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Photopigment and Receptor Properties in *Drosophila* Compound Eye and Ocellar Receptors*

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Abstract. A review of the spectral sensitivity and the rhodopsin and metarhodopsin characteristics in three compound eye receptor types (R1-6, R7, and R8) and ocellar receptors is presented (Fig. 1). Photopigment properties were determined from measures of conversion efficiency. The photopigments of R1-6 were studied using in vivo microspectrophotometry in the deep pseudopupil of white-eyed flies. These studies yielded a refined estimate of the R1-6 metarhodopsin spectrum (Fig. 2). The quantum efficiency relative to the spectral sensitivity estimate of the rhodopsin spectrum was factored out. The quantum efficiency of rhodopsin is about 1.75 times that of metarhodopsin. The peak absorbance of metarhodopsin was estimated to be about 2.6 times that of rhodopsin. The mechanism of the two-peaked R1-6 spectral sensitivity and metarhodopsin spectrum is discussed in terms of evidence that there is only one rhodopsin in R1-6 and that vitamin A deprivation preferentially lowers ultraviolet sensitivity. The prolonged depolarizing afterpotential is reviewed from the standpoint of the internal transmitter hypothesis of visual excitation. A careful comparison of the intensity-responsivity for photopigment conversion and its adaptional consequences is made (Fig. 3).

Key words: Visual pigments – Invertebrate neurobiology – Membrane biophysics – Retina.

Introduction

The visual receptor system of flies has long been a model neural system for studying behavioural integration from sensory input. The spectral sensitivities of the compound eye receptors and their input into behaviour have been particularly well studied (e.g., Bertholf, 1932; Burkhardt, 1962; Eckert, 1971; Harris et al., 1976). In spite of this relatively long history of studies of fly receptor spectra, discoveries are still being made concerning fly receptor function. Recent developments in our understanding of receptor and photopigment spectral mechanisms and physiological consequences of receptor stimulation are reviewed in this synthesis.

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Fly Receptor Spectral Sensitivities

The spectral sensitivities of R1–6, R7, and R8, the eight cells of three receptor types in the compound eye, and ocellar receptors in these so-called "simple" eyes were determined from criterion late receptor potential components of the electroretinogram (ERG) with and without chromatic adaptation (Fig. 1). *Drosophila* whose compound or ocellar eyes were genetically made white provided spectra unconfounded by eye colour pigments. Mutants lacking functional R1–6, R7 or R1–6 + R7 were used to systematically simplify the compound eye ERG to identify receptor-specific spectral sensitivities. These ERG data show that: 1) R1–6 (Fig. 1a) and ocellar receptors (Fig. 1d) have two spectra maxima (in the ultraviolet, UV, and blue); 2) R7 (Fig. 1b) is a UV receptor (perhaps with a blue shoulder); and 3) R8 (Fig. 1c) is a blue receptor (Harris et al., 1976; Stark et al., 1976; Stark, 1977; Hu et al., 1978). These ERG data from white eyes have the advantage of pooling for statistically tenable estimates of spectral sensitivity. However, this combination of ERG with genetic dissection can not detect the spectral multiplicity within a receptor type which has recently been suggested from studies of R1–6 (Moring, 1978; Stavenga, 1979); and R7 receptors (Kirschfeld et al., 1978). Furthermore, since the ERG stimulus is not axial to all receptors in white eyes, this ERG-based data would not reveal the influence of the distal R7 on the spectrum of the axial R8 receptor suggested by the data of Hardie (1977) and Kirschfeld et al. (1978).

