

THE SENSITIVITY OF HOUSEFLY PHOTORECEPTORS IN THE MID-ULTRAVIOLET AND THE LIMITS OF THE VISIBLE SPECTRUM

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In experiments published in his book of 1882, Sir John Lubbock demonstrated that removal of the ultraviolet (u.v.) wavelengths decreased the effectiveness of natural sunlight in stimulating ants to carry their pupae into the dark. An apparently high sensitivity to near-ultraviolet light was subsequently shown in studies of phototaxis in bees (Bertholf, 1931*a, b*; Heintz, 1959) and flies (Bertholf, 1932; Cameron, 1938). More recently electrophysiological techniques have been employed to study selective adaptation of the retinal action potential (Walther & Dodt, 1959; Goldsmith, 1960) and the spectral responses of single reticular cells (Autrum & Burkhardt, 1960; Autrum & von Zwehl, 1962, 1964; Burkhardt, 1962; Autrum, 1965). This work demonstrates that honeybees (*Apis*) have u.v. receptors with peak sensitivity at 340 nm. and the cockroach (*Periplaneta*) at ~ 350 nm. The photoreceptors of flies (*Calliphora*) show two maxima, one of which lies at about 350 nm. The peaks are less well defined, but u.v. receptors maximal at about 350 nm. also seem to be present in Coleoptera, Lepidoptera (Hasselmann, 1962) and probably Odonata (Ruck, 1965). These peaks are not due to secondary excitation of the visual pigments through fluorescence of accessory pigment for (1) both bees and flies are phototactically several times more sensitive to the 365 nm. mercury line than to any longer wavelengths (Bertholf, 1931*a, b*, 1932; Cameron, 1938); (2) in flies the u.v. maxima seen in the retinal action potential are higher than the peaks in the visible region of the spectrum (Walther & Dodt, 1959; Burkhardt, 1962); and (3) bees can be trained to discriminate u.v. from all other wavelengths or mixtures of wavelengths (e.g. Daumer, 1956).

The chromophore of the visual pigments of insects is the carotenoid derivative retinal (Goldsmith, 1958; Goldsmith & Warner, 1962, 1964; Goldsmith, Barker & Cohen, 1964; Goldsmith & Fernandez, 1966*b*). There are as yet no absorption measurements of the u.v.-sensitive pigments, and in the absence of such data action spectra provide important clues to the pigment's molecular structure. Most of the behavioural experiments intended to bear on this matter have not resulted in proper action spectra (cf. Goldsmith, 1961). Although many of the electrophysiological determinations of spectral sensitivity made during the past decade obviate this criticism, none extends to wavelengths significantly shorter than 300 nm.* Possible sensitivity to u.v. light of shorter wavelength remains an open question, for Bertholf's (1932) curve of 'relative stimulative efficiency' for the phototaxis of *Drosophila* shows a minor maximum at

* Behavioural responses of moths to X-rays (Smith, Kimeldorf & Hunt, 1963) and electroretinographic responses of moths to β -radiation (Smith & Kimeldorf, 1964) have been reported.

254 nm. Moreover, Lutz & Grisewood (1934) confirmed that *Drosophila* responded phototactically to the 254 nm. mercury line, and reported that an energy flux of $8\mu\text{W cm.}^{-2}$ at 254 nm. was roughly equivalent to 120 ft.-candles of white light from an incandescent lamp. Although it is impossible to relate these quantities precisely, this much can be said. Visible light has the largest ratio of lumens per watt if it is monochromatic light at 555 nm.; any other spectral distribution will necessitate more energy to provide the same brightness: 120 ft.-candles is 0.129 lumens cm.^{-2} , and at 555 nm. this requires $190\mu\text{W. cm.}^{-2}$. For white light of 120 ft.-candles, the energy content must of course be larger. Lutz & Grisewood's experiment therefore suggests that *Drosophila* is more than 24 times as sensitive at 254 nm. than at any wavelength in the visible region of the spectrum, a conclusion so remarkable and unexpected that it requires verification.

