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Spectral Effects of the Pupil in Fly Photoreceptors

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Summary. Photoreceptors of flies contain pigment granules which upon illumination of the receptors migrate towards the rhabdomere and act as a 'longitudinal pupil'. Data in the literature concerning the effect of the pupil on the spectral sensitivity are contradictory. Therefore spectral sensitivity of *Musca* photoreceptors upon light adaptation was reinvestigated.

The change in spectral sensitivity of fly photoreceptors upon light adaptation as measured by Hardie (1979) was confirmed. Taking into account waveguide optics this change was explained from absorbance spectra of pupillary granules, measured by microspectrophotometry in squash preparations. Furthermore the pupil absorbance spectrum determined in vivo (Stavenga et al. 1973) was interpreted. The absence of a change in spectral sensitivity upon light adaptation measured by pupillary reflexion (Bernard and Stavenga 1979) is explained by a local triggering of the pupil.

Introduction

Fly photoreceptor cells contain tiny pigment granules which upon illumination migrate towards the rhabdomere. This process acts as a light attenuation mechanism, not dissimilar to the pupil of vertebrate eyes (Kirschfeld and Franceschini 1969; Franceschini 1975; Franceschini and Kirschfeld 1976; Stavenga 1979).

While there is a general agreement with regard to the spectral sensitivity of the peripheral photoreceptors (R1–6) of *Musca* and *Calliphora* in the dark adapted state (Burkhardt 1962; Scholes 1969; Dörrscheidt-Käfer 1972; McCann and Arnett 1972; Horridge and Mimura 1975; Hardie 1979; Smola and Meffert 1979) there are different views on how the spectral sensitivity in the light adapted state is influenced by the pupillary pigment.

Firstly, Stavenga et al. (1973) showed spectrophotometrically that in *Calliphora* the rhodopsin-metarhodopsin ratio reached at photoequilibrium with white light depends on the state of the pupil (see also Stavenga 1980). The decrease in metarhodopsin content when the pupil is 'closed' was explained by the strongly blue peaking absorbance of the pupil pigment, in that the effective spectral composition of the incident light changes from white to yellow. Consequently, in the light adapted state a shift of the spectral sensitivity towards longer wavelengths was expected by the authors.

However, Hardie (1979) measured the spectral sensitivity of *Musca* photoreceptors (R1–6) electrophysiologically and found a shift by 40–50 nm of the 490 nm peak towards shorter wavelengths in the presence of an adapting light of an intensity sufficient to activate the pupil mechanism.

Thirdly, Bernard and Stavenga (1979) measured the spectral sensitivity of *Musca* optically by means of the reflectance increase that accompanies the pupillary response. They found no wavelength specific change with light adaptation.

Since all three investigations reached different conclusions a further investigation is obviously called for. In its course we specifically came to realize the distinct influence waveguide effects can have, as the pupillary granules enact their influence at the boundary of the rhabdomeric waveguide. In this report we therefore present a combined study of electrophysiology, microspectrophotometry of the pigment granules and waveguide optical considerations in order to gain a more fundamental understanding of the effect of the intracellular pupil mechanism.

Materials and Methods

Electrophysiology. The experiments were performed on receptor-cells R1–6 (identified by their characteristic double peaked spectral sensitivity during dark adaptation) in the middle lateral eye

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region of female *Musca domestica* (wild type) reared in the laboratory. The preparation was the same as Hardie's (1979) except that the animal was mounted with the epimine-plastic Scutan (Espe) instead of wax. Glass capillary microelectrodes were filled with 2 M KCl, and had resistances of 100–150 megohms in 150 mM NaCl.

Adapting and stimulating lights were both delivered by 75 W Xenon arc lamps (Leitz). Quartz neutral density filters and spectral interference filters (Schott Depal, UV Pil) could be inserted in the collimated beams, which were mixed by a pellicle and focussed onto one end of a quartz fiber-optics light guide. The other end (3° in visual angle in most experiments) was mounted on a Cardan arm device with the insect in the centre of rotation.