Fig. 1. Spectral sensitivities and metarhodopsin spectra for *Drosophila* receptors. Absolute sensitivity (log quanta/cm^2 · s) for (**a**–**d**) are determined by the specific criteria and mutant and rearing conditions in these experiments; however, the spectral sensitivity shapes reasonably accurately resemble the spectra from white-eyed wild-type eyes (see e.g., Harris et al., 1976). For (**a, b,** and **d**), metarhodopsin (dotted lines) spectra were calculated using spectral sensitivity (solid lines) to estimate rhodopsin spectra and using sensitivity measures after photoequilibrium induced by chromatic adaptation to estimate proportion of photopigment in the rhodopsin state. In (**a**), the spectral sensitivity is based on 3.0 mV (R1–6 dominated) ERG receptor component from high vitamin A reared white-eyed (*cn bw*) *Drosophila;* in the chromatic adaptation experiment, intensity required to elicit a criterion small 0.2 mV positive M-potential component was determined (data from Stark et al., 1977). In (**b**), sensitivity is for a 0.5 mV criterion from white-eyed (*w*) *rdg B* mutants (in which R1–6 degenerate); flies were vitamin A deprived (which proved necessary for the chromatic adaptation experiment) and aged (which seemed to minimize the R8 contribution to the otherwise R7 dominated ERG) (data from Stark, 1977). In (**c**), sensitivity is for a 0.5 mV criterion from white-eyed (*w*) *sev; ora* mutants (in which only R8 is functional); lack of significant chromatic adaptation (Harris et al., 1976; recently confirmed by Johnson) precluded the chromatic adaptation based derivation of a metarhodopsin spectrum (data from Stark, 1977). In (**d**), sensitivity is for a 1.0 mV criterion from white (*cn*) lateral ocelli (data from Hu et al., 1978). For (**b** and **d**) photoequilibrium spectra were based on 0.5 and 2.0 mV criterion ERG receptor potentials respectively. (**e**) Action spectra reflecting R480 to M580 conversion and M580 to R480 reconversion in R1–6 in white-eyed *Drosophila.* The squares show in vitro data (from Harris et al., 1976; Harris, 1976). The circles show microspectrophotometric data from the deep pseudopupil of living white-eyed *Drosophila.* The diamonds show data based on sensitivity change; hollow diamonds reflect M580 reconversion data after 370 nm adaptation; filled diamonds after 470 nm adaptation (from Stark, 1975; Stark and Zitzmann, 1976). The triangles show data based on PDA; hollow triangles after 370 nm adaptation; filled triangles after 470 nm adaptation (recalculated from the data of Stark, 1975; Stark and Zitzmann, 1976). The four curves are adjusted to a criterion of $\frac{1}{3}$ of the maximal change (in absorbance difference, sensitivity in linear units and PDA or its repolarization). Standard errors for relative spectra are shown. (**f**) Action spectra for $\frac{1}{3}$ rhodopsin to metarhodopsin conversion (filled circles) and for $\frac{1}{3}$ metarhodopsin to rhodopsin reconversion (hollow circles) in R7 in white-eyed *Drosophila* (from Harris et al., 1976; Harris, 1976)

