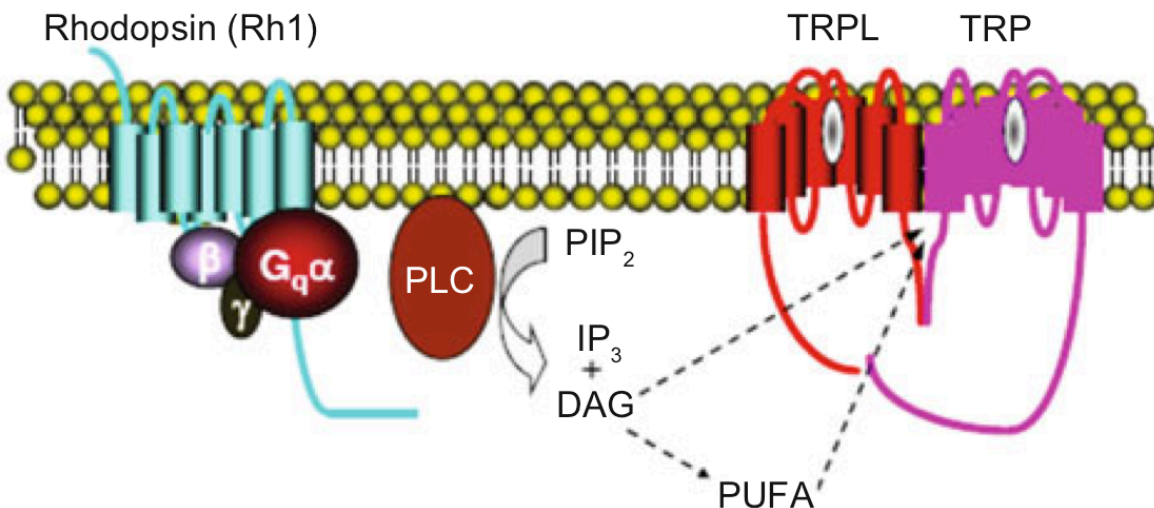


# Electroretinograms of Wild Arthropod Eyes: Electrophysiology meets Behavioral Ecology

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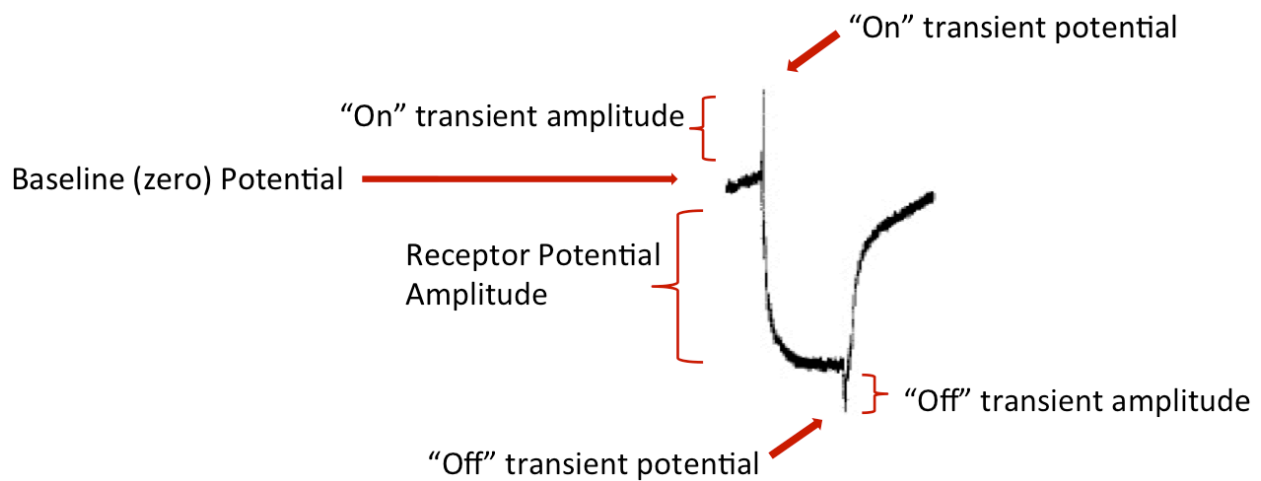
The eye is one of the most important, and most studied, sensory organs in animals. As a teaching preparation, eye electrophysiology provides one of the best combinations of accessibility, ease of use, and richness of analysis. The insect ERG is the combined extracellular response of the photoreceptor cells of the eye (located in the retina), and the evoked potentials in the second order neurons receiving synaptic input from photoreceptors (located in the lamina). The canonical insect ERG system is *Drosophila melanogaster*. First pioneered in the 1960's, the *Drosophila* ERG has been instrumental in developing our understanding of visual signal transduction, and is still used as a fast, reliable physiological output to investigate molecular mechanisms affecting synaptic transmission, ion channel activity, and intracellular signaling.