Measuring procedure: A sensitivity spectrum in the dark adapted state was determined as follows. After adapting with orange (Schott OG 550) light (resulting in a high rhodopsin content) and subsequent dark adaptation (3 min) the spectral sensitivity was measured from the responses to flashes of 30 ms duration with an interval of 10 s. After each dark adapted series an intensity response ($V/\log I$) function was measured at 500 nm. The spectral sensitivity in the light adapted state then was determined as follows: The cell was light adapted by orange light (OG 550) whose intensity was chosen to give a peak response of more than 80% of the maximal response (60 mV). At that intensity the pupil mechanism is approximately saturated as can be estimated from the appearance of the deep pseudopupil. After 1 min, when a stable depolarisation plateau was reached, the superimposed spectral responses and the $V/\log I$ (500 nm) function in the light adapted state were recorded. From these data the spectral sensitivity was calculated. After 4 min of dark adaptation the experiment was repeated. For the measurements of the spectral sensitivity in the dark adapted state we adjusted the intensity of the coloured flashes in such a way that they evoked approximately the same response ($\approx$25 mV). This was accomplished by means of a rotating neutral density wedge. This way of measuring has the advantage that the sensitivity at each wavelength is determined with the same accuracy and, furthermore, in order to convert the responses to sensitivities only a small region of the linear part of the $V/\log I$ curve needs to be used.

Microspectrophotometry (MSP) of Photoreceptor Granules. The measurements were carried out on squash preparations of *Musca* and *Calliphora* wild type eyes. Pieces of the eyes were squashed between two quartz cover glasses and washed with Ringer solution to remove the abundant granules of the secondary pigment cells. Receptor granules were identified by their proximity to the rhabdomere and their yellowish colour in transmitted light. For measurements a field of 5 µm diameter containing clusters of pigment granules was selected by means of a diaphragm in the image plane of the microscope equipped with Zeiss ultrafluar optics. The transmitted intensities $I_1(\lambda)$ between 340 nm and 700 nm (Oriel Monochromator 7240, HW 10 nm) were measured with a photomultiplier (EMI 9558 A). Then a field of the same size was chosen in close vicinity to the latter but without any pigment granules and again the transmitted intensities $I_0(\lambda)$ were measured. The extinction spectra were obtained as

$$E(\lambda)=\log\frac{I_0(\lambda)}{I_1(\lambda)}.$$

Results

Change in Spectral Sensitivity by the Pupil Action

Spectral sensitivities of photoreceptors type R1–6 of *Musca* are presented in Fig. 1. The dark adapted spectral sensitivity shows the well-known two peaks

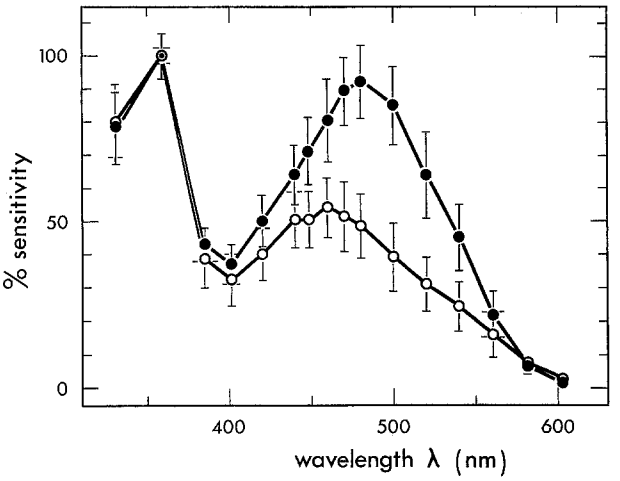

Fig. 1. Normalized spectral sensitivity of peripheral photoreceptors (R1–6) of *Musca* (female, wild type) in the dark adapted *(filled circles)* and in the light adapted *(open circles)* state. Mean values with S.D. of measurements in 10 cells. Note the relative decrease of sensitivity in the bluegreen, and the blue shift of the peak

in the UV and the blue-green respectively, which are similar in height. In the light adapted state compared with the UV sensitivity the sensitivity in the blue-green region is considerably reduced. In the yellow-red region the depression is less or, at the longest wavelength measured, completely lacking. The maximum of the sensitivity in the visible region is shifted toward shorter wavelengths. Due to the relatively high UV sensitivity as well as the suppressed and shifted sensitivity in the visible region the integral sensitivity of the receptors 1–6 is remarkably shifted toward shorter wavelengths.