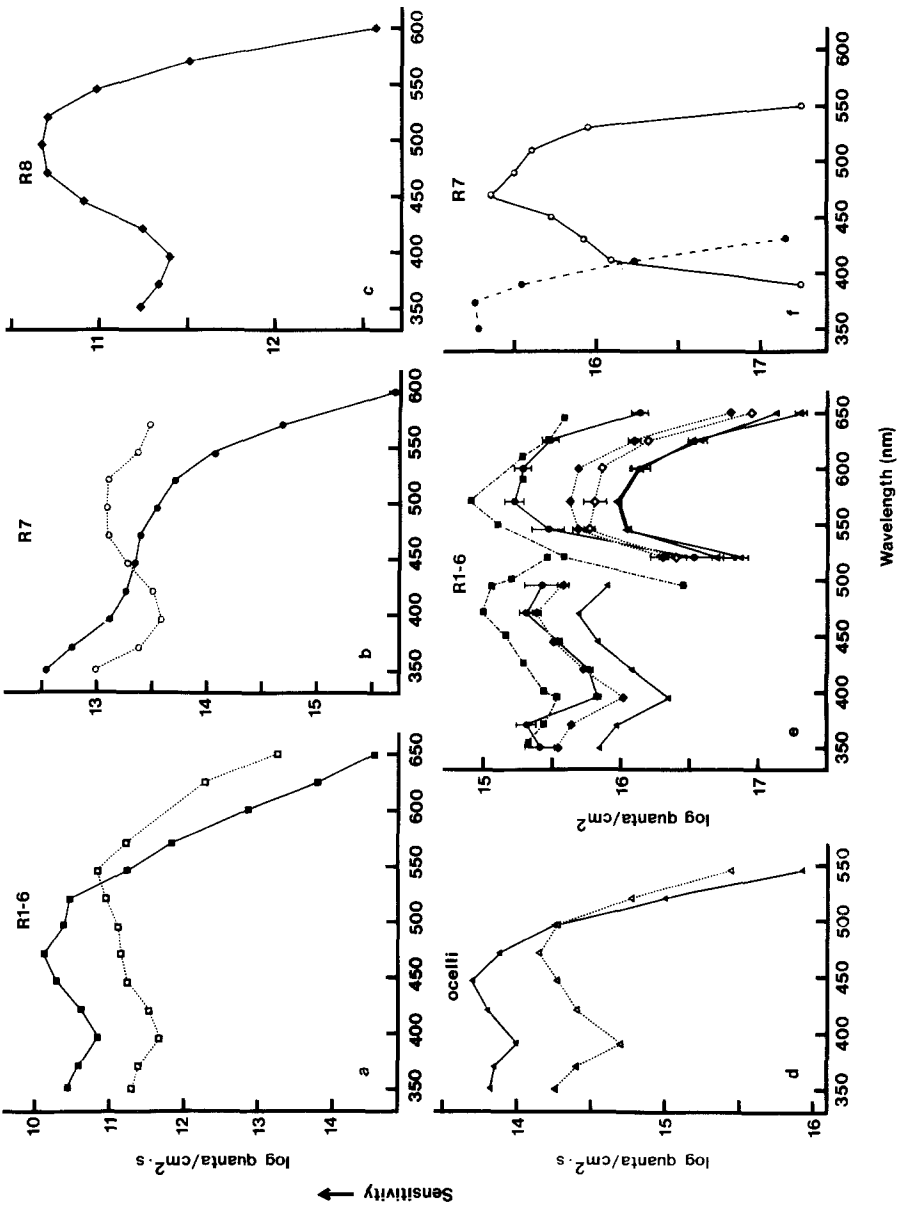

Fig. 1. (Legend see page 198)

Long-Lived Metarhodopsin and Afterpotentials

The discovery of a stable long wavelength metarhodopsin product of rhodopsin in R1–6 receptors of fly eyes (Hamdorf and Rosner, 1973; Stavenga et al., 1973) led to renewed interest in receptor spectra. Early and subsequent work showed the rhodopsin of R1–6 in various fly genera to peak around 450–500 nm (R480) and the metarhodopsin to peak around 540–580 nm (M580). Intense short wavelength

adaptation had been shown to cause an extremely long-lived ERG negative (receptor depolarizing) afterpotential (later called a PDA, prolonged post-stimulus depolarizing afterpotential; Nolte and Brown, 1972; Minke et al., 1975) and sensitivity loss in *Drosophila;* long wavelength adaptation was found to reverse these effects quickly (Cosens and Briscoe, 1972; Stark, 1972). These electrophysiological phenomena were subsequently considered to be adaptational consequences of photopigment conversion in R1–6. Similar afterpotentials were characterized in photoreceptors of *Limulus* (e.g., Nolte et al., 1968; Nolte and Brown, 1972; Minke et al., 1973) and *Balanus* (e.g., Hillman et al., 1972; Hochstein et al., 1973; Brown and Cornwall, 1975b).

Determining accurate photopigment spectra would be difficult since spectrophotometric measurements would have contributions from both R480 and M580 (see Stavenga et al., 1973, and Langer's comment). Ostroy et al. (1974) used selective denaturation of M580, essentially bleaching R480, so that R480 and M580 spectra could be constructed from difference spectra. Yet, simultaneously, models of visual pigment self-screening and waveguide effects (Snyder and Pask, 1973) raised interest in in vivo spectra. Pak and Lidington (1974) estimated R480 and M580 spectra by suppressing vs. eliciting a fast ERG potential, namely the M-potential, initially considered to be like an early-receptor potential (ERP). The M-potential, possibly very different from an ERP, can still be used as a linear manifestation of M580 activation (Minke, personal communication).

Photopigment Spectra Deduced from Conversion Efficiency

Our laboratory has concentrated on constructing photopigment spectra from two types of measures of conversion efficiency, namely 1) criterion action spectra for photopigment conversion and its electrophysiological (adaptational) consequences, and 2) measures of photopigment levels after chromatic adaptation sufficient to induce photoequilibrium using microspectrophotometry or sensitivity for criterion M-potentials or receptor potentials.

