

Sensory Biology: Examination of Electroretinograms from Photoreceptors in Insects

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Introduction

Most animals are capable of detecting light. Photoreception is of central importance in the behavior of animals across the phyla. Many insects have two photoreceptive organs capable of detecting light. These are the *ocelli* and the *compound eyes*. Ocelli are simple, single-lens eyes that detect only the presence of light and appear to play a role in regulating physiological functions that depend on circadian or seasonal rhythms.

Compound eyes are made up of many hexagonally shaped facets. Each facet serves as a corneal lens covering a single photoreceptive structure called an *ommatidium*. Each ommatidium, in addition to containing a cornea, consists of a crystalline cone which helps focus light onto several visual sensory cells (*retinula cells*). The eyes of some insects can have over

25,000 ommatidia (dragon flies) while other can have as few as 300 (some beetles). Cockroaches may have around 3,000. Each retinula cell has a specialized region which contains the photopigment and is analogous to the disks of vertebrate rods. The retinula cells are arranged in a sphere, much like the sections of an orange, with the photosensitive regions arranged around a central axis. This central axis, consisting of the photosensitive regions of all the retinula cells is called the *rhabdom*. Light striking the rhabdom may depolarize the retinula cells eliciting action potentials which travel down axons conveying information back to the central nervous system.

Each ommatidium in a compound eye receives light from a different part of the insect's external world. The insect's perception is apparently one of coarse but recognizable mosaic-like image. Compound eyes may be especially good at detecting rapid movement, being about five times more sensitive than human eyes.

There are several possible ways to examine the physiology of insect eyes. One could record from single photoreceptor cells, either intracellularly or extracellularly. One could also monitor nerve cell activity by recording from a bundle of retinula cell axons. However, for today's exercise you will obtain a measure of the overall electrical activity in the eye in response to light. The measure is called an electroretinogram, or ERG for short. The ERG measures responses from a number of photoreceptors, not the electrical activity of single neurons. The procedure for recording ERGs is very simple and is an excellent means of illustrating some properties of visual adaptation.

Dark adaptation is a phenomenon which should be familiar to all. As we move from a well-lit area to a dimly lit area, we often have difficulty seeing. Think about what happens when you move from the sunlight to a dimly lit movie theater. After a few minutes your eyes become more sensitive to light and you can easily see empty seats that were in darkness previously. In humans, the eyes become fully dark adapted after about 20 to 30 minutes in the dark. In insects, dark adaptation is somewhat faster as you will see. Chemical changes in the receptors account for the phenomenon of dark adaptation. In this exercise, you will plot the course of dark adaptation as reflected by the magnitude of the ERG response.

Materials Per Station

- 2 insect pin electrodes [Insect pins (black steel #00) can be purchased from Carolina Biological.] To make an electrode, cut off the ball at the end of the insect pin and remove the coating with sandpaper. Solder the cut end of the pin to one end of a connector. Solder a piece of hook-up wire, about 18 inches long, to the other end of the connector. Make sure the insect pin tips are cleaned with sandpaper.
- Connectors [e.g. AMP HDP-20 snap-in sockets can be purchased from Newark Electronics (stock no. 66570-3).]
- Hook-up wire (30 AWG) [e.g. Belden PVC insulated hook-up wire, can also be purchased from Newark Electronics (stock no. 37F3198WA).]
- 2 micromanipulators (You could make due with one or, if you are really steady, none.)
 - b. 1 AC preamplifier
 - c. 1 oscilloscope or data acquisition system

- d. 1 dissecting microscope
- e. 1 cardboard box
- f. 1 optical stimulator (fiberoptic light pipe)
- g. 1 cardboard "shutter"
- h. Wax or modeling clay
- i. plastic beaker (100ml)
- j. 1 or more cockroaches (Other insects, crickets, dragonflies, wasps, etc. may also be used.)

