A housefly sensory-motor integration laboratory

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Griff ER, Kane TC. A housefly sensory motor integration laboratory. Adv Physiol Educ 34: 106–110, 2010; doi:10.1152/advan.00068.2009.—Insects have many interesting behaviors that can be observed in an introductory biology laboratory setting. In the present article, we describe several reflexes using the housefly Musca domestica that can be used to introduce students to sensory and motor responses and encourage them to think about the underlying neural circuits and integration of sensory information that mediate the behaviors.

BIOLOGY STUDENTS in introductory courses have an innate interest in animal behavior. Reflexes are relatively simple behaviors that often are used in these courses to illustrate the neural connections and underlying physiological mechanisms linking the sensory stimulus to the motor response. One would hope to find a wide range of laboratory exercises that explore various animal reflexes. However, introductory biology laboratory manuals either do not include experiments to investigate sensory-motor integration (e.g., Refs. 7, 20, and 31) or rely solely on human-based experiments, most commonly the patellar tendon (knee-jerk) reflex and the pupillary reflex (e.g., Refs. 13, 14, 24, and 27). These human reflexes are easy to demonstrate, but the patellar tendon reflex is quite unusual in being monosynaptic, and the circuits underlying the pupillary reflex are very complex, involving neural circuits in several distinct nuclei in the brain. In most laboratory manuals, these human reflex circuits are described as excitation and no examples of inhibitory reflexes are given. Furthermore, the sensory organs for these human reflexes (the muscle spindle and retina, respectively) are quite simple; most laboratory manuals do not describe or discuss this complexity. This article presents alternative experiments that can be used in conjunction with human reflex exercises or in place of them.

The laboratory exercises detailed below let students observe relatively simple behaviors, and they encourage students to hypothesize what neural circuits underlie them, thereby integrating organismal, organ system, and cellular levels. These exercises focus on the housefly, an organism that is easy to obtain and maintain even for large classes (see MATERIALS AND METHODS). Working with a small invertebrate adds a comparative perspective, provides opportunities for experimentation in an inquiry-based learning environment, and presents novel and intriguing anatomic structures. Excitatory and inhibitory responses are observed in the same organism. The exercises below could complement a nervous system, sensory, behavior, or human reflex laboratory in an introductory biology course.

The exercises illustrate general mechanisms underlying sensory motor integration. In addition, this article provides detailed methods and extensive background and references on the studied reflexes so that these exercises can more easily be incorporated into a variety of courses at several levels.

Demonstrating sensory-motor integration in an invertebrate reinforces the important evolutionary principles that all extant organisms have equally long evolutionary histories and use almost identical mechanisms. The presence of taste receptors on a fly’s feet, a proboscis for feeding and drinking, and a vestigial wing called the haltere, which is used for flight stability, also demonstrate unique adaptations in insects that will arouse interest and stimulate curiosity. Houseflies are common, but most students have never looked at them carefully; seeing the compound eye, mouthparts, and sensory hairs at relatively high magnification under a dissection microscope can be an amazing experience. Comparing reflexes and sensory-motor integration in humans and flies should also help students accept the importance of using model systems such as the fruit fly Drosophila melanogaster, the sea anemone Aplysia californica, or the nematode Caenorhabditis elegans to study general principles of animal physiology.

The overall goal is to understand the anatomy and physiology of a reflex by analyzing two examples: the proboscis extension and tarsal reflexes. After completing the exercises, students will be able to 1) identify fly structures such as the proboscis, tarsi, wings and halteres; 2) determine appropriate sensory stimuli for each reflex; 3) hypothesize specific sensory structures and receptors that are required for each reflex; 4) describe the motor responses generated in each reflex; and 5) propose neural circuits that could mediate the reflexes. The objectives can be extended to include the specific structures and muscle groups that participate in the motor outputs.