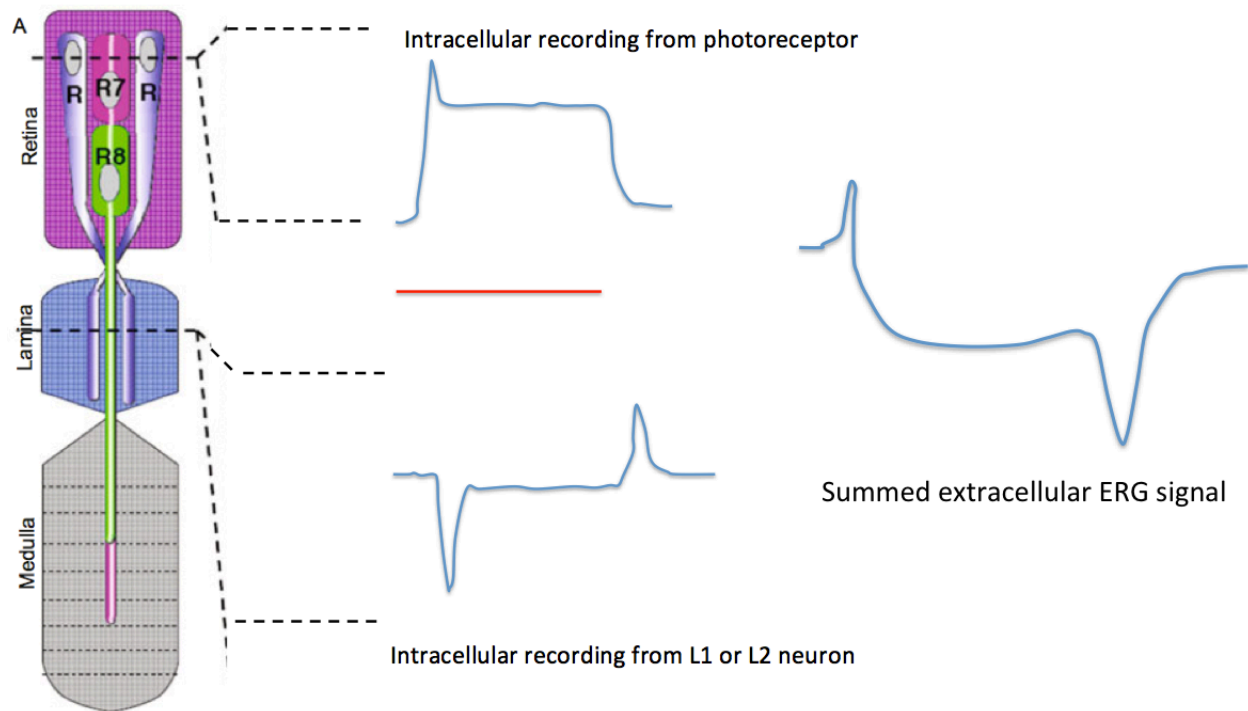
Overview of phototransduction signaling cascade in insect photoreceptors (data from *Drosophila*). From Yao *et. al.*, Phototransduction in *Drosophila*, Sci China Life Science, 2011, 54: 27-34

A wide range of invertebrates, which often are adapted to specific visual niches, can be tested in the same way, with only minor changes in the experimental design. Depending on the eye type and organism, the signal may contain transient “on” and “off” spikes at the start and termination of a light stimulus, a sustained receptor potential, and a characteristic stimulus termination. Different signal components correspond to separate events occurring within the retina and downstream neuropil in response to light. In addition to analyzing ERG waveform and components, one can measure attributes such as relative response strengths to light intensities,

flicker fusion frequency, and relative response to different wavelengths through the use of calibrated colored LEDs. Knowledge of the organism's ecology can inform interpretation of the observed visual neurophysiology. These techniques can form the basis for a wide variety of hypothesis driven (see below), student-designed experiments, which combine literature review, field biology, and electrophysiology. Today we will be measuring the EGR (electroretinogram) of a variety of field-caught insects or arthropods, fresh from local fields and forests.



Components of a representative ERG signal (from *Drosophila*). Amplitudes and other characteristics of each component are potential elements of analysis in a teaching lab.



