Do Flies Have A Red Receptor?

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ABSTRACT (1) The compound eye of *Musca* exhibits characteristics which have heretofore frequently been considered evidence for color receptors: (*a*) The spectral sensitivity curve has several peaks whose relative heights can be altered by selective adaptation to colored lights, and (*b*) the shape of the retinal action potential varies with wave length. (2) The action spectrum for the red enhancement of on and off responses is compared with the "red receptor" calculated by Mazokhin-Porshnyakov from colorimetric data obtained in rapid color substitutions. Both have maxima at 615 to 620 mμ and appear to be different expressions of the same phenomenon. (3) A red receptor is absent. The evidence which suggests different *types* of receptors in the region 500 to 700 mμ can be accounted for by variations in the *numbers* of receptors stimulated. In red light there is a recruitment of additional ommatidia caused by leakage of long wave lengths through the pigment screen, and this spatial summation potentiates the on and off responses. The principal evidence is: (*a*) a white eye mutant which has no accessory screening pigments also lacks the peak of sensitivity in the red, even when adapted to violet light; (*b*) white-eyed flies give identical responses with large on and off effects at all wave lengths from 500 to 700 mμ; and (*c*) reducing the number of excited ommatidia by decreasing the size of the test spot makes the on and off transients smaller relative to the receptor component.

This paper deals with a seeming paradox. The spectral sensitivity functions of certain flies, notably *Calliphora erythrocephala*, have three peaks, in the near ultraviolet, blue-green, and red (Autrum and Stumpf, 1953; Walther and Dodt, 1957, 1959). For apparently good reasons, the 615 to 630 mμ maximum has been interpreted both as a red receptor and as an artifact, and this paper is an attempt to resolve the problem.

The various experimental results on sensitivity of flies to red and green light can be summarized and the problem thrown into sharp focus by stating three hypotheses. The possibility that these hypotheses are not mutually exclusive will be considered briefly in the Discussion.

I. *The Receptor Hypothesis*

One alternative is that there is a receptor cell with maximum sensitivity in the red, perhaps containing a visual pigment with λ_{max} near 620 mμ. The evidence, none of it conclusive, is (*a*) the peak at 620 mμ in the spectral sensitivity

function of the whole eye, (*b*) a wave length dependence in the shape of the retinal action potential (Autrum, Autrum, and Hoffmann, 1961) and (*c*) colorimetric data based on responses to heterochromatic flicker.

Autrum and Stumpf (1953) were the first to study the electrical response of the compound eye to heterochromatic flicker. For certain pairs of wave lengths, the energies could be adjusted so that no transient response occurred when one stimulus light was alternated with another. With other pairs of lights, however, no adjustment of the energies could produce a physiological color match. Yellow could be matched with white, but not with other wave lengths; likewise blue and green could be matched with each other, but either always gave a response when flickered alternately with red. A "color blind" animal was reported which lacked both the 630 mμ maximum in the spectral sensitivity function and "flicker potentials" on alternate stimulation with red and green. Autrum and Stumpf suggested that the eye has separate receptors for red and green, with sensitivity maxima at 625 and 520 mμ.

Mazokhin-Porshnyakov (1960 *a*, 1960 *b*) combined the principles of colorimetry with the technique of rapid color shift and measured the amounts of red and violet in two-color mixtures required to match various test wave lengths. He assumed that his red and violet primaries each stimulated only one type of receptor, and he calculated what he interpreted as action spectra of red and blue-green sensitive elements. The red receptor showed maximum sensitivity at 615 to 620 mμ, close to the peak in the spectral sensitivity curve of the dark-adapted fly. Mazokhin-Porshnyakov (1960 *a*) obtained the same result with *Musca*, a genus in which previous work suggested no prominent red peak in the spectral sensitivity function (Donner and Kriszat, 1950).

II. *The Screening Hypothesis*

A second view is that the red peak in the spectral sensitivity function is caused by the presence of red screening pigments which, because they absorb all but long wave lengths, impart to red light a greater effectiveness, relative to green or blue, than would be predicted from the absorption spectrum of the visual pigments. The evidence is (*a*) absence of a red peak in white-eyed flies which lack the red screening pigments (Autrum, 1955; Hoffmann and Langer, 1961) and (*b*) absence of a red peak in intracellular recordings of spectral sensitivity, except for weakly responding cells whose axes were not directed at the stimulus and which were therefore presumably irradiated only weakly and indirectly by light which had been filtered through the pigment sleeves (Autrum and Burkhardt, 1961; Burkhardt, 1962).

One can grant a screening effect of the accessory pigment, however, and still not be convinced of the absence of a red receptor. White eye mutants are very much more sensitive to green light than wild type (10^3-10^4 for white-apricot; Autrum, 1955), and therefore their spectral sensitivity curve falls

rapidly with increasing wave length. Consequently, a small contribution from a red receptor might be lost on the steep slope. In fact, Autrum's (1955) spectral sensitivity curves of the white-apricot mutant of *Calliphora* have a suggestive shoulder at 630 mμ. This possibility is examined further in the Results.

III. *The Neural Interaction Hypothesis*

As will become apparent below, red sensitivity is reflected prominently in the on and off effects, which in turn have heavy contributions from the optic ganglion. This suggests a third possibility which has not been considered previously. Intracellular recordings indicate several classes of receptors with peaks of sensitivity at 470, 490, and 520 mμ (Autrum and Burkhardt, 1961; Burkhardt, 1962). The spectral sensitivity curves overlap broadly, and one can suppose that maximum on and off effects occur when the eye is stimulated by long wave lengths which excite only the 520 mμ receptor. Shorter wave lengths would excite the other receptors as well, and this might lead to an inhibition of whatever neural mechanisms produce on and off effects. Maximum on and off transients would not only be observed in the red, but their spectral sensitivities would involve in some manner a difference in sensitivities or activities of the 520 mμ and shorter wave length receptors. In a slightly different version of the hypothesis, one can imagine lateral inhibitory interaction between receptor cells also giving rise to a red maximum in the spectral sensitivity curve. In either form, this hypothesis has certain testable predictions which will be described below.

The results of this work provide evidence against hypothesis III and strong support for hypothesis II. Moreover, this paper shows that in the fly the results of color substitution experiments have been incorrectly interpreted. Transient, wave length–dependent electrical responses to color shifts which cannot be eliminated by adjustments in the intensity of one of the stimuli indicate that the two lights excite different *numbers* of receptors rather than different *kinds*. There is therefore no compelling evidence for a red receptor in flies, and all the experimental results obtained to date can be accounted for by the screening hypothesis.