This result is in close agreement with the measurements of Hardie (1979). The main difference between his data and ours is that Hardie measured a relatively weak UV peak in the dark adapted state. This discrepancy most likely is explained by the rather high variation in the UV to visible peak ratio, which is known to depend e.g. on the animal's diet (Goldsmith et al. 1964; Stark et al. 1977).

The dynamic range of the spectral shift was estimated by measuring the sensitivity at 500 nm relative to that at 359 nm at several adapting intensities. The intensity range over which the ratio between the sensitivities changes covers about 2.5 log units and in terms of the dark adapted receptor $V/\log I$ curve (peak response) corresponds to between 20% and 90% V_{max} (Fig. 2).

If the change in spectral sensitivity is assumed to be due to the action of the pupil, its effective absorbance spectrum can be obtained by subtracting the log-sensitivity curves: $\Delta E=\log S_d-\log S_l$. This effective absorbance spectrum (Fig. 3) is valid except for a wavelength-independent constant which can not

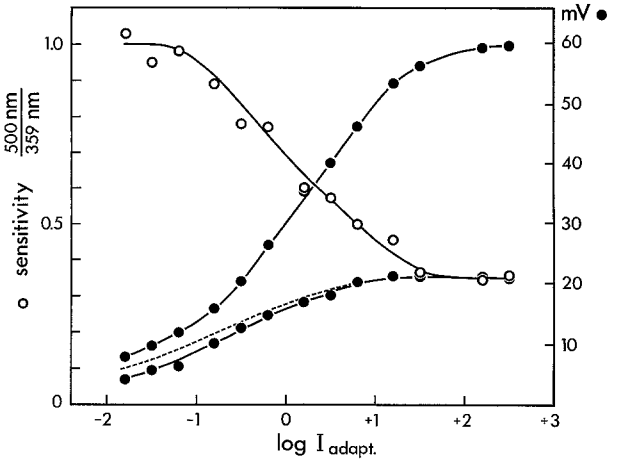

Fig. 2. Change in the sensitivity at 500 nm relative to that at 359 nm *(open circles)* measured by giving test flashes of both wavelengths superimposed on an orange adapting light I_{adapt} of variable intensities. Relative sensitivity is normalized to 1.0 in the dark adapted state. *Filled circles:* Peak response amplitude to the onset of the orange adapting light *(upper curve)* and plateau measured 150 s later *(lower curve). Dotted line* represents the depolarisation 30 s after onset of the adapting light. The deviation from the plateau curve at low intensities indicates the longer time needed to reach an adaptation equilibrium at those intensities. Curves (fitted by hand) represent the mean of repeated (6) measurements of 2 stable recordings. Recordings from other less stable cells are consistent with those shown

be specified from our experiments, because there is, besides the pupil adaptation also membrane adaptation, which cannot be separated from one another. Therefore, an absolute absorption spectrum of the pupil cannot be given. The absorbance spectrum in Fig. 3 can be interpreted as that of a colour filter put in front of the photoreceptor together with a neutral density filter of unknown density.

The data described above were obtained from cells which were orange adapted (leading to a high rhodopsin content in the rhabdomeres). In four cells we measured the spectral sensitivities of both the light and dark adapted state when the visual pigment was put into photoequilibrium with green (495 nm) and orange (OG 550) lights, respectively. The effective pupil absorbance spectra derived from the green and orange adapted state of one and the same cell appeared to be virtually identical. Furthermore, we found that in *Musca* the dark adapted spectral sensitivities (as well as those of the light adapted states) differ due to metarhodopsin screening (see Hamdorf and Schwemer 1975; Tsukahara and Horridge 1977).

Absorbance Spectra of Pupillary Granules

Microspectrophotometry of small clusters of pigment granules yielded the spectra shown in Fig. 4. The data of *Musca* (solid line) averaged from 7 experiments agree with those of *Calliphora* (dotted line). They are not dissimilar to those of Langer (1975) who measured extinction spectra of the receptor granules of *Calliphora*. However, a prominent difference exists between these spectra and the absorbance spectrum determined for the pupil in vivo by Stavenga et al. (1973) (interrupted curve, Fig. 4). The latter spectrum was obtained by measuring the transmission of the rhabdomeres with 'open' and 'closed' pupil, respectively. We presume that the depression in the blue is caused by waveguide optical effects as will be described below.

