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Insect pupil mechanisms

IV. Spectral characteristics and light intensity dependence in the blowfly, *Calliphora erythrocephala*

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Summary. The spectral characteristics of the pupil mechanism in blowfly photoreceptors and their dependence on light intensity have been investigated together with the intensity dependence of the receptor potential. The threshold for the pupil response as measured by reflectance is found at an intensity at which the peak of the receptor potential is about half maximal and the plateau potential starts to saturate. The reflectance saturates at about 3 log-units above threshold. The reflectance spectrum peaks near 620 nm, and its shape is independent of adaptation intensity. The absorbance change, measured by transmission, is extreme in the blue, at about 470 nm. The shape of the absorbance spectrum is slightly intensity dependent, presumably due to optical waveguide effects. The dynamic ranges of the light-induced reflectance and absorbance changes do not coincide. The reflectance change shows saturation at least 1 to 1.5 log units before the absorbance change saturates.

Key words: Pupil – Spectral reflectance – Absorbance – Receptor potential

Introduction

Many insect species live under environmental conditions in which light intensities range over 10 or more log units. The visual system of these species must have sophisticated light adaptation mechanisms in order to stay functional under these varying light conditions. At the level of the visual sense cells, light adaptation is mediated by controlling the phototransduction process, i.e. the process of transducing visual pigment conversion into a change in membrane potential, for instance by decreasing the voltage gain (Laughlin 1975; Laughlin and Hardie 1978) and by decreasing the light flux reaching the visual pigment molecules.

We recall that the visual pigment molecules of fly photoreceptors are contained within the rhabdomeres. These cylindrical structures function as optical wave-

guides, and, therefore, part of the light flux entering a photoreceptor propagates outside the rhabdomere boundary. Furthermore, inside fly photoreceptor cells exist tiny pigment granules (0.1–0.2 μm ; Boschek 1971) which upon light adaptation migrate towards the rhabdomere boundary. The light flux reaching the visual pigment is thus controlled by the mobile pigment granules, because they absorb and scatter light from the boundary wave (Kirschfeld and Franceschini 1969). This intracellular pupil, so-called because of its analogous function to that of the vertebrate pupil, has been demonstrated in the photoreceptors of several insect species (for review, see Stavenga 1989). The pigment migration is under direct control of visual pigment activation (Franceschini 1972; Bernard and Stavenga 1979) and is mediated by an increase of intracellular calcium (Kirschfeld and Vogt 1980).

By comparing the signal-to-noise ratio of the receptor potential in a normal and pupil-less mutant, Howard et al. (1987) concluded that the pupil attenuates the light flux to prevent saturation of the photoreceptor response and to extend the intensity range over which photoreceptors operate with a maximum signal-to-noise ratio. They estimated a maximal effective attenuation of the pupil of about 2 log-units, similar to previous estimates by Muijser (1980) and Leutscher-Hazelhoff and van Barneveld (1983).

The pupil can be studied both by transmission and by reflection measurements (Kirschfeld and Franceschini 1969; Stavenga 1979). Whereas transmission changes can be caused by pigment granules accumulating along the whole length of the rhabdomeres, reflectance changes from pupillary pigment granules originate from the distal part of the photoreceptor only.

Vogt et al. (1982) and Howard et al. (1987) have indirectly estimated the intensity response curve of the pupil response via the receptor potential. In the present study we present simultaneous intracellular recordings and optical measurements on the eyes of the blowfly *Calliphora* (for related measurements on the housefly *Musca*,

see Wilcox and Franceschini 1984). We thus could directly relate the intensity response curves of receptor potential and pupil mechanism. From the optical experiments we derived the spectral characteristics of the absorbance and reflectance changes caused by the pupillary pigment granules, and we obtained new insights in the dynamic range of the pupil mechanism.