Estimates of Photopigment Spectra from Action Spectra

Long vs. short wavelength adaptation were used to create mostly R480 vs. M580 respectively. Action spectra were then obtained for induction of criterion small PDA's and sensitivity losses (with long wavelength adaptation) and for PDA repolarizations and receptor reactivations (with long wavelength adaptation) (Stark, 1975; Stark and Zitzmann, 1976; Fig. 1e). These data should reflect rough approximations of R480 and M580 spectra respectively. The disadvantages of this action spectrum methodology are 1) that data can only be obtained over a limited spectral range (which is why further photoequilibrium studies were undertaken, see next section), and 2) that the spectra are inaccurate, particularly at wavelengths of overlap of pigment absorbance, i.e., expecially in the green in Figure 1e (see Hochstein et al., 1978, for discussion of a more quantitative methodology). The advantage of this action spectrum methodology is that absolute intensities needed for photopigment conversion and its adaptational consequences in the living eye are determined.

Figure 1e also presents the results of our recent microspectrophotometric replications of this experiment as well as the corresponding in vitro spectrophotometric data of Harris et al. (1976) (see also Harris, 1976). All four types of study confirmed that R480 and M580 have blue vs. yellow maxima respectively. These action spectra also showed that R480 has a functional UV maximum in addition to the blue peak just like the spectral sensitivity. Thus, near threshold (from R1–6) ERG spectral sensitivity (i.e., the criterion action spectrum for the late receptor potential) could be assumed to represent the R480 spectrum (as modified by any factors present in the living eye; e.g., self-screening, waveguide effects, accessory pigments, see discussion below). The relationship between sensitivity and pigment spectrum has long been a straightforward assumption in vision research (e.g., see Hecht et al., 1942; Dartnall, 1953).

In vitro action spectra for pigment conversion using mutants lacking R1–6 or lacking R1–6 + R7 also revealed the photopigment systems of R7 and R8 (Harris et al., 1976; Harris, 1976). R7 has a UV-peaking rhodopsin and a blue-peaking metarhodopsin (Fig. 1f). R8 has a different rhodopsin, peaking in the blue as inferred from spectral sensitivity (Fig. 1c). However, no spectrally separable pigments were detected, nor was any chromatic adaptation effect observed in the ERG for R8. Thus, R8 probably has a metarhodopsin with a peak near its rhodopsin's maximum. A less likely alternative would be the possibility of R8 metarhodopsin reconverting to rhodopsin in the dark with a time constant in the order of seconds.

Photoequilibrium Estimates of Photopigment Spectra

A problem with the approach of determining action spectra for substantial conversion is that photopigment spectra are only accurate over a very limited spectral range (see above). To estimate metarhodopsin absorbance spectra denoted by $\alpha_M(\lambda)$, into the UV, spectral sensitivities were used to estimate the effective rhodopsin absorbance spectra, $\alpha_R(\lambda)$. Photoequilibrium spectra, i.e., sensitivities for criterion late receptor potentials or M-potentials after photoequilibrium chromatic adaptation with wavelength λ, were used to estimate the fraction of metarhodopsin $f_M(\lambda)$ relative to an assumed $f_M = 0$ after 600–650 nm adaptation (Stark and Zitzmann, 1976; Stark et al., 1976, 1977; Stark 1977; Hu et al., 1977). Photoequilibrium studies in flies data back to Hamdorf and Rosner (1973) and Stavenga et al. (1973). Photoequilibrium spectra were recently used in studies of barnacle photopigments (Minke et al., 1978). Metarhodopsin spectra have been calculated for R1–6 (Fig. 1a), R7 (Fig. 1b) and ocellar receptors (Fig. 1d) using the equation from photoeqilibrium and bistable pigment assumptions:

$$\log \alpha_M(\lambda) = \log \alpha_R(\lambda) + \log f_R(\lambda) - \log f_M(\lambda)$$

from Stark and Zitzmann (1976) (c.f. Tsukahara and Horridge, 1977).