In my department, these activities were used in the laboratory part of an introductory biology course taught in the freshman year and intended for Biology majors. There were ~500 students in this course with laboratory sections of ~25 students each. The exercises were part of the sensory physiology exercise that also included mapping of sensory receptors on the skin, demonstration of the pupillary reflex, and exercises measuring reaction times under different conditions. If students have already studied some neurophysiology and/or muscle physiology, these reflex examples will reinforce the physiological concepts and apply them to simple behaviors. If not, these reflex laboratories can be used to introduce the nervous and muscular systems.

These laboratory activities can be presented in a traditional format, where students follow written procedures and answer written and/or oral questions based on their observations. The exercises can also be used in a more inquiry-based setting, where students are given questions and/or problems and must develop experiments themselves using equipment and supplies that are provided to answer the questions or test the hypothe-
ses. A few specific suggestions are given below with the description of the procedures.

This laboratory examines a proboscis extension reflex and a tarsal reflex of a housefly, *Musca domestica* (Phylum: Arthropoda; Order: Insecta; Class: Diptera). A reflex is a relatively simple behavior triggered by a sensory stimulus. The sensory stimulus typically excites a sensory neuron that synapses onto one or more interneurons in a ganglion. The interneurons, in turn, synapse onto motor neurons that innervate the muscles involved in the behavior. Motor neurons typically receive hundreds of synaptic inputs, including input from the interneurons involved in the reflex being studied, interneurons involved in the other reflexes, and interneurons from central motor control areas. The activity (frequency of action potentials) of the motor neurons reflects the summation of active inputs from the interneurons (plus any effects of autocrine or other paracrine agents at the synapse), which, in turn, controls the activity of the target muscles. Thus, the sensory stimulus via the interneurons contributes to the level of excitation or inhibition of the target muscles.

The reflexes in this exercise use chemical or mechanical stimuli that interact with receptor proteins that transduce the stimulus into a receptor potential; transduction occurs in the receptor end of the sensory neurons. This depolarizing change in membrane voltage, in turn, triggers action potentials in the axons, which are conducted to the ganglion, where, via chemical synapses, the activity of interneurons and motor neurons is modulated. In some cases, the result of sensory stimulation is a clear excitation of the interneurons and motor neurons (e.g., Ref. 22).

The basic structural and functional unit for mechanoreceptors and chemoreceptors in insects is the sensillum. Gustatory sensilla typically contain two to five sensory neurons, e.g., one responding to a sugar, another to salt, and one to water (5). Mechanoreceptors are subdivided into hair sensilla, which respond when hairs (setae) are moved by contact or air flow, and dome-like campaniform sensilla, which respond when the cuticular dome is deformed, e.g., by movement of the wings. There are also stretch receptors and chordotonal organs (also called scolopophorous or scolopidial organs) (5, 9, 28).

The proboscis is a structure found in insects such as butterflies (Class: Lepidoptera), bees (Class: Hymenoptera), and flies that sucks fluids. It consists of a thin hollow tube or food channel and sometimes a second channel to deliver saliva to liquefy food. The proboscis has three sections and in its resting position is folded (or coiled in butterflies) beneath the head. When food (such as sucrose) is sensed, the proboscis extension reflex is triggered (third section) of the proboscis open, and muscles in the pharynx depolarize the neuron and trigger action potentials. Campaniform sensilla consist of a dome, which is deformed to trigger the overlying cuticle; chordotonal organs respond to distortion of the cuticle during movement (5).

The tarsal reflex involves mechanoreceptors on the tarsi, where there are different types of mechanosensitive sensilla, mostly hair (most often trichoid) sensilla and campaniform sensilla (5). When a hair (or seta) is moved, it distorts the membrane of a sensory neuron, opening ion channels that depolarize the neuron and trigger action potentials. Campaniform sensilla consist of a dome, which is deformed to trigger action potentials. Control of rhythmic wing movements depends on activation of stretch receptors as well as other mechanosensory sensilla such as the chordotonal organs. The latter consist of a sensory neuron, an enveloping cell, and an attachment cell that connects the sensillum to the epidermis of the overlying cuticle; chordotonal organs respond to distortion of the cuticle during movement (5).