Waveguide Optical Interpretation of the Experimental Data

Absorbance Spectrum of the Pupil

In the estimates of the absorbance spectrum of the pupil in vivo (Stavenga et al. 1973) the pupillary granules are assumed to absorb light from the evanescent wave of the rhabdomeric waveguide. It should be recalled here that light in the rhabdomere is propagated in modes, the number of which depends on the way of excitation and the actual value of the waveguide parameter

$$V=\frac{\pi d}{\lambda}(n_1^2-n_2^2)^{\frac{1}{2}}, \tag{1}$$

where d is the diameter of the waveguide, λ is the (vacuum) wavelength, and n_1 and n_2 are the refractive indices of the media within and surrounding the rhabdomere, respectively (review in Snyder and Menzel 1975). For simplicity we consider initially the first mode only.

According to Snyder (1975) the transmission $T(\lambda)$ of a rhabdomere is given by

$$T(\lambda)=T_i(\lambda)\cdot T_e(\lambda), \tag{2}$$

where $T_i(\lambda)$, is the internal transmission of the rhabdomere and $T_e(\lambda)$ is the transmission of the surrounding medium. In the dark adapted state

$$\begin{aligned} T_{i,d}(\lambda)&=\exp\{-\eta_d(\lambda)\,K_i(\lambda)\,L\}\\ T_{e,d}(\lambda)&=1 \end{aligned} \tag{3a}$$

and in the light adapted state

$$\begin{aligned} T_{i,l}(\lambda)&=\exp\{-\eta_l(\lambda)\,K_i(\lambda)\,L\}\\ T_{e,l}(\lambda)&=\exp\left\{-\int_0^L[1-\eta_l(\lambda)]\,K_e(x,\lambda)\,dx\right\}. \end{aligned} \tag{3b}$$

Here η is the fraction of the mode's power within the rhabdomere, K_i represents the extinction coefficient of the pigments inside the rhabdomere (as-

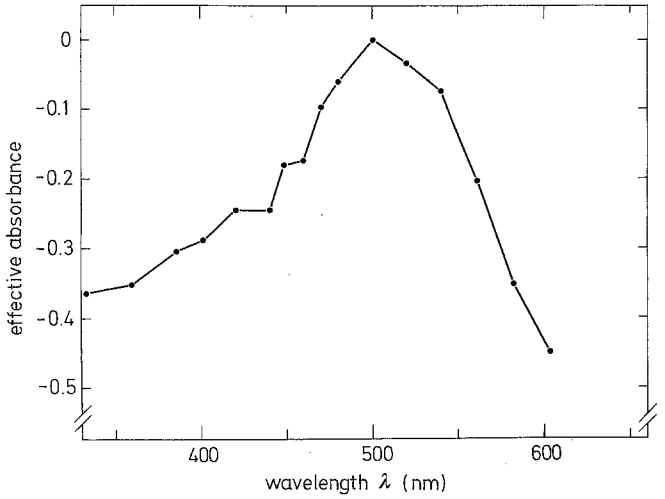

Fig. 3. Spectral filtering effect of the pupil. The difference of the log sensitivities of Fig. 1 is drawn as absorbance decrease relative to the absorbance at 500 nm

sumed to be homogeneous), K_e is the extinction coefficient of the pupillary pigment, x the depth within the rhabdomere and L its total length.

The absorbance difference is calculated by

$$\Delta E = \log \frac{T_d(\lambda)}{T_l(\lambda)}$$

$$= \log e \cdot \left\{ [\eta_l(\lambda) - \eta_d(\lambda)]\, K_i(\lambda)\, L \right.$$

$$\left. + [1 - \eta_l(\lambda)] \int_0^L K_e(x, \lambda)\, dx \right\}. \qquad (4a)$$

Because the pupillary granules are strong absorbers and the dependence of η on state of adaptation probably is only slight, or $\eta \approx \eta_d \approx \eta_l$, the first term may be neglected. So the effective absorbance spectrum of the pupil becomes (assuming one mode)

$$\Delta E \propto [1 - \eta(\lambda)]\, K_e(\lambda) \qquad (4b)$$

$K_e(\lambda)$ is proportional to the spectral absorbance as measured on pigment granules in squash preparation (Fig. 4), where waveguide effects are excluded. Hence ΔE can be calculated when $(1-\eta(\lambda))$ is known.