METHODS

Optical Equipment The test source was a 150 watt xenon arc lamp (Hanovia D-901C-1) operated at 7.5 amp from a direct current power supply. This is an intense 2 mm arc and proxides a continuum of wave lengths throughout the visible and ultraviolet. It was used in conjunction with a monochromator (Bausch and Lomb, 52 mm square grating, 1200 lines per mm) and a filter (Corning 0-53) to remove the far ultraviolet. At 600 mμ and longer wave lengths, the second order spectrum was cleared with a Corning 3-74 filter. White-eyed flies were so sensitive

to blue and green, however, that when recording responses to red light, orange and red filters were necessary at the exit slit to reduce stray light of shorter wave lengths. The filters were Corning 3-67, used at 620 to 640 mμ, and 2-59, used at 660 to 700 mμ. The entrance and exit slits of the monochromator were adjusted for half-band widths of 10 mμ. An image of the exit slit was focused with quartz lenses in the plane of the insect's eye.

For selective adaptation, the eye was irradiated with a low voltage tungsten microscope lamp equipped with a heat filter and incident at an angle of about 30° to the test beam. Red adapting light was obtained with a Zeiss RG2 filter, which cuts off sharply between 605 to 620 mμ; blue (violet), with a pair of Zeiss BG12 filters, which have a broad window centered at 420 mμ and negligible transmission at wave lengths longer than 500 mμ. Intensity was controlled by varying the lamp voltage.

The duration of the test flashes was controlled with a photographic shutter synchronized with the oscilloscope and camera. Intensity was regulated with a pair of annular optical wedges made of films of inconel on quartz and mounted so as to rotate in opposite directions, thereby balancing each other. The wedges had a maximum density of nearly 4 and were continuously variable with a nine-turn rotary dial which could be read to 0.01 revolution. When necessary, the wedges were supplemented with a set of filters of known absorbance.

Calibrations The energy reaching the eye was measured periodically with a calibrated 12 junction bismuth-silver thermopile (Eppley) fitted with a quartz window and connected to a sensitive amplifier (Keithley No. 149) and pen recorder. The density of the wedges was determined over their entire range with a barrier layer photocell and the same amplifier. With the aid of calibrated density filters, it was found that for photocell outputs less than 18 mv the response of the measuring equipment was linear with the logarithm of the intensity. During the calibration of the thinner parts of the wedges, therefore, the intensity reaching the photocell was reduced to this range with filters. The calibration curve of the wedges varied in a systematic way with wave length; consequently, complete curves were made at several wave lengths, and intermediate values determined by interpolation.

Experimental Animals The stock of wild type house-flies was obtained from Dr. Raimon Beard, Connecticut Agriculture Experiment Station. The white eye mutant was a gift of Professor Robert Sokal, University of Kansas. This mutation is recessive (Hiroyoshi, 1961), and homozygous flies have no visible eye pigments. Stocks were maintained on the CSMA medium (Chemical Specialties Manufacturers Association, 1965).

Recording and Analysis Flies were secured on their backs with a low melting point wax blackened with a suspension of finely divided charcoal, and the pro- and mesothoracic legs were removed. A shield of aluminum foil was placed along one side of the animal, with a hole just large enough to admit the entire cornea centered over one eye. The spot of light was larger than the eye, so the entire surface of the cornea was irradiated. The cornea was pierced with a sharp steel pin and a saline-filled capillary with a tip diameter of about 25 μ inserted in the hole. The saline contained 128 mM Na^+, 5 mM K^+, 2 mM Ca^{++}, and 137 mM Cl^-. A cotton wick soaked in saline solution was placed on the opposite side of the head, where it was shielded

from the stimulating beam by the mask of aluminum foil. A chlorided silver wire in the capillary was connected to a high impedance input amplifier (Bioelectric Instruments DS2C) and oscilloscope; a similar electrode in the wick ran to ground through a voltage calibrator. All amplifier stages were direct-coupled. A photocell connected to the second channel of the oscilloscope recorded the opening and closing of the shutter. Responses were displayed on an oscilloscope, photographed, and the film measured by projecting the negatives in a photographic enlarger.

The wild type animals were dark-adapted on the apparatus for 20 minutes or more before recording began. This is sufficient time for recovery of normal flies from the lights to which they were exposed during preparation. White-eyed flies were dark-adapted for at least 30 minutes, and usually for at least 45.

Spectral sensitivity was assessed by measuring complete response-energy functions at one or two wave lengths and small segments of the curves at other wave lengths; distances between the curves, in terms of energy, were then determined graphically. Unless stated otherwise, spectral sensitivity curves are based on responses of about 1 to 2 mv.

RESULTS

For what follows, an understanding of the origins of insect retinal action potentials (Fig. 1) will be helpful. The maintained component reflects largely, if not exclusively, depolarization of the membranes of the retinular cells; therefore, it will be referred to as the receptor component, with the realization that slow, postsynaptic potentials could conceivably make a minor contribution. The evidence that this component originates in the receptors is of several kinds: (*a*) it is the only component that is observed following surgical removal of the optic ganglion (Bernhard, 1942; Jahn and Wulff, 1942; Autrum, Autrum, and Hoffmann, 1961) or in the presence of suboptimal metabolic conditions (Autrum and Hoffmann, 1960; Goldsmith, 1960); (*b*) it can be recorded in virtual isolation with a microelectrode among the retinulae and a reference electrode on the cornea—that is, when the somata of the retinular cells are the only neural elements between the electrodes (Ruck, 1961); and (*c*) intracellular records from retinular cells show a maintained drop in membrane potential in response to light (Burkhardt and Autrum, 1960; Naka, 1961; Naka and Eguchi, 1962 *a*, 1962 *b*). The same experiments also show that the off effect is postsynaptic in origin. The cellular basis for the on effect is less certain; it may represent a response of the sense cell axons, or postsynaptic units, or both.

Wave Length Dependence of the Retinal Action Potential

Fig. 1 shows that the shape of the retinal action potential of *Musca* depends on the wave length; the on and off effects become relatively more prominent as the stimulus changes from green to red. A similar result has been reported in *Calliphora* (Autrum, Autrum, and Hoffmann, 1961).

Fig. 2 is a more detailed analysis of the wave length dependence of the retinal action potential. Here are plotted response-energy curves for the three measurable parameters—on, off, and receptor components—for blue-green (500 mμ) and red (620 mμ) light. For any value of the receptor component, the on and off transients are smaller for green light than for red.