The most interesting feature of all of these metarhodopsin spectra, which are derived from spectral sensitivities, is that they all have functional UV absorbance peaks in vivo. The finding that R1–6 M580 has such a UV peak has been confirmed directly using microspectrophotometry (Kirschfeld et al., 1977; Schwemer, personal

communication). The metarhodopsin UV peaks are particularly curious: metarhodopsin's chromophore should be all-trans, lacking the "cis" peak (see Dartnall, 1972, discussion for β-carotene). A secondary rhodopsin "cis" peak, perhaps enhanced by in vivo factors (Snyder and Pask, 1973), had been thought to account for two-peaked receptor spectral sensitivities (see discussion below).

Using sensitivity measures for the receptor potential to estimate $f_M(\lambda)$ assumes linearity between sensitivity and rhodopsin level (Razmjoo and Hamdorf, 1976; Harris et al., 1977; Stark et al., 1977). Relative metarhodopsin spectral shapes should be reasonably accurate, though slightly confounded (see next section) from these photoequilibrium spectra. The quantum efficiency ratio of rhodopsin to metarhodopsin conversion over the efficiency for metarhodopsin to rhodopsin conversion (γ_R/γ_m) is not factored out of these spectra. This ratio, known only if the isosbestic point, determined from difference spectra, is analysed together with such photoequilibrium spectra (see next section), would alter the level of the metarhodopsin spectrum relative to the rhodopsin spectrum by log (γ_R/γ_m) on these logarithmic plots (Tsukahara and Horridge, 1977; Hu et al., 1978).

Refining the Estimate of the R1–6 M580 Spectrum

Small errors in the photoequilibrium spectra will produce large errors in the derived metarhodopsin spectra at long wavelengths. For R1–6, this makes the derived M580 curve (Fig. 1a) inaccurate especially near the peak of M580. We have determined the M580 spectrum at long wavelengths (Fig. 2) from microspectrophotometric difference spectra by 1) estimating the ratio of peak absorbances of M580 and R480 (α_M/α_R); and 2) adding the appropriately weighted rhodopsin spectrum ($\alpha_R(\lambda)$, estimated from spectral sensitivity) to the difference spectrum. To perform our calculations, we obtained difference spectra, photoequilibrium spectra, intensity-responsivity for photopigment conversion (e.g., see below, Fig. 3), and criterion action spectra (see Fig. 1e) for conversion from single R1–6 receptor types in the deep pseudopupil of white-eyed *Drosophila*. The major steps in this calculation were as follows: 1) f_M after maximal 470 nm adaptation was estimated to be about 0.87 from the photoequilibrium spectra for criterion electrophysiological responses (Stark et al., 1977); 2) the isosbestic point, determined from difference spectra to be about $\lambda = 502$ nm, was then applied to the photoequilibrium spectra to estimate quantum efficiency ratio $\gamma_M/\gamma_R = 0.57$ using equations presented by Stavenga (1976); 3) peak absorbance ratio was then estimated to be about $\alpha_M/\alpha_R = 2.6$ by analysing intensity-responsivity data according to the exponential equations (for approach of a bistable pigment to photoequilibrium) presented by Hochstein et al. (1978). The major assumptions were that $f_M = 0$ after 600–650 nm induced photoequilibrium, that difference spectrum data at and above 570 are based on M580 alone (not R480), and that the M580 spectrum roughly fits the Ebrey and Honig (1977) nomogram from 470–570 nm; the latter assumption is fairly safe as seen from Figure 2.

The deep pseudopupil is a good technique for microspectrophotometry. It has been previously used for studies of visual pigments in other fly genera (Stavenga et al., 1973; Stavenga, 1976). One views seven magnified superpositions of the virtual