In principle $\eta(\lambda)$ can be directly obtained because η is a unique function of V, quantitatively given by e.g. Snyder (1975). In real fly rhabdomeres V has been determined by measuring their refractive index (n_1) (Beersma 1979), by measuring their birefringence (Kirschfeld and Snyder 1975) and also by measuring intensities transmitted by a rhabdomere using different sized diaphragms (McIntyre and Kirschfeld 1981). At $\lambda = 500$ nm the range of experimental V values for rhabdomeres type R1-6 is $2 \leqq V \leqq 3$.

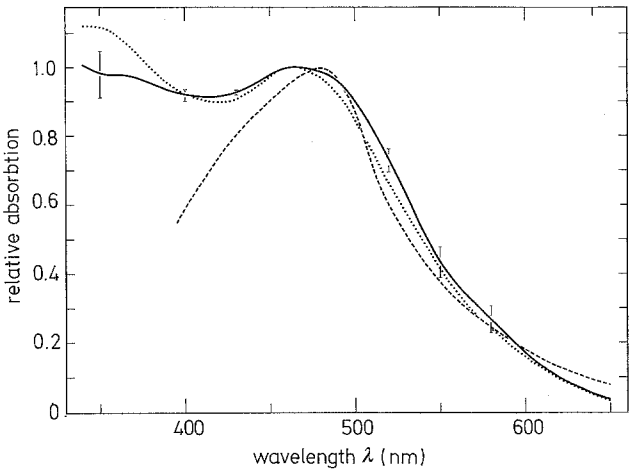

Fig. 4. Absorbance spectra of pupillary pigment granules. Microspectrophotometrical measurement of squash preparations of *Musca* (female, wild type) yielded the continuous line (mean of 7 measurements). *Dotted line:* one measurement on *Calliphora*. The interrupted line was taken from Stavenga et al. (1973) and is the absorbance spectrum of the pupil as determined in vivo. Note the strong depression of the latter spectrum in the blue

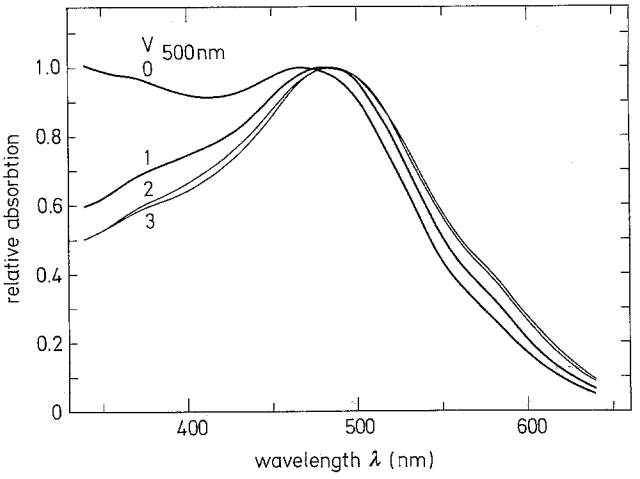

Fig. 5. Absorbance spectra of pupillary granules ($V=0$) modified by waveguide factor $(1-\eta)$ assuming different waveguide parameters V (Eq. 1) at $\lambda = 500$ nm. Curves normalized to their extreme value. The relative magnitudes of absorbance at $\lambda = 500$ nm are 1, 0.80, 0.28, 0.11, for $V=0$, 1, 2, 3, respectively

We calculated $[1-\eta(\lambda)]$ for three different cases, namely with $V=1$, 2 and 3 respectively at $\lambda = 500$ nm and multiplied the three functions by $K_e(\lambda)$. The obtained curves are presented normalized to their extreme value in Fig. 5. Interestingly the curves for $V=2$ and $V=3$ hardly differ from each other. Therefore the effective absorbance spectrum of the pupil is insensitive to the actual value of V. It appears that the effective spectrum is substantially suppressed in the blue and shifted towards longer

wavelengths with respect to the pure absorbance spectrum ($V=0$).