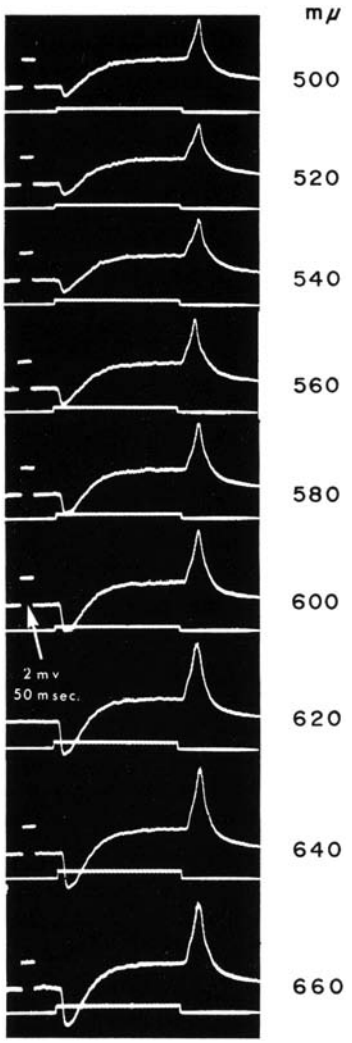

FIGURE 1. Dependence of the retinal action potential of a wild type housefly on the wave length of the stimulus. Energies of the stimulating lights were adjusted to evoke approximately equal receptor components; however, the relative prominence of the on and off effects clearly increases with wave length. Negativity of the subcorneal electrode is indicated by an upward deflection. Photocell response (lower trace of each pair) is about 0.5 sec. and indicates the time of stimulation.

Fig. 2 also makes clear why it is impossible to stimulate alternately with red and geen lights without the eye responding to each transition. Even when the energies have been adjusted to produce equal receptor components, there will be on and off responses to the red phase of the stimulating cycle.

This suggests that the red receptor of Mazokhin-Porshnyakov's colori-

metric analysis is related to the enhancement of on and off transients by red light. This idea is further supported by the experiment shown in Fig. 3. The small filled circles and the curve show the red-sensitive mechanism of *Calliphora* which was calculated by Mazokhin-Porshnyakov (1960 *b*) on the basis of color matches in rapid color substitutions. Similar results were obtained with *Musca* (Mazokhin-Porshnyakov, 1960 *a*). The large circles are my measurements of spectral sensitivities of on and off responses of *Musca* to 0.5 second flashes. The criterion responses were several millivolts, and the on effects were larger than it was possible to elicit with green light. The large points therefore

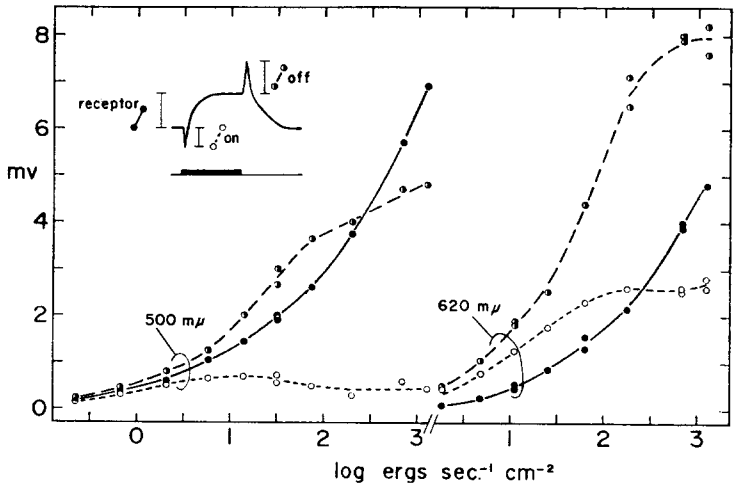

FIGURE 2. Typical response-energy curves for the on, receptor, and off components of the retinal action potential of a dark-adapted red-eyed fly. The three parameters which were measured are shown in the insect; each is plotted without regard to sign. The responses to blue-green and red light are qualitatively different at all levels of stimulation.

show the spectral sensitivity of the red enhancement of the on and off transients. The correspondence of the three sets of points strengthens the conclusion that the method of rapid color substitution is based on an inability to shift alternately from long to short wave lengths without evoking on and off effects. That red and green light do not have identical effects on the eye is therefore clearly substantiated.

Spectral Sensitivity and Selective Adaptation of Normally Pigmented Eyes

The upper curve in Fig. 4 is the average spectral sensitivity between 500 to 700 mμ of eight dark-adapted eyes. Sensitivity, plotted in logarithmic units on the ordinate, is the reciprocal of the relative number of photons required to evoke a constant receptor component in the retinal action potential. There is

a maximum in the near ultraviolet which is not shown, another in the blue-green, and a small peak in the red near the position of the red peak of *Calliphora.*

This observation is evidence against the first version of hypothesis III, for if the red maximum were a product of neural interaction in the optic lobe, one

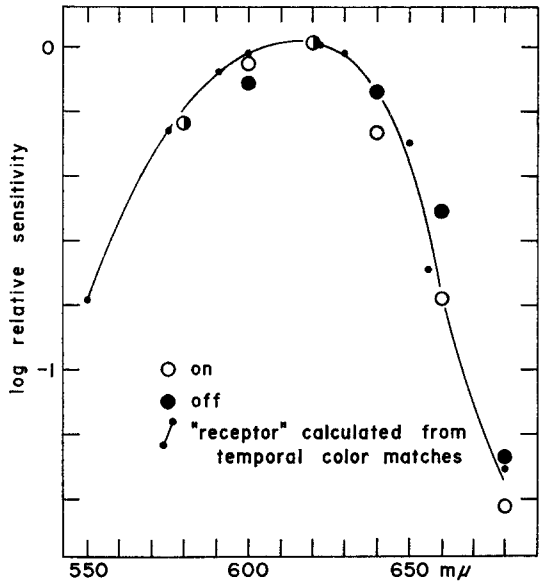

FIGURE 3. The red receptor of *Calliphora* calculated by Mazokhin-Porshnyakov (small filled circles, solid line) compared with the spectral sensitivity of the red enhancement of on and off effects (large circles) of a housefly, *Musca.* Ordinate is the log of the reciprocal number of photons for a constant effect. In the case of the on responses this was 2.3 mv, or more than two times larger than could be obtained from this animal with blue-green light. Other individual *Musca* also showed a peak at 620 mμ; however, the shape of the spectral sensitivity curve depends to some extent on the criterion response, for with increasing intensity of stimulation the relative sensitivity increases on the long wave length side of the maximum.

would not expect to encounter it at the receptor level. On the other hand, the experiment is perhaps equivocal because the receptor component may not arise exclusively from the retinulae and because of the possibility of lateral inhibition at the receptor level.

