The initial exercise, as described above, was done on the laboratory bench with the room’s light on. The eye was therefore in a light-adapted state. A dark-adapted state can be accomplished by surrounding the preparation, light source, and electrode in aluminum foil to make a quasi-light-tight housing. Repeating the series of stimuli at different intensities will produce another intensity-response function that is shifted due to the increased sensitivity of the dark-adapted eye (see Ref. 10).

Finally, the temporal dynamics of the basic ERG response can be investigated by stimulating at different frequencies using the digital-to-analog output function of a software package or driving the LED with an adjustable square-wave generator, such as is available from Grass Telefactor (West Warwick, RI) or could be fabricated following circuit diagrams readily available on the internet. The ERG response is fully modulated from the baseline by single flashes, but, as the interstimulus interval shortens, the response becomes smaller and eventually fuses at high frequency (Fig. 7). The precise frequency at which modulations fuse will vary depending on the intensity of the stimulus, adaptation state, etc. Nevertheless, it will be obvious that the eye of the fly is faster than the human eye, as the stimulus will look fused, i.e., constantly illuminated, to stu-

Fig. 6. Intensity-response function. A: superimposed electroretinograms (ERGs) from an adult house fly. Each trace (gray) is the ERG response to a 1-s pulse of green light. Black lines are low-pass-filtered (Butterworth) averages (10 trials each) of the steady-state portion of the ERG. ERG voltage increases with proximity to the light stimulus (inset: distance from the eye, corresponding to the top-down order of traces, respectively). ON and OFF indicate transients likely generated by first-order interneurons (see text). B: ERG amplitude plotted as a function of stimulus distance from the eye (intensity).

Fig. 7. Temporal dynamics. A: effect of stimulation frequency on the amplitude of the ERG. Three examples of stimulus rate are given: 2, 10, and ~20 Hz (top to bottom). The time between light pulses, or interstimulus interval (ISI), is shown in gray, as is the ERG amplitude. B: ERG amplitude plotted as a function of ISI.
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students at a much lower frequency than occurs for the fly. A 40-ms pulse of illumination is long enough to unambiguously identify the ERG response but short enough to test frequencies up to nearly 25 Hz, which exceeds the human flicker-fusion frequency.

Data analysis is relatively straightforward. Data are plotted as amplitudes of the ERG versus independent variables (Figs. 6B and 7B). Intensity-response functions are typically plotted as voltage versus the log of intensity. We have chosen to show the controlled (independent) variable on a linear axis (Fig. 6B). This is because the students manipulated distance rather than intensity in our setup. The plot can be converted to a standard intensity-response plot by knowing that the intensity drops off as the square of the distance. Temporal responses can be plotted simply as the amplitude versus the interstimulus interval (Fig. 7A). It may be of interest to more advanced or computational courses to view the temporal data as relative amplitude of the response, or gain (in decibels), plotted over the frequency (in Hz). Stimulus frequency is the reciprocal of the combined interstimulus interval plus stimulus duration. Gain is 20 times the base 10 logarithm of the quotient of the measured response at a given stimulus frequency divided by the maximum response measured at the slowest frequency. The resulting log-log plot of gain (in dB) over frequency (in Hz) is referred to as a Bode plot and can be used to characterize the frequency response properties of any system (11), not just sensory systems. Students can be encouraged to employ such a systems approach in their thinking about the retina. For instance, the temporal dynamics of the ERG demonstrate that slow changes in light intensity are faithfully reproduced and faster ones are attenuated. Thus, the retina acts as a low-pass filter of light signals, similar to a setting on their amplifiers or software that attenuates electrical signals. Students might also be encouraged to think about what the world of the fly looks like as it flies through an environment of light and dark objects, such as vegetation, that are flickering on its retina. At slow speeds, it will resolve the world adequately, but, at higher flight speeds, the retinal response to such flickering will be attenuated and the fly rendered “blind.” Finally, with all exercises, it will be useful to analyze the amplitudes of the transients separately from those of the plateau since they are derived from separate cells that may have different response properties (e.g., Fig. 7A, compare top and middle traces).

