

Spectral Sensitivity of Larval Mosquito Ocelli

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ABSTRACT The spectral sensitivity of lateral ocelli in both wild-type and white-eyed larvae of the yellow fever mosquito *Aedes aegypti* L. (reared in darkness) was measured by means of the electroretinogram. The spectral sensitivity is maximal at about 520 nm, with a small secondary peak near 370 nm. When allowance is made for some screening and filtering by the eye tissues, the spectral sensitivity is in reasonable agreement with the absorption spectrum of ocellar rhodopsin ($\lambda_{\max} = 515$ nm).

INTRODUCTION

The visual pigment in larval ocelli of the mosquito *Aedes aegypti* has recently been measured by microspectrophotometry (Brown and White, 1972). The four ocelli contain a typical rhodopsin, with λ_{\max} at 515 nm. Associated with it is a stronger absorption band in the ultraviolet, with λ_{\max} about 345 nm, that appears spectrophotometrically to be light stable. One purpose of the present measurements was to see whether one or both of these pigments contribute to the electrical response.

The spectral sensitivity of the larval ocelli was measured by means of the electroretinogram (ERG). Though the ERG of the compound eye of adult *Aedes* has been recorded (Brammer and White, 1961), these are the first such measurements in the larval eye.

METHODS

The equipment was modified from that of Wald and Seldin (1968). Light from a 100 w zirconium arc was projected with quartz lenses through a Bausch & Lomb grating monochromator (250 mm, 2100 lines/mm; Bausch & Lomb, Inc., Rochester, N.Y.), transmitting a wave band 5 nm wide throughout the spectrum. A shutter at the exit slit regulated the duration of test flashes, usually 18 msec. A pair of contrarotating circular quartz neutral wedges provided gradations of intensity over a range of 3.4 log units. This range could be extended with calibrated neutral filters. The emergent light beam was concentrated with a quartz condenser into a 3 mm spot on a quartz

slide on the stage of a compound microscope. A dark-adapted mosquito larva was mounted on the slide, its body in a well of insect Ringer (see Brown and White, 1972), its head outside, centered in the spot of light. The well was made by sticking a ring of 1 cm o.d. polyethylene tubing, 5 mm tall, on the slide by heating over a gas flame. A "V" notch was cut through the wall of the ring, its apex at the outer surface. The larva was held with its neck in this notch, its head outside the well, its body inside, with the caudal respiratory papillae free to function.

The active electrode was a glass pipette, tip diameter 2-5 μm , filled with insect Ringer or with 1 M KCl (each gave similar results). This was inserted into the head of a larva so that the tip lay adjacent to the group of four ocelli at one side of the head. The indifferent electrode, a nonpolarizing Ag-AgCl wire, dipped into the insect Ringer in the well. A Grass P-18 DC preamplifier (Grass Instrument Co., Quincy, Mass.) was used, with the electrodes connected for push-pull operation, coupled with a Tektronix 502-A dual beam oscilloscope (Tektronix, Inc., Beaverton, Ore.).

The spectral sensitivity was determined by measuring at each wavelength the relative number of photons per 18 msec flash required to elicit a 2.5 or 5 μv response. The sensitivity is the reciprocal of that number.

The mosquitos (*Aedes aegypti*) used in this study were grown in the dark from eggs kindly supplied by Professor George B. Craig, Jr., Department of Biology, University of Notre Dame. See Brown and White (1972) for details of rearing methods.

RESULTS AND DISCUSSION

Two typical ERG responses are shown in Fig. 1. The first (Fig. 1 *a*), recorded with DC coupling between preamplifier and oscilloscope, shows that the ocelli respond to sustained illumination with a sustained DC potential that returns to the base line when the light is turned off. The second record (Fig. 1 *b*) with AC coupling, shows a typical response to an 18 msec flash. After a latency of about 20 msec, a rapid rise to peak potential is followed by a slower return (about 0.1 sec) to the base line. The largest ERG potential recorded was about 10 μv . The ERG varied in amplitude with stimulus intensity and wavelength, but there was no change in waveform with wavelength. All four ocelli appear to have identical electrical characteristics.

We determined the spectral sensitivities of both wild-type larvae, whose ocelli contain granules of dark accessory pigment, and white-eyed mutants, whose ocelli are relatively transparent (see Brown and White, 1972).