Experiments on selective adaptation to colored lights, however, rule out hypothesis III as the explanation of the red maximum. Fig. 4 also shows the spectral sensitivities of two animals (A and B) in different states of adaptation to red and blue background lights. Although a blue adapting light raises the threshold throughout the spectrum, the eye becomes more sensitive to 620 than to 500 mμ; that is, a blue adapting light enhances the red maximum. Red

light, on the other hand, if it has any effect makes the red peak somewhat less prominent than in the dark-adapted eye. These results are obviously consistent with the receptor hypothesis (I), and they are equally, but perhaps less obviously, consistent with the screening hypothesis (II) as well. They are at variance with hypothesis III, however, for the following reason. If high

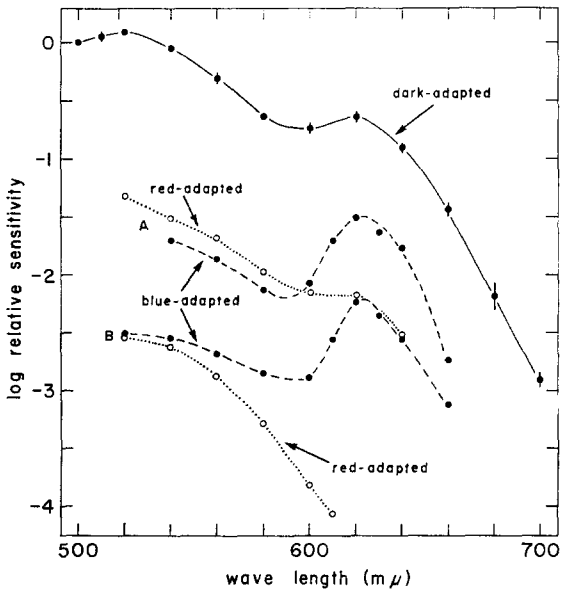

FIGURE 4. Spectral sensitivity of the receptor component of the retinal action potential of dark-adapted, red-eyed *Musca* (filled circles, solid line). Points represent averages of eight animals; standard errors, when larger than the points, are indicated by vertical lines. The effects of red and violet adapting lights are shown for two animals (A and B). Blue light enhances the 620 mμ peak. Ordinate, log reciprocal photons for a constant receptor component. Abscissa, wave length of the stimulus, in millimicrons.

sensitivity to red light depended on the absence of inhibitory processes which were activated by short wave lengths, a blue adapting light would depress the red peak, not make it more conspicuous.[1]

To recapitulate, the red peak arises in the receptor layer, for it is present in both receptor and optic ganglion components of the retinal action potential;

[1] One might argue that this experiment is not a critical test of hypothesis III if the inhibitory mechanism fatigued at lower intensities of background illumination than were employed here, thereby freeing the long wave length receptor from inhibition and enhancing the long wave length peak. That this too is not consistent with the observed result can be appreciated by considering the limiting case—complete absence of inhibitory capacity. The spectral sensitivity of the long wave length receptor would then be undistorted and its maximum would appear in the green at 520 mμ. Therefore, depending on the extent of fatigue of the short wave length receptor responsible for inhibition, the long wave length peak could lie anywhere from 520 to 620 mμ. What is observed, however, is that the red peak remains at 620 mμ regardless of its relative prominence.

moreover, it is increased in relative prominence by a blue adapting light, which would not be expected if it were the result of neural inhibitory processes triggered by short wave lengths.

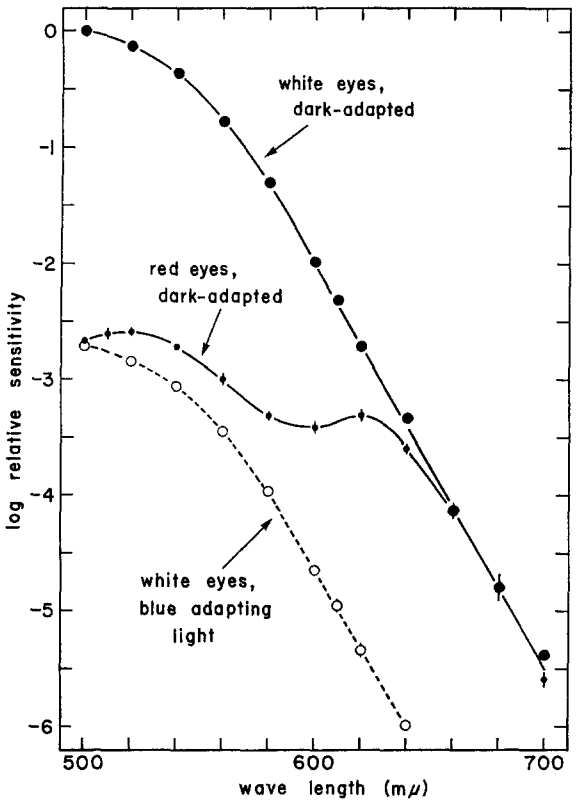

FIGURE 5. Average spectral sensitivity of nine dark-adapted, white-eyed flies (large filled circles, solid curve) compared with normal red-eyed flies (small filled circles, solid curve) and the average sensitivity of the nine white-eyed flies in the presence of a violet adapting light (open circles, dashed curve). Axes as in Fig. 4. See the text for discussion.

Spectral Sensitivity and Differential Adaptation of White Eyes

A choice can be made between the receptor and screening hypotheses on the basis of measurements on a white eye mutant which lacks both ommochrome and significant amounts of other screening pigments as well. The large filled circles in Fig. 5 represent the average spectral sensitivity of nine dark-adapted animals. For comparison, the spectral sensitivity of red-eyed flies has been plotted on the same graph. The curves were plotted to coincide at 660 and 680 mμ, giving a difference in sensitivity at 500 mμ of 2.68 log units. The measured average difference in sensitivity at 500 mμ, based on a total of nine white and thirty-two normal flies, was 2.61 ± 0.32 log units. The two figures

agree well within the limits of experimental error. The white-eyed flies are therefore nearly 500 times as sensitive to blue-green light as the wild type animals, but both strains have the same absolute sensitivity at 660 mμ and longer wave lengths. This result is qualitatively similar to what has been found for *Calliphora* (Autrum, 1955) and supports the screening hypothesis (II).

The open circles in Fig. 5 show the spectral sensitivity of the same white-eyed flies in the presence of a blue adapting light. If a red receptor were present, it should be accentuated by this treatment; however, the curve has the same shape as the dark-adapted animals' and is merely transposed downward along the ordinate. By suitable displacement, the two curves can be superimposed within ±0.05 log unit over their entire length.

Not only is the white eye mutant more sensitive than the wild type to short wave lengths, but lower energies of the blue adapting light are required to depress the sensitivity. The adaptation in Fig. 5 was produced with a background flux of about 10^3 ergs sec.$^{-1}$ cm^{-2}, only about 15 per cent the value of 6.7×10^3 ergs sec.$^{-1}$ cm^{-2} required with animal B in Fig. 4. In each case, the fall in sensitivity at 500 mμ was approximately 2.5 log units.