Procedure

1. Prepare an insect for use by first immobilizing it with CO₂ and securing it to an upside down beaker (a wooden block or other platform may be substituted) using wax or modeling clay. Position the head of the insect so that it extends above the top edge of the beaker using "wax or clay worms" (wax or clay rolled out into thin worm-like bands). Make sure the head is secure and not likely to move when the electrodes are inserted into the insect's eye. (See Figure 1.)
2. Position the upside down beaker under the dissecting microscope so the insect is facing you. Make sure the insect's eye from which you intend to record is oriented toward your stimulator and free of excess wax. You can use wax or clay to secure the beaker to the table.
3. If you have two manipulators per setup you should attach each electrode to a manipulator. Position the manipulators so they can easily be used to insert electrodes into the insect's head and yet be out of the way of the box which will have to be placed over the entire set-up. Also make sure the microscope can swing freely out of the way once your electrodes are in place or make sure that the box will fit over the entire preparation including the microscope. If you only have one manipulator, attach the active electrode (the one that will be placed in the eye) to it.
4. If you have two manipulators, use one to insert the ground/reference electrode into any area of soft tissue. Do not attempt to insert an electrode through the exoskeleton of the insect as this may damage your electrode tip. The base of the antenna is a good location for the electrode. If you only have one manipulator carefully insert, by hand, the ground reference electrode into the antenna base and secure it to the platform with wax or clay. (See Figure 1.)
5. The recording electrode should be placed into either a compound eye or one of the ocelli (simple eyes: white spots near the compound eye). Avoid penetrating the eye too deeply with the electrode. Make sure your electrode tips are clean and sharp. You may have to guide the electrode in using forceps. (See Figure 1.)
6. After placement of the electrodes is completed, turn off the microscope illuminator and carefully move the microscope out of the way. (If it is not possible to move the microscope, make sure the cardboard box will cover it and the preparation.)

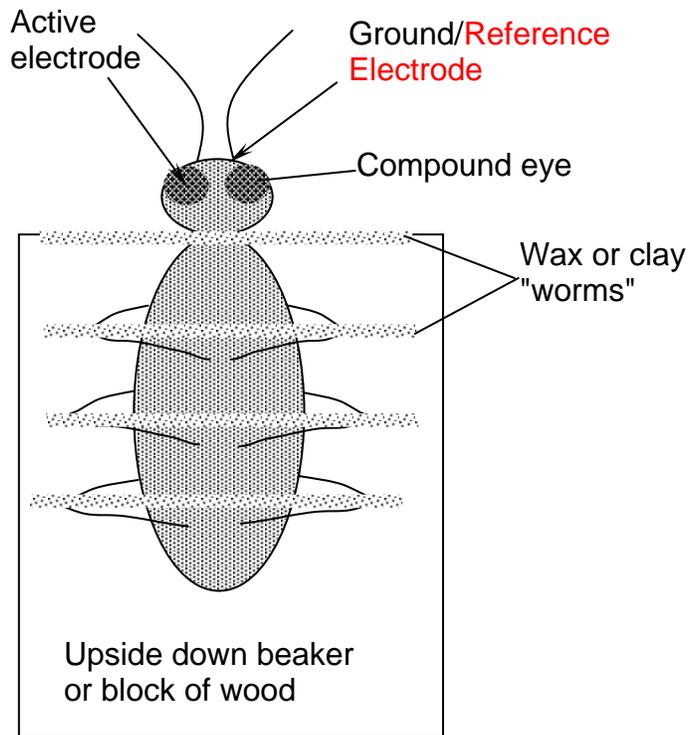


Figure 1. Diagram of electroretinogram set up.

7. Make sure the electrode wires are attached to the pre-amplifier. Tie the ground and reference input jacks together and connect the ground/reference electrode to one of the jacks. Connect the active recording electrode to the active input jack.
8. Use the following settings on your equipment:

Amplifier:

Band-pass filters:

Low filter: 0.1 Hz

High filter: 10 kHz

Mode: AC recording

Gain: 1000

Oscilloscope:

Time/Div: 0.2 secs

Volt/Div: 2 mV

Trigger Source: INT

Mode: auto

Make sure the Trigger Mode setting corresponds to the oscilloscope channel you are using. You can use the ERG response to trigger the oscilloscope trace.

Some minor adjustments in the filters of the preamplifier or sensitivity (volts/div) of the oscilloscope (data acquisition system) may be necessary depending on the insect used and the proximity of the eye to the stimulator.