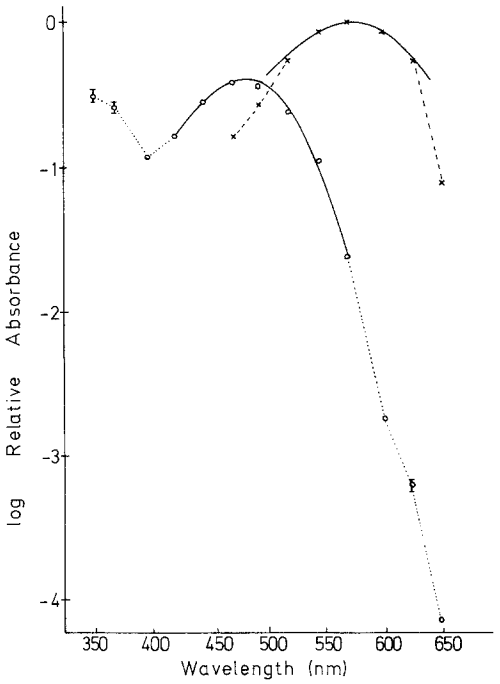

Fig. 2. Spectral sensitivity and M580 spectrum. Spectral sensitivity (circles, dotted line) for a 3.0 mV ERG receptor potential from white-eyed wild-type *Drosophila.* Standard errors for relative sensitivity are shown ($n = 4$). The spectral sensitivity, typically expressed in log quanta/cm^2 · s, has been normalized at 470 nm to the log absorbance of R480 relative to that of M580 at 570 nm. The M580 spectrum, calculated from difference spectra and spectral sensitivity is shown (X's, dashed line). Ordinate is log relative absorbance where 0 corresponds to 0.20, close to the average absorbance difference at 570 nm for single R1–6 rhabdomere type spots in the deep pseudopupil. Nomograms peaking at 485 and 570 nm (Ebrey and Honig, 1977) are drawn with solid line through the data points

images of numerous R1–6 and R7/8 rhabdomere tips respectively in a pseudopupil formed deep beneath the cornea (Kirschfeld and Franceschini, 1969; see Franceschini, 1975, for review). Absorbance differences from photopigment conversion are high since measurements are taken from the whole rhabdomere length. Hence, we obtain peak absorbance difference values near the peak of M580 of over 0.30 (for 470 vs. 600 nm adaptation); such differences, easily seen by eye, are much higher than absorbance difference values obtained from whole eye measurement (e.g., Ostroy et al., 1974; Pak et al., 1976) in which the measurement area includes non-aligned rhabdomeres and the remainder of the eye. Lo and Pak (1978) suggested that transmission changes in the deep pseudopupil include contributions from intracellular adaptation events. However, we have determined that absorbance differences are well correlated with sensitivity and vitamin A level, substantiating the idea that microspectrophotometry of the deep pseudopupil is a good photopigment measure. A more detailed presentation of this work is in preparation.

Vitamin A Deprivation and Spectral Sensitivity

An interesting manipulation to study the UV peak in the R1–6 spectral sensitivity and M580 spectrum came from the rediscovery of Goldsmith and Fernandez's (1966) finding that vitamin A deprivation selectively lowered the UV sensitivity in R1–6 (Stark et al., 1976, 1977; Kirschfeld et al., 1977). By contrast, vitamin A deprivation does not change the spectral sensitivity shape in R7 or R8 (Stark, 1977). Sensitivity and photoequilibrium studies of vitamin A deprived *Drosophila, Calli-*

phora, and *Musca* showed that vitamin A deprivation also lowered the UV peak of M580 in R1–6 (Stark et al., 1977). Thus, some feature or consequence of the vitamin A level affects spectral sensitivity and metarhodopsin spectrum shape in the living eye. For instance, R1–6 could have UV and visible rhodopsins, with the hypothetical "visible opsin" having preferential affinity for the limited vitamin A supply during development in deprived animals. However, chromatic adaptation data suggested that a one rhodopsin (R480) pigment was the "final common pathway" in mediating both UV and visible sensitivity and adaptation effects (Stark et al., 1977). Microspectrophotometry (Stavenga et al., 1973; Kirschfeld et al., 1977) and action spectra for resensitization and PDA suppression after UV vs. visible adaptation (Stark, 1975, Fig. 1e) suggested that M580 was the sole metarhodopsin. Although the presence of two rhodopsins had been suggested as a possibility in R1–6 (Horridge and Mimura, 1975; Rosner, 1975) the present consensus is that there is not a second (UV) rhodopsin (Burkhardt, 1977; Kirschfeld et al., 1977; Stark et al., 1977; Tsukuhara and Horridge, 1977).