These experiments urge the following tentative conclusions. Hypothesis II is correct; the eyes of flies lack a red receptor; and the peak of sensitivity in the red as well as large on and off transients in the retinal action potential is caused by the leakage of red light through the pigment sleeves. A peak of sensitivity in the red must therefore be caused by the excitation of peripheral ommatidia, whose responses contribute to the retinal action potential and make red light more effective than one might anticipate from the wave length sensitivity of the receptor pigment. If this be true, large on and off effects are a function of the number of active ommatidia.

Absence of Wave Length Effects in the Retinal Action Potential of White-Eyed Flies

The correctness of these conclusions is subject to additional experimental test. White-eyed flies should give identical electrical responses to red and green lights, provided only that the energies are properly adjusted. Fig. 6 shows that this is in fact the case.

More extensive results are shown in Fig. 7, which should be compared with the response-energy curves for the red-eyed fly shown in Fig. 2. In Fig. 7, the set of three curves at 500 mμ can be superimposed on the set at 620 mμ. By contrast, in the red-eyed flies, only the receptor components come close to superimposition by displacement along the abscissa. Thus in the absence of screening pigment, the wave length dependence of the retinal action potential disappears.

The significance of this comparison does not end here, for either set of curves for the white eye mutant can be nearly superimposed on the set

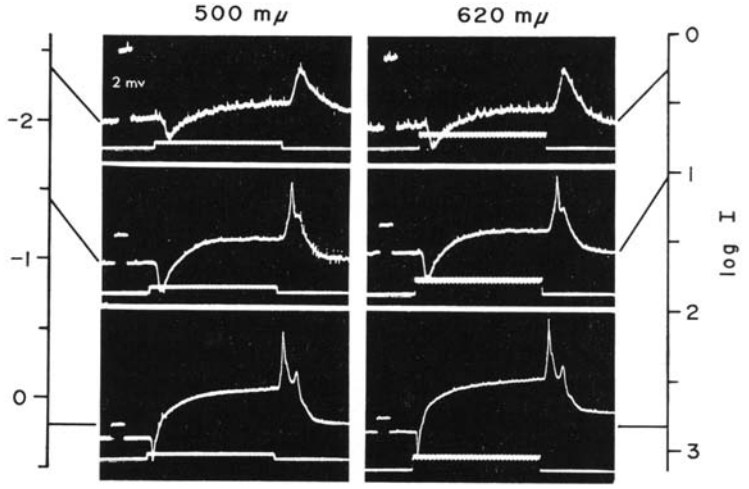

FIGURE 6. Retinal action potentials from a white eye mutant of *Musca*, showing that responses to blue-green light can be matched by responses to red. Negativity of electrode in the illuminated eye is shown by an upward deflection. Calibration pulses preceding each response are 2 mv. Intensity scales to the right and left give log ergs sec.$^{-1}$ cm^{-2}. Test flashes (lower trace in each frame) were 0.5 sec. duration.

measured at 620 mμ in the red-eyed fly. The common feature of these three measurements is that the stimulus was able to penetrate throughout much of the eye.

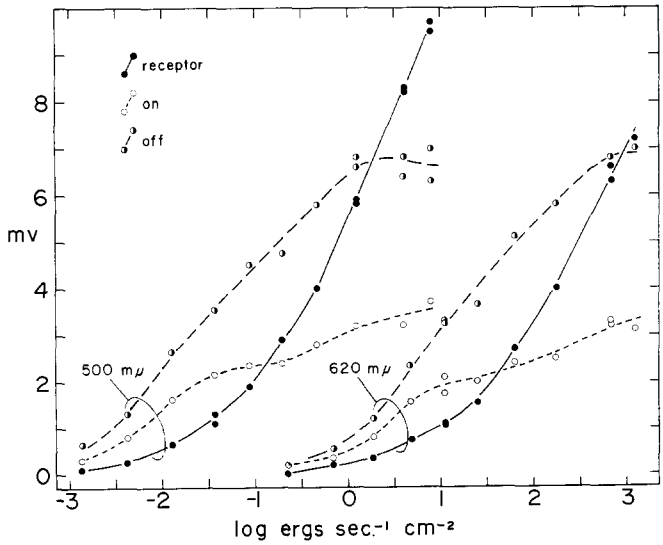

FIGURE 7. Typical response-energy curves for the on, receptor, and off components of the retinal action potential of a white-eyed fly. Compare with the red-eyed strain shown in Fig. 2. In the white eye mutant, wave length dependence of the response is absent, and the response-energy curves are similar to those obtained from red-eyed flies with red light.

Response-Energy Curves for the Receptor Component

The response-energy functions recorded for the receptor component of the retinal action potential bear further inspection. Assume a population of receptors with identical response characteristics to axial illumination. When the eye is irradiated from one direction, as in these experiments, the thresholds of these receptors will not all be the same; units oriented at a large angle to the stimulus will be less sensitive.[2] As the intensity of the stimulus is raised, more cells will be excited significantly and contribute to the mass response. The more cells whose thresholds are exceeded with each increment in intensity, the steeper will be the slope of the response-energy function.

The effect of the pigment screens on the distribution of thresholds will vary with wave length. With green or blue light which is absorbed by the accessory pigment, there will be a large difference between the thresholds of central and peripheral ommatidia. With red light which penetrates through the pigment sleeves, this difference will be smaller. Thus the number of additional receptors excited per unit increase in intensity will be greater for long than for short wave length stimuli; consequently the response-energy function should be steeper for red light than for green or blue.

That this prediction is fulfilled is shown by Fig. 8. The magnitude of the receptor component is plotted as a function of log I for five animals. Filled circles and solid lines indicate responses to blue light; open circles and broken curves, to red. Each pair of curves is for a single animal, and in order that the responses to red and blue can be easily compared, the pairs of curves are plotted so as to intersect at their upper ends. So that each animal can be distinguished from the others, each pair of curves has also been separated from the next by another arbitrary displacement along the x-axis. The abscissa can therefore not be labeled in units of absolute energy. The point of this figure is that for each animal the slope of the response curve to red light is steeper than for blue, as expected on the basis of the screening hypothesis.

It the difference in slopes is caused by the screening pigment, it should not be present in white-eyed flies. This prediction too is borne out by experiment. Fig. 9 shows the same kind of measurements as those in Fig. 8 repeated on five white-eyed flies. The solid curves were drawn through the filled circles (500 mμ); however, the same curves fit the open circles (620 mμ) nearly as well. One can therefore conclude that in white-eyed flies the slopes of the response-energy functions are invariant with wave length.

Enhancement of On and Off Effects through Spatial Recruitment of Ommatidia

A final corollary to the screening hypothesis is that the size of the on and off transients depends on the number of ommatidia that are excited. That this is a consequence of hypothesis II has been recognized previously (Autrum,

[2] Threshold here refers to the threshold of detection in the retinal action potential.