Comparing Figs. 4 and 5 we conclude that the depression in the blue of the effective pupil spectrum of Stavenga et al. (1973) has its basis in waveguide effects.

Change in Spectral Sensitivity upon Light Adaptation

If the spectral sensitivity of photoreceptors is assumed to be proportional to the number of conversions of rhodopsin into metarhodopsin, the spectral sensitivity of a dark adapted photoreceptor with pure rhodopsin content (review Snyder 1975; Hamdorf 1979), is

$$S_d \propto 1-e^{-\eta K_i L} \tag{5}$$

Here $\eta=\eta(\lambda)$ and $K_i=K_i(\lambda)$.

In the light adapted state, when the pupil is closed, the number of rhodopsin conversions is reduced. In the simplified view that the pupil is located in front of the photoreceptor, as is the case in vertebrate eyes, this reduction equals the reduction in transmission. The spectral sensitivity then becomes

$$S_l \propto T_e\{1-e^{-\eta K_i L}\}, \tag{6}$$

where $T_e=T_e(\lambda)$ represents the spectral transmission of the pupil. The drop in transmission of fly rhabdomeres due to the action of the pupil can be up to two log units or even more (see Stavenga 1975). However, the pupil consists of pigment granules distributed along the rhabdomere and thus the most distal part of the rhabdomere is less screened than the more proximal parts. Therefore the reduction in the conversion rate may be much smaller than the decrease in transmission (see Muijser 1980).

Nevertheless, the amount of rhodopsin conversion in the presence of the longitudinal pupil and thus the spectral sensitivity can be formally described.

In the Appendix we present a general formula for the spectral sensitivity of a photoreceptor cell with an inhomogeneous distribution of the pupillary granules and an inhomogeneous distribution of membrane adaptation. A special case is the homogeneous pupil, i.e. $K_e(x,\lambda)=K_e(\lambda)$, and a homogeneous membrane adaptation. Then,

$$S_l \propto \frac{\eta K_i}{\eta K_i+(1-\eta)K_e}\{1-e^{-[\eta K_i+(1-\eta)K_e]L}\}. \tag{7}$$

The change in spectral sensitivity with respect to that in the dark adapted state may be regarded as an effective absorbance (ΔE) given by the difference $\log S_d-\log S_l$; a simple expression then results (from Eqs. 5 and 7) by assuming a strongly absorbing pupil and a weakly absorbing visual pigment, i.e., $\eta K_i L \ll 1 \ll (1-\eta)K_e L$

$$\Delta E = C+\log(1-\eta)K_e L \tag{8}$$

where C is a constant (wavelength independent) factor. With Eq. (8) the effective absorbance curve deduced in Fig. 3 can be interpreted qualitatively. Again, as concluded above for the case of the transmission measurements the depression in the blue will be due to the waveguide factor $(1-\eta)$.

In the analysis applied so far we assumed the proportionality of sensitivity and rhodopsin to metarhodopsin conversions. This holds as long as two conditions are met: The contribution of a single quantum absorption to the receptor potential has, firstly, to be independent of the location in the rhabdomere. Secondly, it must be independent from the local density of quantal absorptions (see Hamdorf 1979). The above conditions may be fulfilled approximately in the dark adapted receptor but they certainly will not be met in the light adapted state. In the latter case a steep gradient will exist in the intensity of the adapting light along the rhabdomere (especially in the distal part of the photoreceptor where the pupil is working). Therefore membrane adaptation, if it does not spread over the length of the rhabdomere will strongly depend on the location in the rhabdomere. The adapting light will desensitize the rhabdomeric membrane and this desensitization to incremental light will be most severe distally. A realistic expression for the spectral sensitivity in the light adapted state therefore should contain a factor (depending on the depth within the rhabdomere and the intensity and the wavelength of the adapting light) accounting for the local adaptation effect (see Appendix).