Spectral Mechanisms in R1–6

Kirschfeld et al. (1977), from microspectrophotometric and other evidence, suggested that a UV sensitizing (non-rhodopsin) pigment serves as an antenna pigment, "harvesting" UV quanta and transferring energy to the R480 by inductive resonance (migration of molecular excitation). The discovery of a long wavelength fluorescence from UV excitation of R1–6 rhabdomeres (Franceschini, 1977; Stark et al., 1977) is consistent with this hypothesis. The further findings that vitamin A deprivation eliminates this fluorescence (Stark et al., 1977) and that it also eliminates a high rhabdomeric absorbance in the UV (Schwemer, personal communication) suggests that a vitamin A derivative could serve as this light-harvesting antenna. On the other hand, if the fluorescence source were R480, rather than the sensitizing pigment, the elimination of fluorescence by vitamin A deprivation would result from vitamin A deprivations's well established reduction of photopigment (Razmjoo and Hamdorf, 1976; Harris et al., 1977; Stark et al., 1977).

Stark et al. (1977) suggested that such an "antenna" may also transfer energy to M580 accounting for its functional UV peak. Proposed spectral mechanisms involving concentration and waveguide effects (Snyder and Pask, 1973) should not apply in the ERG from white-eyed flies (Kirschfeld, 1979); furthermore, such mechanisms were judged to be inadequate to completely account for the vitamin A deprivation effect on UV sensitivity (Stark et al., 1977). Thus, Kirschfeld et al.'s energy transfer hypothesis seems a likely explanation for two-peaked pigment spectra in fly R1–6 receptors at the present time.

Mechanisms Governing the Adaptational Consequences of Photopigment Conversion

The PDA, a phenomenon perhaps unique to invertebrates, has been of extreme interest as a powerful manipulation of adaptation level. The PDA, generally consid-

ered to be closely related to the late receptor potential, has been alternatively explained:

1) in terms of excitor and inhibitor processes of intracellular message separated from rhodopsin and metarhodopsin processes (Minke et al., 1973; Minke, 1979) or

2) as a direct consequence of photopigment states (Hamdorf and Razmjoo, 1977, 1979; Wright and Cosens, 1977).

While the mechanisms of generating the PDA remain controversial, most of our laboratory's results are consistent with the transmitter hypothesis. For instance, Stark and Zitzmann's (1976) discovery that vitamin A deprivation eliminated the PDA in R1–6 in *Drosophila* (while not eliminating R480 to M580 conversions) is consistent with this model. Harris and Stark (1977) characterized a defect in the PDA in a mutant (*rdg B*) with hereditary light-induced retinal degeneration in