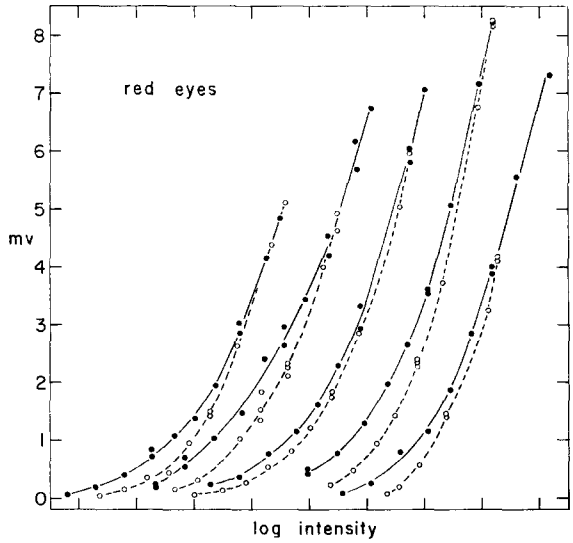

FIGURE 8. Comparisons of the slopes of the receptor component *vs.* log *I* for long and short wave lengths in five red-eyed flies. Filled circles and solid curves, blue light (Corning 5-60, tungsten source); open circles and broken curves, red light (Zeiss RG-2, tungsten source). Abscissa is marked in intervals of one log unit relative intensity. To facilitate comparison the curves have been moved parallel to the x-axis by different amounts.

Autrum, and Hoffmann, 1961), but not tested. Stimuli which excite a small patch of ommatidia should produce smaller on and off effects than stimuli which excite the whole eye, even if the energies are adjusted for equal receptor

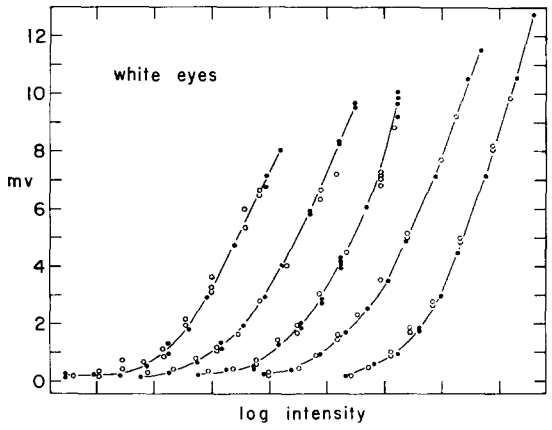

FIGURE 9. Response-energy curves (receptor component) for long and short wave lengths in five white-eyed flies. Filled circles and curves, blue-green light (500 mμ); open circles, red light (620 mμ). Abscissa is marked in intervals of one log unit relative intensity.

components. Fig. 10 (upper half) shows the results of such an experiment on a white-eyed fly. The 30 μ spot was produced with a microscope objective. Most of the light was concentrated on about five facets, but stray light was obvious on surrounding regions of the eye. Nevertheless, the spatial pattern of stimulation was very different from a uniform spot of light on the entire eye. The result is clear; with white-eyed flies, decreasing the size of the spot decreases the

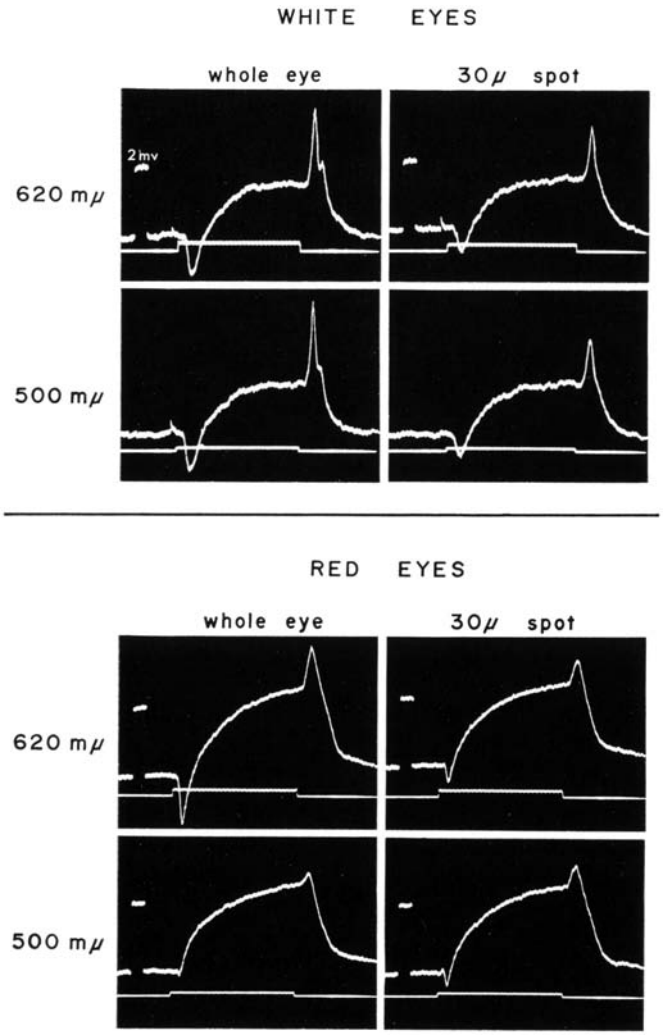

FIGURE 10. The effect of the size of the spot on the relative prominence of the on and off effects in two animals, white- and red-eyed. In each animal, energies were adjusted for approximately equal receptor components. Test flashes were 0.5 sec. and calibration pulses 2 mv throughout. An upward deflection indicates negativity of the illuminated eye.

prominence of the on and off transients. Moreover, the effect is about equal with green and red light and is therefore independent of wave length. Moreover, it can be demonstrated in either the dorsal or ventral parts of the eye.

With red-eyed flies, decreasing the size of the test spot should decrease the on and off responses to red light. The effect should be much less marked with green light, however, for even when the whole eye is irradiated, the excitation should be confined to those ommatidia whose axes are oriented within 8° of the stimulus, this being the angle of acceptance for individual ommatidia in the fly (de Vries and Kuiper, 1958; Autrum and Wiedemann, 1962). An example of an experiment on red eyes is shown in the lower half of Fig. 10. For red light, when a small area of the eye is illuminated, the on and off effects are decreased relative to the receptor component. For blue-green, decreasing the area illuminated has little effect on the wave form of the retinal action potential, and in the example shown in Fig. 10 the on and off transients were actually increased slightly.

The Pigment Screen

For a single receptor in the eye, the effect of the pigment screen on sensitivity depends on the absorbance of the shielding pigment;

$$\log E_\lambda^r - \log E_\lambda^w = \alpha_\lambda \beta$$

where E_λ^r is the energy at wave length λ required at the cornea to elicit a criterion response from the receptor in a red-eyed fly, E_λ^w is the energy required to excite the same receptor in a white eye mutant, α_λ is the absorption coefficient of the pigment screen, and β is equivalent to the product of concentration of accessory pigment times path length, and varies with the position of the receptor in the red eye.