Origins of the ERG signal in relation to insect compound eye anatomy. Omatidial schematic adapted from Yao *et. al.*, Phototransduction in *Drosophila*, Sci China Life Science, 2011, 54: 27-34

## Procedures:

### A. Setting up the rig

1. Connect the amplifier output (usually a 10X output) into input channel 1 of the A/D board. Connect the analog output to the BNC cable terminating in a two-slot header plug. Run a parallel connection from this output into input channel 2 of the A/D board.
2. Fill a sharp, broken off electrode with 0.9% NaCl. This will be the ground electrode. Insert a thread about halfway into an unpulled electrode capillary, and cut it off leaving 1-2 mm of thread protruding from the tip. Fill this by capillary action through the wick with 0.9% NaCl. This will be the recording electrode.
3. Mount electrodes on holders and fix into micromanipulators.
4. Turn on A/D boards and start LabChart software.

5. The visual stimulus consists of light from LEDs (white and colored), driven by the analog output channel of the A/D board. Acquisition settings should be as follow:

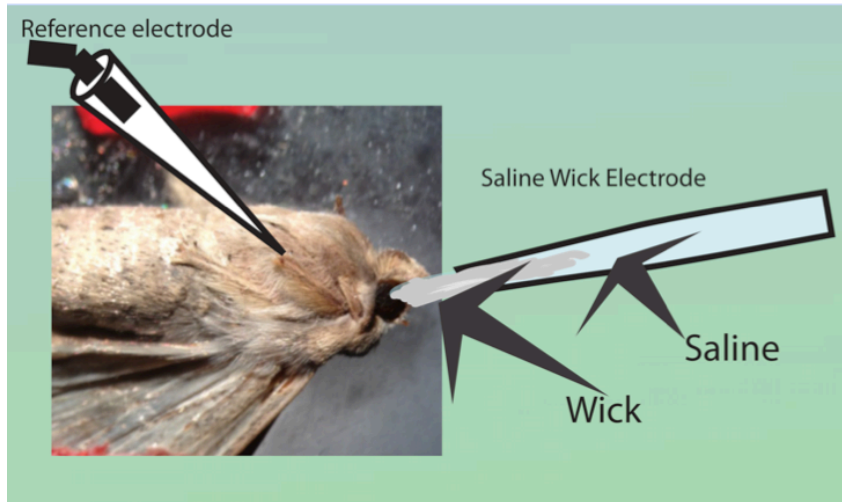
- Two channels, sampling at 10-20KHz. Ch1 should be set to a range of 500 mV, Ch2 should be set to a range of 5 V.
- Stimulator should be set to Pulse, Single Pulse, Manual Start, Frequency 0, Pulse duration 1000 msec, pulse amplitude 5 V.

## **B. Preparing the animal**

1. Place subject in a vial or tube, cold- anesthetize by placing the vial in an ice bucket. Meanwhile, set up an ice container with a plastic petri dish on top. Place the assembly under the dissection scope.
2. Put the anesthetized insect in the plastic petri dish, and tack down body and mobile appendages with wax, as described below.

Fixation of insects to a base can be done by a combination of rolled and molded wax threads, and drips of melted wax strategically connecting the insect to the underlying dish. The animal must be constrained, but not so covered in wax that it dies from suffocation, and care should be taken not to damage the animal too much with molten wax. Leave much of the abdomen and thorax free, tacking down only the abdomen tip and the underside of the thorax, abdomen, and head. If necessary, tack down the proboscis. Make sure the animal is lying on its left side (if the recording electrode is to approach from the right), with the eye facing up free of any wax. Keep light levels down to a minimum during the procedure to maximize ERG amplitude.

3. Take the petri dish on which the animal is mounted off the ice, and allow the subject to come to room temperature in the rig with the light off.
4. Place the magnetic base with the fiber optic guide in a position such that the tube approaches the exposed eye as closely as possible.



Schematic showing typical placement of sharp ground electrode and saline wick recording electrode on a moth.

### C. Recording ERGs

1. Insert the sharp ground electrode into the thorax of the insect, making sure that the tip penetrates through the cuticle.
  2. Advance the recording electrode until the wick patches on to the exposed eye. Probably the most important component for getting a good ERG is the contact between the recording electrode and the eye surface.
  3. Place the white LED into the header plug that connects to the analog output, making sure that the longer wire goes into the positive (red) connection. Thread the attached fiber optic through the guide tube until it just protrudes, placing it right next to the eye.
  4. Start the recording, and trigger a one second pulse of white light using the settings described above. The resulting recording should show the ERG on channel 1, and the stimulus on channel 2.
- Wait about a minute between ERG pulses to prevent excessive light adaptation.