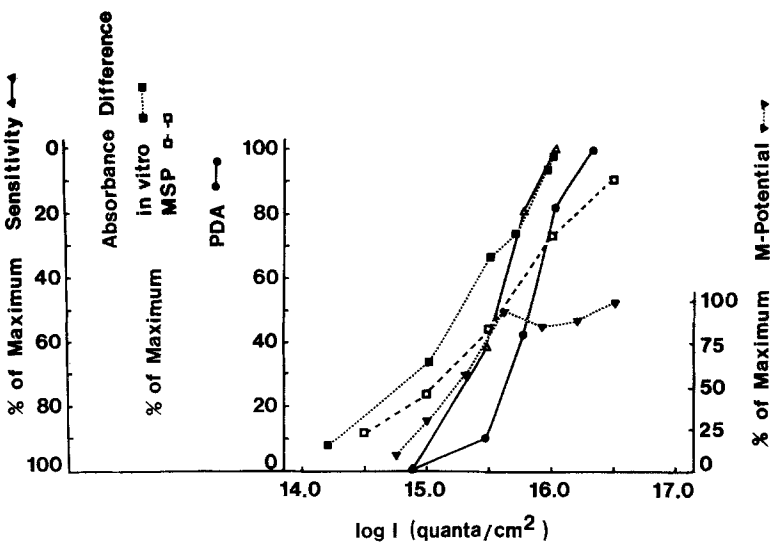

Fig. 3. Intensity-responsivity for R480 to M580 conversion and its adaptational consequences. The solid squares show absorbance difference relative to maximum for homogenates from white-eyed wild-type flies as a function of 469 nm adaptation intensity (in vitro, from Harris et al., 1976; Harris, 1976). The remainder of the data is from living eyes plotting response against intensity of 470 nm adaptation intensity (log quanta/cm^2) directed into the eye in the physiologically normal (orthodromic) direction; all these data were collected from apparatus with similar optical geometry and components and with calibrations from the same photodiode. The hollow squares show microspectrophotometric 470 nm induced absorbance differences measured at 578 nm averaged from seven preparations (MSP, each a single rhabdomere type in the deep pseudopupil). The inverted triangles show the magnitude of the second (positive) M-potential component (relative to maximum, averaged from six preparations) as a function of 470 nm adaptation intensity. The scale (right ordinate) has been adjusted to the microspectrophotometric data at the M-potential's initial saturation to reflect the fact that the function reaches saturation at about half R480 to M580 conversion (assayed microspectrophotometrically). The triangles show the near threshold (from R1–6) 3.0 mV ERG receptor potential sensitivity loss (% maximum sensitivity plotted downward) caused by increasing intensities of 470 nm adaptation (averaged from six preparations). The circles show the negative ERG potential (PDA) relative to maximum (17.25 mV) measured 1 min following the termination of the 470 nm adapting stimulus (averaged from 10 preparations)

R1–6. Studies of the interaction of *rdg B* mutants with no receptor potential A (*norp A*) mutants suggested that activation of the *norp A* gene product is associated with excitation and that the *rdg B* gene product inactivated this process or excitor. Thus, Harris and Stark's (1977) results are also consistent with an excitor-inhibitor model and tentatively identify these processes with specific interactions among gene products.

Recently, we have obtained intensity response data for microspectrophotometric R480 to M580 conversion with bright 470 nm adaptation in the deep pseudopupil of live white-eyed flies. We have compared these data with electrophysiological measures reflecting conversion (Fig. 3). The PDA is only induced at high conversion levels. Coincident with PDA induction is a precipitous sensitivity decrease (in the limit, inactivation). Membrane conductance channels were shown to be open during the PDA (Hochstein et al., 1973; Muijser et al., 1975; Brown and Cornwall, 1975a). Thus, it is possible that these open conductance channels of the PDA block the receptor potential. Coincident with the intensity level for PDA induction is a supra-saturation limb of the M-potential induction curve. Minke and Kirschfeld (Minke, personal communication) have also observed this unexplained phenomenon but only when high intensity stimuli were used to generate the M-potential. The results of Figure 3 can be used to quantify the relation between photopigment conversion and its adaptational consequences. Interesting issues remain, however, concerning the mechanism of PDA generation and its relation with transduction or adaptation processes.

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Note added in proof. Further microspectrophotometry has yielded refined estimates of $\frac{\gamma_M}{\gamma_R} = 0.71$ and peak extinction ratio $\frac{\alpha_M}{\alpha_R} = 1.15$ to 1.35 (the latter in closer agreement with an early estimate, Hamdorf and Rosner, J. Comp. Physiol. **86,** 231–292, 1973). From dark adaptation studies, sensitivity as a function of photopigment level was estimated (with neural adaptation factored out). Our microspectrophotometric photoequilibrium spectrum could thus be better scaled to metarhodopsin fraction yielding the improved estimate of $\frac{\gamma_M}{\gamma_R}$. Along with more extensive microspectrophotometric data on approach of a bistable pigment to photoequilibrium as a function of adaptation intensity, we have revised our peak extinction ratio estimate. We have extended such intensity-response measures across wavelengths from 350 to 650 nm to derive a spectrum representing $\alpha_M(\lambda)\,\gamma_M + \alpha_R(\lambda)\,\gamma_R$ which has a substantial UV peak. Calculations from spectral sensitivity suggest that this UV conversion efficiency is transferred to rhodopsin and metarhodopsin. Finally, microspectrophotometry shows the excitation spectrum of the vitamin A dependent fluorescence in R1–6 rhabdomeres to peak in the UV. Fluorescence does not change during a monitored rhodopsin to metarhodopsin conversion. These observations reinforce the possibility that rhabdomere fluorescence is related to UV sensitivity and that it may in fact be generated by the hypothesized sensitizing antenna pigment (Stark, Kruizinga, Stavenga: Invest. Ophth. Vis. Sci. Suppl. 1979, in press).