We have measured the spectral sensitivity of the housefly, *Musca domestica*, another species known to be strongly phototactic to near-ultraviolet wavelengths (Cameron, 1938) and to be attracted to light traps emitting primarily the 254 nm. mercury line (Deay & Taylor, 1962). In order to minimize distortion of the action spectrum by either absorption or fluorescence of the accessory screening pigments, a white-eye mutant was used. The absorption of individual corneal facets also was measured, and its effect on the spectral sensitivity curve was calculated.

A preliminary account of this work has appeared in abstract (Goldsmith & Fernandez, 1966a).

METHODS

The white-eye mutant of the housefly which we employed is recessive (Hiroyoshi, 1961), and homozygous flies have no visible eye pigments. Both wild-type and mutant flies were maintained on the CSMA medium (Chemical Specialties Manufacturers Association, 1965). In addition to minimizing distortion of the spectral sensitivity function, the mutant flies have the additional advantage of being more sensitive than wild-type animals.

The stimulating source was the intense 2 mm. arc of a 150 W. xenon lamp (Hanovia D-901 C-1) operated at 7.5 A. d.c. For measurements of spectral sensitivity in the u.v., light was passed sequentially through a pair of monochromators, a Bausch and Lomb 52 mm. square grating, 1200 lines/mm., and a Perkin-Elmer Model 83 Universal prism monochromator. Slits on the grating monochromator were adjusted for a 6.6 nm. band width. Slits on the prism monochromator were set for a linear dispersion at the exit of 7 nm. at wavelengths 290–340 nm.; dispersion increased to 10 nm. by wavelength 400 nm., but fell to 4.2 nm. as the wavelength was decreased to 250 nm. Measurements at wavelengths longer than 400 nm. were performed with the grating monochromator and appropriate blocking filters alone. The energies of the various wavelengths were measured with a thermopile, and during the experiments they were adjusted with a pair of optical wedges made of Inconel deposited on quartz.

The retinal action potential was recorded with silver: silver chloride electrodes. One contacted the illuminated eye through a micropipette broken off at the tip to a diameter of about $25\mu\text{m.}$, filled with physiological saline, and inserted through a pilot hole in the cornea. The reference contact was a saline-coated wick on the dark side of the head, shielded from the stimulus by a mask of aluminium foil. The electrical responses were

recorded with a high-impedance d.c. amplifier, displayed on an oscilloscope, and photographed. Further details of the calibration and recording procedure can be found in Goldsmith (1965).

Spectral sensitivity was measured by determining the relative quantum flux required to elicit a standard response of about 1 mV. or less from the dark-adapted eye. The test flashes were about 0.5 sec. in duration, which is long enough for the development of the maintained component originating in the receptors (cf. Goldsmith, 1965), and it is this feature of the response that was measured. The transient on-and-off effects were not studied. In practice, responses yielding small segments of the response-energy function on either side of the criterion were recorded at each wavelength, and the energies required to produce the criterion negativity were determined graphically after the film was measured.

Absorption of individual corneal facets was measured with a dual-beam recording microspectrophotometer slightly modified from the design of Liebman & Entine (1964). Light from a 500 W. deuterium lamp was passed through a Bausch and Lomb grating monochromator to illuminate a pair of apertures. These were in turn demagnified and imaged in the specimen plane with a $\times 10$ quartz ocular and a Zeiss $\times 32$ neofluar objective (N.A. 0.4). Pieces of cornea were placed in slightly diluted glycerin (refractive index 1.455) between quartz coverslips and positioned so that the sample beam passed through a single facet. The collecting optics focused the light on to the cathode of a photomultiplier tube (EMI 9558Q). The reference and sample beams were separated in time by a rotating sector disk, and the output of the photomultiplier in response to the former was used in a feedback loop to control the voltage applied to the dynodes and so to maintain the reference output constant with wavelength.