### D. Variations of the light stimulus

1. After taking several sample ERGs, you can measure the Flicker Fusion Frequency (FFF) of the eye, which gives a rough estimate of the speed of visual response and motion sensitivity of that animal. Set the stimulator to send multiple pulses at various frequencies. A good approach is to begin at about 20 Hz and go up in 10 Hz increments. A standard criterion for determining when FFF has been reached is to take the frequency at which responses to individual light pulses begin to be skipped. At this point, the eye is starting to see fewer distinct pulses and eventually only sees one long light stimulus, rather than the actual pulse train.

One good way to set this up in a manner that allows easy comparison is to choose an overall stimulus duration (say 5 seconds), and set the number and frequency of pulses to fill this time. To keep overall light levels constant, make sure to use a "square" stimulus, with the light pulse

taking up half of each cycle. So, for example, a 5 second, 20 Hz stimulus would require 100 pulses of 50 msec duration.

As you test your insect, you can test your own FFF, and you won't even have to patch an electrode to your eyeball. Just look at the flickering LED during each stimulus, and note when the light appears to be just "on" to you. For example, *Drosophila* eyes can often respond to flicker frequencies of up to 80 Hz. How do you compare? What about your insect?

2. Another variation is to change the wavelength of the light stimulus. We have provided you with an array of narrow band LEDs of different colors. After allowing your animal to recover from light adaptation, change the white LED to either the Ultraviolet or the Red LED. The guide tube allows for consistent placement of each light stimulus. To control for intensity, we have calibrated the voltage required for each LED to emit approximately the same number of photons per second. Due to the properties of the assorted LEDs, this overall intensity will be lower than what is emitted by the white LED, so don't be surprised if you never see a response as large as you obtained from white light at maximal amplitude. The appropriate voltages for each LED are:

Red:	1.74
Orange:	1.84
Yellow:	2.12
Green:	2.81
Blue:	2.72
Long UV:	3.05
Short UV:	3.22

## **E. Interpreting results**

The initial ERG response to a single pulse of white light can be very informative, especially when two or more insects are compared. Aspects to compare would include:

- Presence/ absence or relative amplitudes of the on or off transients
- Time to peak receptor potential and the time to recovery to baseline
- Amplitude of receptor potential
- Time to recovery from light adaptation with closely spaced pulses

Transient response can vary with the strength of synaptic transmission between photoreceptors and lamina neurons, with the distance of the lamina from the recording electrode, and with the resistive value of the basement membrane separating the retina from the lamina (forming a structure somewhat analogous to the vertebrate blood-brain barrier). Receptor potential amplitude can vary with the light sensitivity of the photoreceptors, the number and size of the photoreceptors, and intrinsic photoreceptor properties such as rhodopsin type and distribution and channel content.

FFF comparisons can indicate which insects are better at seeing fast-moving objects or tracking the flow of visual fields. Results from the colored LEDs can give an approximation of the spectral sensitivity of the insect, indicating the number and characteristics of the rhodopsin

variants that could be present in the eye. Once the electrophysiological tools are mastered by students, they can use them to address questions of how visual physiology varies with the behavioral ecological niche of the organism. Projects can range from a simple "atlas" of ERG waveforms in different insects, to more intensive studies comparing carefully chosen subjects. Interesting comparisons can include:

- Flying vs. non-flying (or seldom-flying): Typically insects that are flying need to be able to quickly integrate visual inputs, and hence their eyes tend to be faster.
- Diurnal vs. crepuscular vs. nocturnal : Typically nocturnal insects are limited by light, and therefore they tend to integrate visual input more strongly, both spatially as well as temporally. The latter leads to very slow FFFs. In addition, nocturnal animals frequently lose color vision capacity.
- Arthropods found in fields vs. those found under leaf litter or other darker habitats: This comparison leads to similar expectations as does the diurnal vs. nocturnal comparison, to varying degrees.
- Predacious vs. herbivorous: Predacious animals typically need to move faster than herbivorous animals, and accordingly are expected to have larger FFFs
- Larger vs. smaller eyes: Small eyes typically are able to capture less light, so they too tend to be slower. Overall ERG amplitude is often greater in larger eyes.

In our experience, insect or arthropod choices are mostly constrained by local and seasonal availability, the time it takes to obtain specific specimens, and the robustness of the subject in the face of captivity and electrophysiological recording. Of course it is best to choose subjects that occupy different niches but are otherwise as closely phylogenetically related as possible. Students can combine literature research on the enormous diversity of insects, formulation of testable hypotheses about eye function, and applied experimentation. Very often students are extremely excited even by just succeeding in obtaining an ERG; after all, chances are pretty good that they are the first ones to ever have recorded ERGs from that species!