DISCUSSION

We believe that the cost-benefit relationship of these manipulators makes them an enabling technology for the teaching laboratory. In our experience, operation of these manipulators is transparent and can be grasped quickly by students. Students trained to use the manipulators discussed here required no explicit instruction and effectively utilized the devices promptly.

The preparation of interest and the tissue being observed or recorded from are primary considerations when choosing a manipulator. These manipulators are not designed for demanding and sensitive experiments or research neuroscience. However, in our hands, the floating design provides sufficient stability and versatility for research-grade recordings from insect muscle fibers. The potential applicability of these manipulators is broad. Combining sliding base and floating design manipulators with a standard high precision 3-D manipulator, for just the most delicate electrode(s), still offers a considerable cost savings. A well-equipped rig might consist of two sliding base manipulators and two floating manipulators, which can be assembled for about $180 total: nearly an order of magnitude less expensive than four conventional micromanipulators (more than $1,600; Table 1). Moreover, outfitting teaching laboratories with the less-expensive manipulators will reduce wear on expensive commercial manipulators, if these are already present.

Fabricating devices in the laboratory interests some undergraduates and illustrates one of the practical considerations of physiology research: designing and/or generating custom equipment. Production of these manipulators is at a reasonable level of complexity for undergraduates to tackle in a single week’s laboratory period (3–4 h); once students are experienced, ~1 h is required to make each manipulator. For the sliding base design, Plexiglas may be substituted for aluminum since it may be cheaper or easier to cut and glue together. We have produced two manipulators using Plexiglas, each of a different size. They offer nominally reduced stability, and their production requires more cutting than aluminum, but are otherwise comparable. A consideration when using Plexiglas is the means of joining small pieces. Methylene dichloride is a solvent commonly used to join Plexiglas. It can be purchased at local hobby stores or from a chemical supply company such as Fisher Scientific (item no. D138-1).

Most commercial micromanipulators use a dovetail slide to control unwanted movement outside the axis of extension. The sliding base design described here uses a simple laterally restricted slide for this purpose, whereas the floating design has implicit restrictions upon extra-axis movement: the spindle advances along a single axis. Making corrections in position while approaching a tissue is helpful in recording from small preparations. This is accommodated by approaching tissue slowly when using either of the manipulators discussed here. Since most preparations used in teaching exercises (e.g., insect, crayfish, turtle, or frog) offer fairly long periods of viability, spending extra time placing electrodes is unlikely to be a major limitation. Additionally, the design of the slide is easily modified to suit a particular application and/or movement parameter. Its size and shape can be changed by adjusting the size and/or aspect ratio of the plans described here. For example, a steeper and taller sliding base manipulator may be more effective for larger crustacean preparation dishes.

The fly ERG is a valuable preparation for exploring the physiology of sensory systems. It is incredibly robust and can last for more than 24 h, given occasional feeding of the fly with a 12% (wt/vol) sucrose solution, thus allowing the investigation of circadian rhythmicity in addition to more typical properties of sensory systems. In a conventional laboratory session, much time is spent on technical aspects of the preparation, especially when small organisms are studied. With the fly visual system, however, most students acquired a good recording in minutes and then devoted the rest of the laboratory session to developing competency with the equipment and making discoveries about sensory physiology.

Flies are comparatively smaller in size than mainstays of physiology and/or neuroscience laboratory education, but some, such as D. melanogaster, offer powerful and novel insights into the role of genetics in physiology. As students...
become comfortable with the small scale required of adult fly recordings, a natural extension is to introduce fruit fly mutants. There are many mutants of the visual system that offer robust phenotypes and varying degrees of gene ontology documentation (for a review, see Ref. 13). The eye color mutants (Carolina Biological Supply) are one example that, when combined with varied wavelength stimuli, illustrate a fundamental concept of visual transduction. These offer students the opportunity to bridge concepts from genetics to physiology and behavior. We are currently developing such exercises.

The manipulators and exercises described here offer accessibility to fundamental characteristics of sensory physiology at a small fraction of the cost typically incurred using commercially available equipment.

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REFERENCES