RESULTS

The average spectral sensitivity of ten white-eyed flies in the spectral region 250–400 nm. is shown by the filled circles in Fig. 1. Standard errors are indicated by vertical lines through the points. The curve shows the familiar peak at 340–350 nm., continually falling sensitivity at shorter wavelengths, and a pronounced shoulder at about 280 nm. There was insufficient energy to extend the curve below 250 nm.

To reach the receptor pigment light must obviously pass through the dioptric apparatus, the cornea and cone. Being composed of organic materials these structures are likely to absorb in the u.v. and attenuate the light reaching the receptors. The pseudocones of Diptera are soft, fragile structures and not easily handled; however, a partial correction for the filtering action of the dioptric structures is readily obtained from measurements of corneal absorption. Figure 2 shows the mean absorption of eight individual corneal facets. There is virtually no absorption through the visible and near-ultraviolet, but there is a sharp peak at 277 nm. With one exception, the standard errors in Fig. 2 are for the average curve after normalizing the individual spectra; however, the error bar at the maximum is calculated from the absolute values of absorption at the peak. Its larger size indicates that the shape of the curve is somewhat better defined than the absolute magnitude of absorption. From the shape it appears likely that this curve reflects the presence of tryptophan and perhaps tyrosine residues in the protein which is associated with the chitin of insect cuticle (Fraenkel & Rudall,

1947). This curve is similar in shape to our earlier measurements on *Sarcophaga* (Goldsmith & Fernandez, 1966*a*) and to those on moths (Bernhard, Miller & Møller, 1965), but the absolute absorption is less, probably because the cornea of *Musca* is thinner.

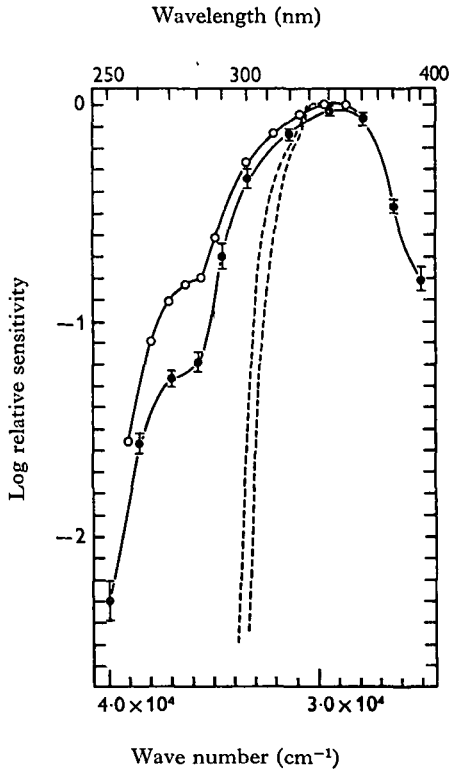


Fig. 1. Spectral sensitivity of housefly photoreceptors in the ultraviolet. Ordinate is the log of the reciprocal of the relative number of photons required to elicit a constant electrical response from the reticular cells of a white-eyed mutant. ●, Sensitivity of the intact eye. ○, Sensitivity corrected for corneal absorption. Dotted curves: atmospheric attenuation of natural sunlight at wavelengths shorter than 340 nm.

The open circles in Fig. 1 indicate the spectral sensitivity of the receptors, corrected for energy loss in the cornea.

Figure 3 shows the spectral sensitivities of white-eyed (above) and wild-type (below) houseflies throughout that part of the spectrum visible to these insects. The effect of the red screening pigment is to depress the sensitivity throughout all but the red end of the spectrum and to create a minor maximum at 620 nm (Autrum, 1955; Hoffmann & Langer, 1961; Burkhardt, 1962; Goldsmith, 1965; Langer, 1967). Maxima are still observed in the green and near-ultraviolet.