Fig. 2 shows three representative sensitivity curves from dark-adapted larvae of both types. The sensitivity of the fourth instar ocelli was measured at the same stage as the rhodopsin was measured microspectrophotometrically. Since the rudiment of the *compound eye* develops in the fourth instar, a second instar animal, with no formed elements of the compound eye, was also measured, to make certain that the ERG responses were generated exclusively from ocelli. All the curves are nearly symmetrical between about 450 and 600 nm, with λ_{max} about 520 nm. In each case there is a minor secondary peak at about 360-370 nm.

Fig. 3 compares these spectral sensitivity measurements with the absorption band of ocellar rhodopsin (Brown and White, 1972). When all the curves are brought together at long wavelengths, the white-eye sensitivity curve falls below the rhodopsin absorption at short wavelengths, the wild-type curve much more so. This indicates that the large absorption band at 345 nm in the rhabdom (Brown and White, 1972) cannot be contributing appreciably to the ERG spectral sensitivity.

Differences between absorption and sensitivity curves are common. The human rod spectral sensitivity curve lies well below the absorption spectrum of human rhodopsin at short wavelengths, mainly because of filtering by the

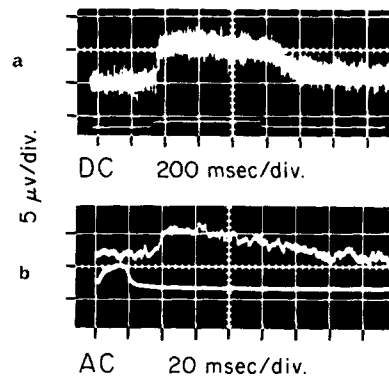


FIGURE 1. *Aedes aegypti* ERG. Electroretinograms in response to 520 nm light. Each ERG is accompanied by a time marker taken from a photocell monitoring the test flashes. (a), response to a 0.6 sec exposure recorded with 200 msec/division sweep and DC coupling between preamplifier and oscilloscope. (b), a typical ERG in response to the usual 18 msec test flash recorded with AC coupling and sweep set at 20 msec/division. In both records sensitivity is $5 \mu\text{v}/\text{division}$. Preamplifier rise time was 1 msec. An upward deflection signifies active electrode negative with respect to indifferent electrode. The ERG's were taken from two different animals.

yellow lens. Also the spectral sensitivity of the lobster, *Homarus americanus*, lies lower in the blue than the absorption spectrum of lobster rhodopsin (Wald and Hubbard, 1957). One could cite more examples, all probably coming out of filtering of short wavelength light by eye tissues interposed between the light and the photoreceptor organelles. In mosquito larvae such selective filtering of short wavelength light probably occurs in the cuticle, hemolymph and cytoplasm, to which the wild type adds the reddish screening pigment. Our microspectrophotometric measurements (Brown and White, 1972) show that even clear areas of cytoplasm in the larval head absorb light at short wavelengths.

The rhodopsin measured microspectrophotometrically seems therefore to be the visual pigment of the larval ocellus. No ERG is associated with the photostable absorption band maximal at about 345 nm. However, selective

adaptation experiments were not carried out. The secondary sensitivity maximum near 370 nm may represent the β band of rhodopsin, which we would expect to lie at about 360 nm.

Similar results were obtained by Langer and Hoffman (1966) comparing the spectral sensitivity of *Calliphora* with the spectrum of its rhodopsin. The