The receptor component of the retinal action potential represents summed activity of a number of sense cells, each shielded to a different extent by the pigment screen. It is possible, however, to equate the difference in log sensitivity of white- and red-eyed flies to the average absorbance of the pigment screen, provided there is (*a*) direct proportionality between the magnitude of the receptor component of the retinal action potential and the sum of the extracellular currents generated by the increased conductance of the sense cell membranes, and (*b*) a linear relation between change in membrane potential and the log of the stimulating intensity. The first condition implies that the resistance between the recording electrodes is essentially the same for currents originating anywhere in the retinular layer and is fulfilled if most of the voltage drop occurs across a distributed structure such as the basement membrane. The second condition is met by cells which are moderately or strongly excited (Fuortes, 1963) but probably not by the weakly excited units. The latter, however, may make a minor contribution to the mass response.

With these assumptions, the condition for a constant response from a red-eyed fly is therefore

$$\sum_{j=1}^{n} \log E'_{\lambda j} = \sum_{j=1}^{n} (\log E^{r}_{\lambda} - \alpha_{\lambda}\beta_j) = k$$

where n is the number of receptors responding, $E'_{\lambda j}$ is the energy reaching the jth receptor, and k is a constant. $E'_{\lambda j}$ is related to the absorbance of the pigment cells by $\log E^{r}_{\lambda}/E'_{\lambda j} = \alpha_{\lambda}\beta_j$. For the same response from a white-eyed fly containing an equal amount of visual pigment, $N \log E^{w}_{\lambda} = k$, where N is the total number of receptors, which are assumed to be uniformly excited. The condition for equal responses from red- and white-eyed flies can therefore be written by eliminating k.

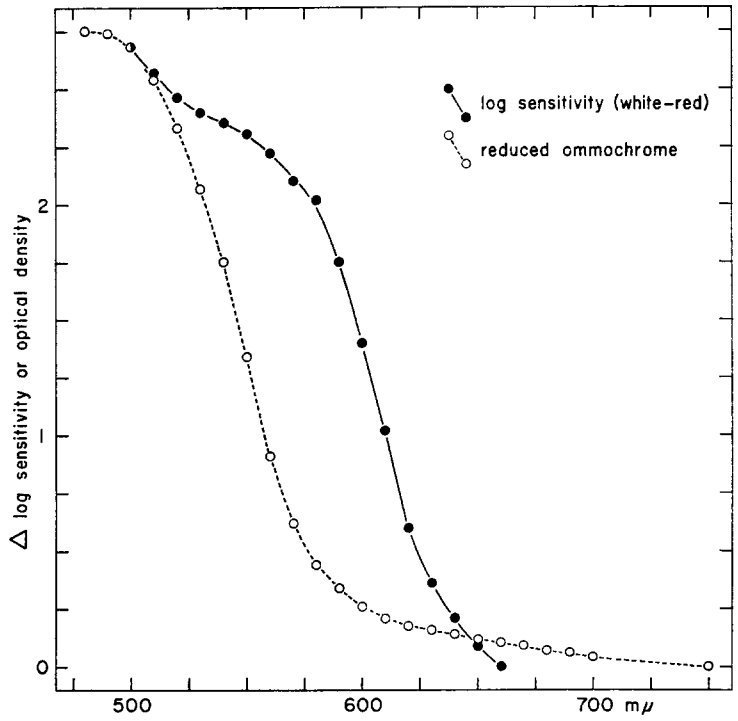

FIGURE 11. Log sensitivity of the white eye mutant less log sensitivity of the wild type strain (filled circles, solid curve) compared with the absorbance in acid alcohol of an extract of reduced ommochrome from *Musca* (open circles, dashed curve). See the text for discussion.

$$\frac{\sum_{j=1}^{n} \log E^{r}_{\lambda}}{N} - \frac{\sum_{j=1}^{n} \alpha_{\lambda}\beta_j}{N} = \log E^{w}_{\lambda}$$

or

$$\overline{\log E^{r}_{\lambda}} - \overline{\log E^{w}_{\lambda}} = \overline{\alpha_{\lambda}\beta}.$$

In other words, the difference in the *average* values of log threshold of red- and white-eyed flies is equal to the *average* absorbance of the pigment screen.

Fig. 11 (filled circles, solid curve) shows the difference in log sensitivity of white- and red-eyed flies at wave lengths longer than 500 mμ, obtained by subtracting the two uppermost curves in Fig. 5. This is the average absorption spectrum of the pigment screen seen by the sense cells of red-eyed flies under the conditions of these experiments. Interestingly, this simple cut-off filter is all that is required to produce the 620 mμ maximum in the spectral sensitivity function of normal flies.

The open circles and broken curve in Fig. 11 show the absorption spectrum of a crude extract of ommochrome from the heads of wild type *Musca*. The pigment was extracted with 1 per cent HCl in ethanol and reduced with $Na_2S_2O_4$. Extracts of heads in ammonium hydroxide solutions followed by paper and thin layer chromatography showed that the house-fly has no pterins absorbing at these wave lengths. This spectrum in Fig. 11 is therefore chiefly the result of absorption by dihydroxanthommatin (Butenandt *et al.*, 1960).

There is a clear discrepancy between the absorption of the pigment extract and the calculated absorption of the pigment screen. The most likely reason is that *in vivo* the pigment undergoes a spectral shift as a result of binding to protein. Unfortunately, the native pigment, attached to protein granules, is virtually insoluble.

DISCUSSION

The Rationale for Using White Eye Mutants

The use of white eye mutants in this and previous experiments on spectral sensitivity requires the assumption that white-eyed flies differ from wild type only in the absence of accessory screening pigments. In *Musca* and *Calliphora* the mutation *w* is a single recessive gene which blocks the synthesis of ommochrome and also leads either to an absence of pterins or a conversion of colored pterins to colorless forms. This pleiotropic effect on two apparently unrelated synthetic pathways is not well understood, but there is evidence that both types of pigment undergo their last synthetic steps in association with the protein granules to which they are finally bound and that the gene *w* acts at this level (*cf.* Ziegler, 1961). One might also suppose that in addition *w* causes the absence of a red receptor—a retinal-based pigment which one would expect to be in the rhabdom and not part of the accessory pigment granules—while sparing other retinaldehyde pigments with λ_{max} at shorter wave lengths. Although this is possible, it does not seem probable.