DISCUSSION

Site of excitation

The retinal action potentials recorded at wavelengths below 300 nm. are no different from those evoked in the near-ultraviolet or blue. The excitation is therefore

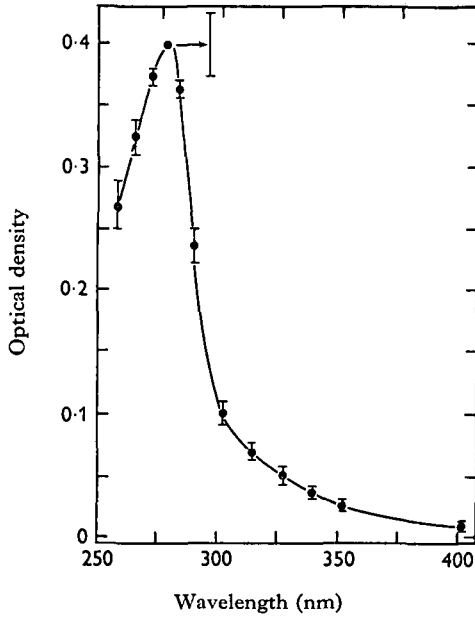


Fig. 2. Average absorption of eight individual corneal facets of the housefly, *Musca domestica*.

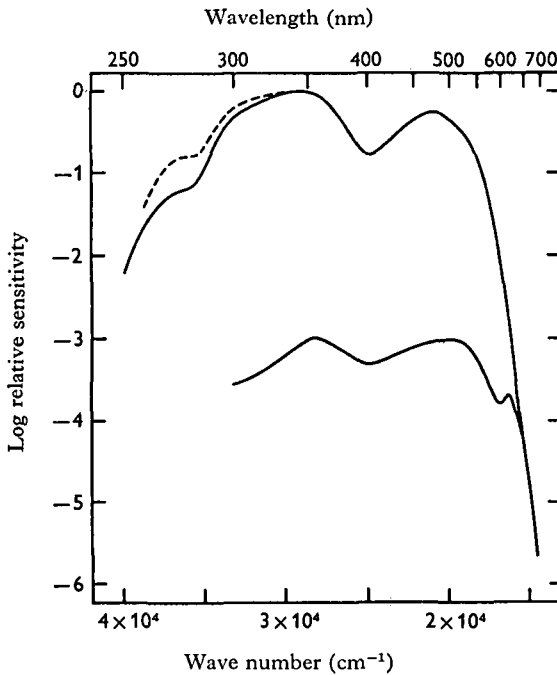


Fig. 3. Spectral sensitivity of white-eyed (upper curve) and wild-type (lower curve) houseflies. The section of the upper curve lying to the left of 400 nm, is a replot of Fig. 1. Ordinate: log of the reciprocal of the relative number of photons for a constant response. Wild-type and white-eyed flies have the same absolute sensitivity to deep red light (Goldsmith, 1965).

believed to originate in the receptor cells. This point takes on importance because of the recent suggestion (Carlson, Smith & Stanley, 1968) that 270 nm light excites the optic ganglion of the moth *Manduca* directly. It seems to us, however, that the differences in the retinal action potentials which these authors recorded at 270 and 310 nm are simply effects of intensity.

Energy transfer

There are two possible explanations for the shoulder observed in the spectral sensitivity curve at 280 nm. The first is that the primary absorption at this wavelength is by some other molecule and energy is passed secondarily to the visual pigment. For example, a residual fluorescence in the eye could produce a spurious elevation in sensitivity that would not be reflected in the absorption spectrum of the visual pigment. The alternative is intramolecular energy transfer—that energy absorbed by the protein component of the visual pigment has a significant probability of leading to visual excitation. This view is quite credible, for Kropf (1967) showed that 254 and 280 nm. light absorbed by vertebrate rhodopsin produces isomerization of the chromophore and bleaching. The quantum efficiency is 0.26 in the u.v. as opposed to 0.66 in the visible, but because of the greater molar extinction at 280 nm., the photosensitivities at 280 and 500 nm. are similar. If the corrected action spectrum in Fig. 1 signifies the properties of the visual pigment, the photosensitivity at 480 nm. is only 3.6 times greater than at 280 nm. Moreover, the correction applied in Fig. 1 is conservative, for it does not take into account inert proteinaceous material either in the pseudocones or the rhabdomeres themselves. Correction for additional u.v. filtering can only make the 280 nm. shoulder more prominent.