If this effect becomes significant the pupil will tend to function more as an end-on filter. In an extreme model assuming complete local desensitization over a distal range x and assuming that the pupil works only in this range the effective absorbance is (from Eqs. 5 and 6)

$$\Delta E = C+(1-\eta)K_e x \log e \tag{9}$$

(with weak absorption by the visual pigment). Also with this very simplified model we recognize the prominent effect of the waveguide factor $(1-\eta)$ and can interpret the effective absorbance curve (Fig. 3) qualitatively. However, comparing the maxima of

this curve (Fig. 3) and the waveguide-corrected pigment absorbance curves of Fig. 5a spectral shift of 15–20 nm exists.

Discussion

We have related MSP measurements of pupil pigment granules in squash preparations to the absorbance spectrum of the pupil in vivo and the change in the spectral sensitivity of the photoreceptors during light adaptation. We concluded that the existing spectral discrepancies can be at least qualitatively explained from waveguide optics.

In the presented analysis we considered the first order mode only. As in the electrophysiological measurements axial illumination was used the first order mode is expected to be the dominant one. Nevertheless, the second order mode can also be propagated in fly rhabdomeres (R1–6) as has been demonstrated by Franceschini and Kirschfeld (1971) and by Pick (1977). This is particularly relevant for the interpretation of the in vivo pupil spectrum, because for a given wavelength $(1-\eta)$ of the first order mode is distinctly smaller than that of the second order mode. Hence the latter mode can substantially contribute to the in vivo pupil spectrum. A quantitative treatment of the relative contributions of the two modes is not attempted here because of the many unknowns; however, the description of the blue filtering effect of the waveguide will be essentially similar to the analysis given for only the first mode.

We thus fully attribute the distinct change in spectral sensitivity of the photoreceptor upon light adaptation to the influence of the pupil.

The electrophysiological experiments must be compared with the optical measurements of Bernard and Stavenga (1979), who measured the spectral sensitivity by means of the reflectance change associated with pupil action. Although the adapting intensities were about 0.2 and 2 log units above the pupil threshold they found no wavelength specific change (in *Musca*). According to reflection measurements the pupil in *Musca* saturates about 2 log units above threshold (Franceschini and Kirschfeld 1976). Since the dynamic range of changes of both reflection and transmission due to the pupil coincides (*Drosophila*, Franceschini and Kirschfeld 1976; *Calliphora*, Stavenga, unpublished) we conclude that the experiment at 2 log units above threshold approximates the saturated state. The conjecture of Bernard and Stavenga that at higher adapting intensities distortion of the spectral sensitivity due to the pupil still could occur seems unlikely. In line with this conclusion is Hardie's (1979) finding, that the time course of the spectral shift is comparable to that of the pupil closure as measured by Franceschini (1972), and Stavenga's (1980) who found changes in rhodopsin/metarhodopsin ratio due to filtering by the closing pupil, as well as our finding that changes in spectral sensitivity are already observable at low adapting intensities (Fig. 2).

We note that in the optical measurements of Bernard and Stavenga (1979) the reflection of the pigment granules located in the most distal part of the cell was monitored. From the absence of a spectral change upon light adaptation in the reflection measurements we thus conclude that the optical method does not probe the spatially integrated response of the receptor cell and that the determined spectrum represents the local spectral sensitivity of the distal part of the cell. There spectral filtering by the pupil will be minor whereas in the proximal parts of the cell it becomes more and more important.

Beersma (1979), from both reflection and transmission measurements, concluded that the migration of the pupillary pigment granules is most effectively triggered by that particular part of the rhabdomere where the maximum amount of light is absorbed by the visual pigment. His result that the angular acceptance as measured by pupil action is much larger than that measured by the receptor potential agrees with the above conclusion that the triggering of the granules occurs locally. The view that the triggering of migration of pigment granules represents a localized process within a receptor cell is supported by the finding that the pupil action is not controlled by the receptor potential but by the intracellular free Ca^{++} concentration (Kirschfeld and Vogt 1980), which is likely to be locally different. A localized desensitization produced by intracellular calcium ion injection has been demonstrated by electrophysiology (Fein and Lisman 1975) in *Limulus* ventral photoreceptors. We know from Muijser (1979) that also in fly photoreceptor cells a desensitization is caused by Ca^{++}, and furthermore from Hamdorf (1970) that the desensitization by local illumination remains local.