In Diptera, the hind wing is modified to a structure called the haltere, which is used to provide sensory inputs to maintain stability during movement and in flight. In French, haltere means barbell or dumbbell, referring to the shape of this small organ (see Fig. 1). At its base are groups of campaniform sensilla. During flight, the halteres beat at the same frequency as the wings (but out of phase); the end of the haltere moves in an arc so that the halteres act as miniature gyroscopes. The
sensory neurons near the base detect yaw (rotation about the vertical axis) and pitch (rotation about the transverse axis) as the insect flies and provide feedback to stabilize flight (e.g., Ref. 5). The halteres also provide sensory input to maintain the head and eye position during walking and/or flight (26).

MATERIALS AND METHODS

Materials. The following materials are needed for each group:

- A 4-lb test monofilament line, 4-in. length
- A paper clip
- A votive or household candle
- A 50-mm plastic petri dish with cover
- A 85-mm plastic petri dish or slide with wells (such as a blood-typing slide)
- Water and sugar solutions in dropper bottles
- Live and dead flies (M. domestica or other larger flies)
- Frozen 4 × 6-in. gel ice packs or ice in bowls

Tethering a fly. These laboratory activities use live flies, and to control them, they first must be tethered by attaching a length of monofilament line to the thorax; we have found that wax works better than glue, since the fly is covered with setae. Live flies must first be immobilized, and this is accomplished by putting them in the freezer for a few minutes; they can also be anesthetized with CO2. Individual cooled flies are then transferred to small petri dishes and kept cold on ice or frozen gel ice packs, which are colder. The tethering is best done with the fly in the petri dish on the ice pack. Cooling both anesthetizes and immobilizes the flies.

The key is to place a small amount of wax on the thorax without getting wax on the wings or head and then to insert monofilament into the wax as it is remelted and then rehardens. Wax can be transferred from a burning candle using a paper clip that has first been partially unbent and heated in flame. Students should first practice transferring wax from the candle to any solid surface such as the cover of a petri dish; using a dissection microscope will help ensure that the amount of wax transferred is small (<1 mm in diameter). To increase the surface for attaching the monofilament, tie a single overhand knot in the end of the line and cut the extra line very close to the knot; this is easiest under the dissection scope. With the paper clip, transfer a small amount of wax to the knot.

Next, one can attach the monofilament to the dot of wax on the petri dish cover. Reheat the end of the paper clip, and, holding the heated paper clip in one hand and the monofilament in the other, bring the paper clip close enough to just melt the wax on the petri dish cover while inserting the knotted and wax-covered end of the monofilament into the melted wax. Again, this is best accomplished under the dissection scope. The wax rehardens quickly, and the monofilament becomes attached. Students should practice on dead flies, first putting a dot of wax on the fly thorax without getting wax on the wings or head and then attaching the monofilament to the thorax. When the technique is mastered, students can tether a live fly. If the heated wire wakes up the cooled fly before the tether is attached, the fly must be put back in the freezer again before attempting to attach the monofilament. A tether of a few inches is sufficient, although students often prefer longer tethers. At the end of the laboratory, flies can be euthanized by putting them in the freezer.

Tarsal reflex demonstration. To demonstrate the tarsal reflex, tethered flies are carefully removed from the small Petri dish and placed on a substrate such as a pencil or finger. When this substrate is removed, the fly should start to beat its wings and fly. Flies that have just emerged from pupation may not fly well. Continued flight requires gently blowing on the fly or letting it actually move through the air so that body setae are stimulated. When the tarsi contact the substrate, the wings stop beating.

Proboscis extension demonstration. To demonstrate the proboscis extension, hungry (starved) flies will feed on sucrose solutions. To distinguish between a chemosensory reflex and a thirst reflex, flies are first allowed to drink pure water until sated. A few drops of water are placed on a petri dish or in a slide well. Holding the fly by its tether, the fly is brought to the water so the tarsi touch it. In an inquiry-based setting, students could be asked how one could distinguish whether the fly is drinking from thirst or hunger and design experiments to test their hypothesis.