9. Make sure the free end of the stimulator light pipe is as close as possible to the eye without touching it.
10. Place the cardboard box over the entire preparation. There should be a small hole in the cardboard box through which the insect's head is visible. The insect should be close to the hole.
11. Turn on the preamplifier and note any noise in the signal. If it is excessive, adjust the sensitivity (volts/div) on the oscilloscope and/or the filters on the preamplifier.
12. Stimulate the eye. A piece of cardboard held in front of the hole can be used as a shutter. Hold the piece of cardboard in front of the eye while the light stimulus is on. Remove it to stimulate the eye. You can trigger the oscilloscope or data acquisition system to start the trace each time the eye is stimulated. If the trigger is set properly and the insect and electrodes have been prepared correctly, you should see a trace start across the screen each time the eye is stimulated. It is not necessary to trigger the trace, especially if you are using a data acquisition system. Untriggered responses should be similar to the ones seen in Figure 2.
13. Once you have determined that your preparation is working, collect dark adaptation data using the following procedure:
 - a. Light adapt the insect for 30 seconds to 1 minute using the stimulator. The insect must be light adapted before each dark adaptation trial. Why? In order to save time and make sure you are able to collect all your data, you might not light adapt after the 90 sec dark adaptation period. Instead, stimulate as soon as each of the remaining periods are reached. The amount of light adaptation which occurs with the brief amount of light from the stimulator should be negligible at these longer intervals. [Note: Be sure to turn off the projector between the longer intervals to conserve the projector bulb.]
 - k. Dark adapt the insect for varying time periods: 1 second, 2 seconds, 3 seconds, 5 seconds, 7 seconds, 10 seconds, 15 seconds, 20 seconds, 30 seconds, 60 seconds, 90 seconds (It is after this period that it may not be necessary to light adapt the eye), 3 minutes, 5 minutes, and every 5 minutes thereafter, until there is no appreciable change in the response magnitude.

After each dark adaptation interval stimulate the eye and record the magnitude of the response. (Remember to use the same light intensity for all stimuli.) (See #14 below.)

14. Measure the strength of the neural response (the voltage). This can be done by measuring from peak to peak.
15. Typical ERG responses should be progressively larger with more time spent in the dark, up to a point. Then there should be relatively little change in response (an asymptote). Examine your data **before removing the box or electrodes**. If there are any responses which seem unusual, collect another data point for that (those) dark adaptation interval(s).

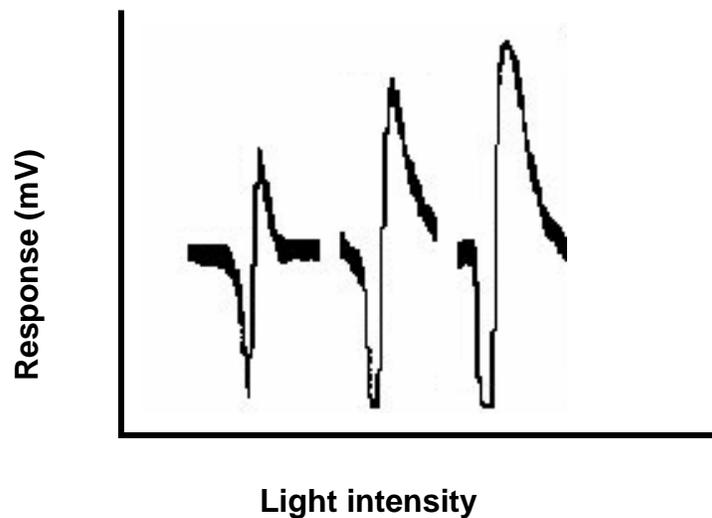


Figure 2. Typical ERG responses.

Other Experiments

1. Repeat the ERG procedure using a different type of eye (compound vs. simple) or a different type of insect (e.g. diurnal vs. nocturnal, or herbivorous vs. carnivorous, etc.)
2. Construct stimulus-response curves for insect eyes by varying the intensity of the light and measuring the effect on the magnitude of the response. A light meter is useful to determine light intensities.
3. You can also use the ERG to determine the spectral sensitivity of the insect eye. This can be accomplished by placing different color filters between the light source and the eye.

References

- Brown, P E.; Anderson, M. (1996) Spectral sensitivity of the compound eye of the cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae). *Bulletin of Entomological Research*, 86:337-342.
- Kugel, M. (1977) The time course of the electroretinogram of compound eyes in insects and its dependence on special recording conditions. *Journal of Experimental Biology*, 71:1-6.
- Ruck, P. (1958) Dark adaptation of the ocellus in *Periplaneta americana*: a study of the electrical response to illumination. *Journal of Insect Physiology*, 2:189-198.
- Ruck, P. (1958) A comparison of the electrical responses of compound eyes and dorsal ocelli in four insect species. *Journal of Insect Physiology*, 2:261-274.

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