In conclusion, Ca^{++} appears to be important for both triggering the pupil and local adaptation (see Kirschfeld and Vogt 1980). Our interpretation of the measurement of Bernard and Stavenga (1979) suggests that the optical measurements on the pupil can provide information on steps of the transduction process, as e.g. absorption by the visual pigment, but the results must be interpreted with caution when considering the information reaching the first synapse, i.e., considering what the animal could see.

The overlap of the effective absorbance spectrum of the pupil and the rhodopsin spectrum makes the pupil a well adapted device for controlling the light-flux in the rhabdomere, especially for avoiding the formation of too high fractions of metarhodopsin which would lead to severe desensitization by the so-called prolonged depolarizing afterpotential (reviews Hamdorf 1979; Stavenga 1980).

Our result that in contrast to the absorption of the pigment granules the pupil is relatively transparent in the UV because of the waveguide properties of the rhabdomere does not contradict this interpretation: in this respect the pupil spectrum should not be compared with the sensitivity spectrum of the receptors but with the photoequilibrium spectrum between rhodopsin and metarhodopsin. This has only a single maximum in the blue spectral range and decays in the UV. Thus the similarity of the effective absorbance spectrum of the pupil with the equilibrium spectrum, taking into account the UV region as measured by Minke and Kirschfeld (1979), illustrates best the pupil's protective function.

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Appendix

For an infinitesimal element dx of the rhabdomere we have for the absorbed intensity dI

$$dI = -[\eta K_i + (1-\eta) K_e] I\, dx$$

or, after integration (A1)

$$I(x) = \exp - \int_0^x [\eta K_i + (1-\eta) K_e]\, dx.$$

When the visual pigment is the only absorber in the rhabdomere and all molecules are in the rhodopsin state the absorption by the rhodopsin then is

$$dI^R = -\eta K_i I(x) dx \quad \text{(A2)}$$

and its contribution to the spectral sensitivity is

$$dS = A\, dI^R \quad \text{(A3)}$$

where A is a factor of proportionality. The spectral sensitivity of the complete cell then is

$$S = \int_0^L dS = \int_0^L A \eta K_i I(x)\, dx. \quad \text{(A4)}$$

In the general case A is a function of the local state of adaptation, which will vary along the rhabdomere. Furthermore $\eta = \eta(\lambda)$, $K_i = K_R(\lambda)$ and $I(x)$ is given by Eq. (A1), with still $K_e = K_e(x, \lambda)$.

The spectral sensitivity S is very difficult to estimate for the general case where K_e, representing the pupil absorption, is strongly varying along the rhabdomere and A, the local adaptation factor is also greatly dependent on the location in the rhabdomere.

The simplification introduced by taking K_e and A constant along the rhabdomere is considered in Results.

We make three comments here. First, assuming that the absorption inside the rhabdomere is only due to rhodopsin, indicated by $K_i(\lambda)$ the existence of a sensitizing pigment in the UV is neglected. However, when the efficiency of energy transfer is high the above assumption does not seriously affect the analysis, if $K_i(\lambda)$ then is considered as representing the extinction spectrum of rhodopsin and sensitizing pigment.

Second, we point to the analogy of ηK_i and $(1-\eta) K_e$ to $\alpha_R \cdot f_R$ and $(1-f_R)\ \alpha_M = \alpha_M \cdot f_M$, respectively; the latter terms emerge in the formalism used by Hamdorf and Schwemer (1975) and Tsukahara and Horridge (1977) (see also Hamdorf 1979).

Third, I_0 in Eq. (A1) represents the intensity at the entrance of the rhabdomere. The power P_0 of modes launched into the rhabdomeres depends on the waveguide properties. When η varies due to the accumulation of pigment granules near the distal tip of the rhabdomere $P_{0,d}(\lambda) \neq P_{0,l}(\lambda)$. i.e., the power is not the same for the dark and light adapted state respectively, and I_0 in Eqs. (A1) and (A4) has to be replaced accordingly (see Snyder and Horridge 1972).

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