Water-sated flies can be tested with different concentrations of sucrose to determine the threshold for the chemosensory proboscis reflex and the change in responsiveness with increasing concentrations. The tarsi must be washed between each test solution by bringing the tarsi in contact with water. It is important not to let the fly actually feed on the sucrose solution or it will become satiated. Repeat each test solution twice; suggested concentrations are 0 (water), 0.01, 0.1, and 1.0 M sucrose solutions. Students record the fly’s responses (see Table 1 for sample data).

Table 1. Sample data

<table>
<thead>
<tr>
<th>Sucrose Solution</th>
<th>0.01 M</th>
<th>0.1 M</th>
<th>1.0 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fly 1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Fly 2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fly 3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Fly 4</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fly 5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fly 6</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Fly 7</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fly 8</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Means ± SD</td>
<td>0.5 ± 0.93</td>
<td>1.4 ± 0.92</td>
<td>1.9 ± 0.35</td>
</tr>
</tbody>
</table>

Values are numbers of fly responses.
RESULTS AND DISCUSSION

Once the fly is tethered, students can carefully observe the fly under a dissection microscope to identify various structures. In my department, these fly activities are part of a 3-h sensory system laboratory in the third quarter of our first-year general biology course. Students examine and answer questions about fly sensory structures such as the compound eye, antennae, tarsi, sensory hairs (setae), and halteres (see Fig. 1). Questions can be adapted for traditional or inquiry-based learning environments. Protruding from the antenna is a feathery structure called the arista; this sensory structure may contain hygroreceptors. The halteres are observed more easily when the fly is oriented horizontally while it holds onto a vertical substrate such as the side of the petri dish; the halteres beat periodically even when the fly is not in flight. In addition to sensory structures, students can review (or be introduced to) morphological characteristics of Arthropoda, Insecta, and Diptera. For example, mouthparts differentiate arachnids from the other major groups within the traditional classification of the phylum Arthropoda.

After observing the tarsal reflex, students can be asked to hypothesize what type(s) of sensory receptors, neural circuits, and muscles are required for this reflex. Since the stimulation of the tarsal sensory receptors causes inhibition of wing movement, the concept of an inhibitory interneuron can be developed. This is also a good time to lead a discussion about how the fly adjusts its wing movements to steer via the stimulation of motor neurons that control muscles. The discussion can consider additional sensory inputs that are involved such as from the eyes, halteres, and setae that are moved by the air as well as sensory inputs from receptors associated with the movement of the wings themselves. Students can be asked to predict and compare the sensory information analyzed by a still fly and one in flight. A goal here is to start to begin to understand the complexity of the nervous system using the principles established for the relatively simple tarsal reflex. Variability in these additional factors can also explain why some flies that appear normal and were carefully tethered may not fly. There are several excellent reviews on insect flight (e.g., Refs. 5 and 8). To expand the tarsal reflex exercise, students could be asked to research the number and types of muscles and mechanoreceptors that in some way control the movement of the wings and compare this with human arm movement at the shoulder joint.

If an old CRT monitor is available, students should observe flight in front of the screen. The refresh rate of the CRT is of the same order of magnitude as the wing beat frequency. Acting as a strobe light, the wings therefore appear to beat in the same order of magnitude as the wing beat frequency. Students can be asked to determine if there is sensitivity to the rate of change of length (dynamic) than the secondary afferents (static). These afferents synapse directly onto α-motor neurons, which innervate the extrafusal muscle fibers (e.g., Ref. 11).

Invertebrate muscle stretch receptors probably have been best studied in the crayfish abdomen, where there are two pairs of muscle receptor organs between segments (9). A muscle receptor organ consists of muscle fibers contacted by fine dendrites of a single, large mechanoreceptor. The static or slowly adapting type of muscle receptor organ responds as long as the muscle fibers are stretched. The tonic or rapidly adapting type produces only a short burst of action potentials. It is not clear whether these muscle receptor organs are found in insects, where chordotonal organs have been more widely studied. In the locust leg, some afferents from the chordotonal organ respond with a static or tonic discharge while others respond with a dynamic or phasic, rapidly adapting discharge (30).