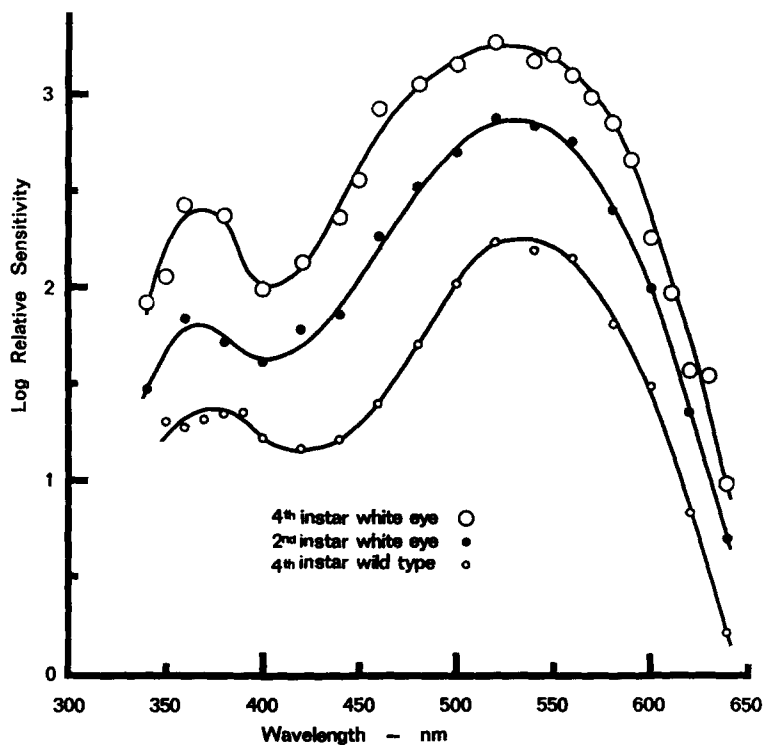


FIGURE 2. Spectral sensitivities of three animals reared in darkness. All experiments measured the relative number of photons per 18 msec flash required at 10- or 20-nm intervals to elicit a 2.5 or 5 μ v criterion amplitude in the ERG. The spectral sensitivity is the reciprocal of the number of photons.

λ_{\max} of *Calliphora* rhodopsin is about 510 nm, while its ERG spectral sensitivity maxima lie close to 500 and 360 nm.

Brown and White (1972) found no regeneration of rhodopsin in the dark for at least an hour after mosquito ocelli had been bleached with a flash of yellow light. We find, however, that following a similar flash exposure (Sylvania M3 flash bulb, GTE Sylvania, Inc., Mountain View, Calif., plus Corning 3484 filter, Corning Glass Works, Corning, N. Y.), the ERG recovers rapidly. In one experiment, the initial dark-adapted sensitivity was regained 12 min after the ERG had been depressed 3 log units by a yellow flash.

Dark adaptation in vertebrates consists of an initial rapid component fol-

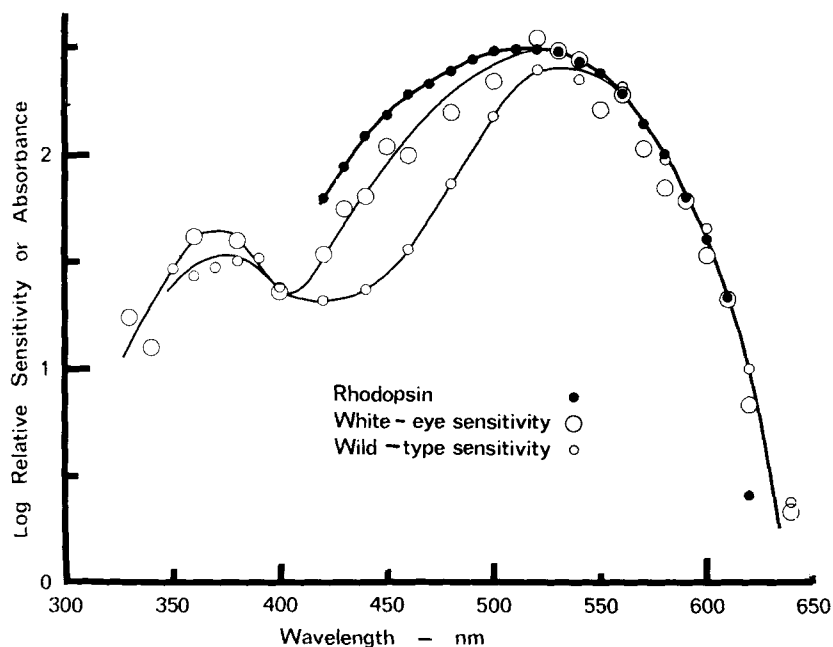


FIGURE 3. Spectral sensitivity curves compared with the averaged rhodopsin difference spectrum. The curve for white-eye larvae represents the average of data from five animals, all spectral sensitivities being set equal at 520 nm. The single curve for wild type represents data from one larva. The spectral sensitivity and the rhodopsin spectrum were superimposed at long wavelengths where they coincide.

lowed by a slow component. The fast component is independent of rhodopsin regeneration and appears to be mediated by nonphotochemical or "neural" mechanisms. The slow component appears to depend directly upon rhodopsin regeneration (Dowling, 1967; Weinstein et al., 1967).

Our results suggest that dark adaptation in the mosquito ocellus does not depend upon rhodopsin regeneration. We seem to be dealing with a relatively rapid neural adaptation that runs far ahead of the regeneration of visual pigment and may principally govern the level of the ERG threshold.

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