Comparison of Musca and Calliphora

Several of Autrum's (1955) observations support the thesis that these findings on *Musca* are also applicable to *Calliphora*. A wave length dependence of the

on effect was present in red-eyed *Calliphora*, for the response-energy curves had steeper slopes the longer the stimulus wave length. In the mutant white-apricot, moreover, the response-energy curves were parallel at all wave lengths longer than 511 mμ, indicating the absence of a specific wave length effect. Although the response-energy function for blue light (449 mμ) had a shallower slope than for green, yellow, and red, this too can be accounted for by the screening hypothesis. The mutant, white-apricot, although devoid of ommochrome, has a yellow pterin screening pigment with peak of absorption in the blue (Burkhardt, 1962).

The red maximum of *Calliphora* (Autrum and Stumpf, 1953; Walther and Dodt, 1957, 1959) seems to be much more conspicuous than the corresponding peak in the spectral sensitivity curve of *Musca*. This is largely illusory. The measurements on *Calliphora* were based on the height of the on effect, and because the on effect increases more rapidly than the receptor component with the number of ommatidia excited, the red peak is most in evidence when the on effect is employed as the criterion response (*cf.* Autrum, Autrum, and Hoffmann, 1961). The 630 mμ peak of *Calliphora* decreases in prominence as the intensity is lowered (Autrum, 1955; Walther and Dodt, 1959), and in rapid color substitutions only a single primary is necessary at low energies to match any monochromatic radiation (Mazokhin-Porshnyakov, 1960 *a*, 1960 *b*). Both these observations also can be accounted for by the fact that below saturation the on and off effects increase as a function of energy more steeply than the receptor component.

The possibility that the receptor and screening hypotheses might not be mutually exclusive alternatives was alluded to in the introduction. Autrum, Autrum, and Hoffmann (1961) state that in *Calliphora* the red enhancement of ganglionic potentials is not entirely an area effect due to the screening pigment but indicates a heterogeneity of receptors. Their evidence is that in the eye mutant white-apricot some of the wave length effect persists. This observation differs from my measurements on *Musca*, but perhaps because white-apricot is not completely devoid of screening pigments.

Implications for the Study of Color Vision

Perhaps the most important result of this work is that it forces a reevaluation of several neurophysiological criteria which have previously been suggested (*e.g.* Goldsmith, 1961) as evidence for more than one kind of color receptor in compound eyes. Burkhardt (1964) has raised similar questions. The first of these criteria is the inability to match retinal action potentials in different regions of the spectrum. The present experiments show that in the house-fly prominent on and off effects can result from the excitation of relatively large numbers of ommatidia, and this can come about as a result of leakage of long wave lengths through the pigment sleeves. The generality of this explanation, however, remains to be determined. In the honeybee there is a similar en-

hancement of the off effect with increasing wave length (Goldsmith, 1960), but the pigmentation of the eye appears so dark as to be almost black, and there is no peak of sensitivity in the red. In this species further work will be required to assess the extent of leakage through the pigment cells.

The second equivocal criterion is the widely employed technique of changing the shape of the spectral sensitivity function by selective adaptation of the eye to different wave lengths. The usual interpretation is that one is isolating color receptors by this maneuver. The ambiguity which now complicates these experiments is a result of the morphological organization of compound eyes. In the fly, a green test spot, even though as large as the eye, fails to excite peripheral ommatidia whose axes are oriented at a wide angle to the stimulus. Red light, on the other hand, passes through the accessory pigment and reaches these receptors. A blue adapting light depresses the sensitivity of the central ommatidia while sparing the peripheral units; consequently these latter cells, which are reached only by red test lights, dominate the responses of the blue-adapted eye, with the result that the 620 mμ maximum becomes relatively more conspicuous.

Again, the extent to which light leakage complicates work on other species will have to be established by experiment. On hindsight, however, it seems unlikely that the results obtained on the bee by the method of selective adaptation (Goldsmith, 1960) are much in error on this account. The spectral sensitivity of the ultraviolet receptor was measured by adapting the eye to yellow light. If the 340 mμ maximum which is revealed in this way existed for the same reason as the 620 mμ peak in flies, it would be necessary that the screening pigment be more transparent to ultraviolet than yellow, and this seems exceedingly unlikely. Moreover, the existence of the ultraviolet receptor has been confirmed by intracellular recording (Autrum and von Zwehl, 1962).

These results on flies have even more serious and immediate implications for the method of deducing the spectral sensitivities of receptors from experiments on rapid color shifts. This technique can be doubly misleading. The 620 mμ peak of flies is not a receptor but is a fortuitous difference between the action spectrum of the photoreceptors and the absorbance of the pigment sleeves. Furthermore, the method fails to detect the different receptors which have been found by measuring the spectral sensitivities of single receptor cells (Burkhardt, 1962). The results which were obtained on dragonflies by color substitution (Mazokhin-Porshnyakov, 1959) should therefore be reexamined by other methods.

In this regard, some of Autrum and Stumpf's (1953) results with heterochromatic flicker require further comment. Although the largest flicker potentials were obtained by substituting red for blue-green, small responses could frequently be obtained on shifting from one short wave length to another. For each wave length in the region 500 to 560 mμ, however, there

was a corresponding one at 400 to 450 mμ with which a color match could be made. In order to account for these equivalent wave lengths to either side of 490 mμ, Autrum and Stumpf postulated that the "red receptor" had a secondary peak of sensitivity in the violet.

The screening hypothesis provides a possible alternative explanation for these equivalent wave lengths. The peak of absorption of reduced xanthommatin lies at 490 mμ, and there is a minimum at shorter wave lengths. In the blue or violet region of the spectrum, therefore, there may be a small window in the pigment sleeves which is not completely shaded by pterins and which permits a small amount of leakage equivalent to that encountered in the green.

Where does this leave the status of color vision in muscid flies? There is evidence for a heterogeneity of receptors in the report (Autrum and Burkhardt, 1961; Burkhardt, 1962) of cells with maximum sensitivities at 470, 490, and 520 mμ. Nevertheless, the area effects which are described here also could provide the basis for a limited and crude form of wave length discrimination. (For example, only objects which are not red might appear to have sharp contours.) Critical evidence is lacking, however, for unlike the honeybee there is no behavioral demonstration that these animals have color vision.

On the other hand, the presence of stray light might be expected to be disadvantageous, and one wonders why, if ommatidial isolation is important, these flies have a pigment sleeve that leaks so badly in the red? On this point it is relevant to compare the light energies used in these experiments with those present in the normal habitat of the fly. If the experimental intensities were very much higher than normal sources, it might be argued that the ommatidia coaxial with the stimulus were saturated and that the recruitment of peripheral units by red light is an artificial phenomenon of little consequence to the fly. The very highest energy fluxes of blue-green light employed in this work, however, are equivalent to about 100 lumens per square foot, or what one might encounter on the surface of a brightly lighted desk. This value of corneal illumination is certainly exceeded when the animals fly in direct sunlight.

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