The short-wavelength limit of the visible spectrum

The ommochrome screening pigments of the wild-type eye show strong absorption in the middle u.v. (Butenandt & Neubert, 1955; Butenandt, Biekert & Beckmann, 1957); therefore, although spectral sensitivity measurements on red-eyed flies have not been extended to wavelengths shorter than 300 nm., it is likely that the fall in sensitivity in the middle u.v. is even sharper than for the white-eye mutant. Ecologically, however, the question is academic, for there is essentially no solar energy reaching the earth's surface at wavelengths shorter than 300 nm. The principal reason for this is the filtering effect of ozone in the earth's atmosphere. The pair of dotted curves in Fig. 1 show the attenuation of solar energy with wavelength, relative to 340 nm. The upper curve is $\log I = \log I_0 - \alpha l$, where I_0 is the measured value of solar energy above the atmosphere, α is the absorption coefficient of ozone, and $l = 0.25$ cm., a reasonable mean value. The lower curve represents measured values of solar energy—skylight and sunlight—reaching the earth at Davos-Platz, Switzerland, with the sun at an altitude of 50° and a vertical ozone column of 2.5 mm. The upper curve was calculated on the basis of data from various sources summarized by Robinson (1966); the second is due to P. Benes and is taken from the same source. Quite clearly, the visual apparatus of insects such as the housefly is designed to exploit the shortest wavelengths available in the environment with no interference from the cornea. By contrast, in the vertebrate eye the short-wavelength limit of the visible spectrum is usually set by absorption of the lens (e.g. Wald, 1952; Kennedy & Milkman, 1956).

The long-wavelength limit of the visible spectrum

The long-wavelength limit of the visible spectrum, as in the vertebrate eye (e.g. Griffin, Hubbard & Wald, 1947), is determined by the steady fall in absorption of the visual pigment. By 700 nm. the sensitivity has decreased nearly 6 log units from the major peak at 340–350 nm. Those who would have us believe that insects' eyes are infrared detectors and that attraction of insects to u.v. light-traps is based on something other than the presence of u.v. wavelengths (Callahan, 1965) will have to argue from direct measurements of receptor sensitivity to infra red light before their case can be regarded seriously.

SUMMARY

1. The spectral sensitivity of the photoreceptors of a white-eye mutant of the housefly *Musca domestica* has been measured to 250 nm. in the mid-ultraviolet. Maximum sensitivity is at 340–350 nm., as in the wild-type eye, and decreases at shorter wavelengths with a distinct shoulder at 280 nm.

2. Microspectrophotometric measurements of individual corneal facets show little absorption at wavelengths longer than 300 nm. but a sharp band (peak density about 0.4) at 277 nm. Adjustment of the spectral sensitivity curve for the filtering effect of the cornea makes the 280 nm. shoulder more prominent, suggesting the presence of energy transfer from the protein component of the visual pigment to the chromophore.

3. The short-wavelength limit of the housefly's visible spectrum is determined by the availability of ultraviolet light and is about 300 nm. in nature. The long-wavelength limit is set by the falling absorption of the visual pigment in the red.

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Note added in proof

It has been suggested to us that the 280 nm. shoulder in the spectral sensitivity function reflects an absorption band of the chromophore. We do not consider this the likely explanation because: (1) free retinal (all *trans* or 11-*cis*) apparently lacks an adequate peak at 280 nm.; (2) if the 11-*cis* isomer showed such absorption when combined with opsin, one would expect the bleaching of rhodopsin to be accompanied by decreases in absorption around 280 nm. Such spectral changes are not observed with vertebrate visual pigments.

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