Contrasting this reflex with fly reflexes, the proboscis extension reflex produces movement, the tarsal reflex inhibits movement, and the stretch reflex produces negative feedback to help stabilize movement. The sensitivity of the vatebrate stretch reflex can be controlled by activity of the γ-motor neurons and via presynaptic inhibition of the sensory afferents (4–6). The sensitivity of the chordotonal organ is via presynaptic inhibition. The circuits underlying the tarsal, proboscis extension, and chordotonal reflexes are simpler and more typical in that interneurons integrate signals between the sensory afferents and motor neurons.

After observing flies drinking water, students can hypothesize how the fly senses that water is present and the circuits required for this reflex. In an inquiry-based learning environment, students could devise experiments to test the hypothesis that flies are responding to a hypotonic solution via osmoreceptors on the tarsi. In fact, the mechanisms by which flies sense water are still being investigated (16, 18, 25). Note that when the proboscis is extended, one can easily observe the maxillary palps, feeler-like organs at the distal end of the rostrum, the first (proximal) section of the proboscis (2).

The proboscis extension reflex can be evoked by stimulation of the receptors on either the tarsi or proboscis. Several recent studies with Drosophila have used this reflex to screen for genes involved in feeding (e.g., Refs. 17 and 21). There are several possible aspects of this part of the laboratory that students could pursue, such as investigating the response to other sugars (mono- and/or disaccharides) or the inhibition of the extension reflex by salts (21). There are several excellent review articles on gustation in insects (e.g., Refs. 25 and 29).

To expand the proboscis extension exercise further, students could learn about different sugars and then search the Flybase (http://flybase.org), a database of fly genes, to see which taste genes have been described. Using Flybase with a premed query for a biological function and specifying “taste” returned eight hits, mostly taste receptor genes. For example, Ishimoto et al. (17) identified a putative gene for a trehalose receptor in Drosophila. Disruption of this gene reduced the proboscis extension response evoked by trehalose to ~25% of control flies. Flybase also provides information about where to obtain the fly strains.

Conclusions. The activities presented above provide novel exercises for students in a laboratory course to study sensory-motor integration. The use of flies adds phylogenetic diversity.
to standard vertebrate exercises and provides an interesting diversity of sensory receptors. The responses are reasonably easy to observe and simple enough for the underlying circuits to be understood by first-year students. For more advanced students, the activities can be expanded without having to write the animal protocols required for vertebrate experiments.

After completing these exercises, students should be able to discuss the number and different types of tarsal receptors and the different neural circuits required for the proboscis extension and tarsal reflex. Students can be asked about other animal and/or human reflexes that they know; examples include sneezing and coughing, eye blink, pupillary reflex, patellar tendon reflex, and eye movement (nystagmus). The discussion could emphasize sensory receptors, integration via interneurons, and motor outputs. One could also discuss the different ways that sensory information about the motor responses is obtained by animals. Vertebrates monitor the length and tension of the muscle using the muscle spindles and Golgi organ; invertebrates rely primarily on mechanoreceptors that measure distortion of the cuticle via chord stretch receptors and/or chordotonal organs (e.g., Refs. 6 and 30); there are also muscle stretch receptors (9). The exercises could be used as a basis for an indepth discussion of motor control.

The fly exercises described above provide a relatively simple demonstration of reflexive responses to specific sensory stimulation. They could lead to additional experiments combining or altering sensory inputs or experiments using mutants. These exercises with invertebrates could encourage a more detailed investigation of invertebrate sensory-motor integration structures. Either by themselves or in conjunction with other sensory and/or motor exercise, these activities should excite students and provide insights into the physiology underlying animal sensation and motor responses.

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DISCLOSURES

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