Material and methods

Preparation. Females of the blowfly, *Calliphora erythrocephala*, wild type, were taken from the laboratory stock (7–14 days after emergence). They were immobilized with wax and mounted on a goniometer in a microspectrophotometer. A small hole was made at the backside of the head, through which a light guide was inserted for antidromic illumination. For electrophysiology, conventional glass electrodes were used, filled with a mixture of 3 M KAc and 0.05 M KCl (resistance 150–200 M Ω). Photoreceptor recordings were made via a window in the cornea, a small piece of which was removed.

Setup. The microspectrophotometer was constructed from an Ortholux 2 microscope (Leitz). Antidromically transmitted or orthodromically reflected light was measured by imaging the deep pseudopupil in the plane of a diaphragm in front of a photomultiplier (EMI 9862) by an NPL10 objective (0.20, Leitz). Illumination from 150 W Xe lamps was controlled by shutters (Uniblitz). The intensity of the illumination was controlled by neutral density filters and a neutral density wedge. Stimulus control as well as sampling of the receptor potential (1 kHz) and the photomultiplier signal (20–500 Hz) were performed by an intelligent laboratory interface (1401, Cambridge Electronic Design). Data, after transfer to a computer, were stored on hard disc for further analysis.

Intensity response curve of photoreceptor potential and pupillary reflection. The eye was light adapted (OG570, Schott) for 45 s during which time the receptor potential was measured. 50 ms after this adaptation period the reflection from the deep pseudopupil (Franceschini 1972) was tested at a wavelength of 580 nm (interference filter, bandwidth 15 nm). After 2 min dark adaptation the stimulation sequence was repeated at a higher adaptation intensity.

Reflectance spectra of the pupillary pigment. The eye was light adapted (OG570 or white light) for 3 min after which the light-adapted reflection from the deep pseudopupil was measured in a sequence consisting of 100 ms (low intensity) orthodromic test flashes at different wavelengths (interference filters, bandwidth 10–15 nm) and 5 s adaptation illumination. This sequence of test and adaptation illumination did not alter the state of adaptation of the pupil. In order to determine the dark-adapted reflection, the eye was dark adapted for 2 min followed by the test flashes. This sequence was repeated for 9 adaptation intensities.

The reflectance change of the pupillary pigment granules was calculated at different light adaptation states using Eq. (1)

$$\Delta R(\lambda) = \frac{(R_1(\lambda) - R_d(\lambda))}{R_{st}(\lambda)} R_{st}^*(\lambda) \quad (1)$$

where λ is the wavelength of the test light, $\Delta R(\lambda)$ change in reflectance with respect to the dark-adapted state, $R_1(\lambda)$ reflection signal from the light-adapted eye, $R_d(\lambda)$ reflection signal from the dark-adapted eye, $R_{st}(\lambda)$ reflection signal from a reflectance standard (NG1, Zeiss) as measured under the same conditions as the reflection from the eye, $R_{st}^*(\lambda)$ absolute reflectance of the reflectance standard.

In order to check whether the reflectance spectra had a uniform shape, a least squares linear fit was performed on the spectra using

a template reflectance spectrum, obtained by averaging all experimentally obtained reflectance spectra.

Absorbance spectra of the pupillary pigment. The stimulus sequence was equal to that used for determining the reflectance spectrum with the exception that the transmission measured from the deep pseudopupil was tested with (low intensity) antidromic test flashes. The absorbance spectra of the pupillary pigment granules were calculated at different states of light adaptation from the transmission measurements using Eq. (2)

$$\Delta E(\lambda) = -\log_{10} \left(\frac{T_1(\lambda)}{T_d(\lambda)} \right) \quad (2)$$

with $\Delta E(\lambda)$ absorbance change at wavelength λ , $T_1(\lambda)$ transmission measured in the light-adapted eye, $T_d(\lambda)$ transmission measured in the dark-adapted eye.

Because the background contributes to the transmission signal, an estimate of this background signal was first subtracted before calculating the absorbance spectra. This estimate of the background signal was made by measuring the transmission through the eye just outside the deep pseudopupil, with a diaphragm size identical to that used in the deep pseudopupil measurements.

Results

The receptor potential in response to 45-s light pulses of varying intensities of red light (OG570, Schott) was measured simultaneously with the induced pupillary response in *Calliphora*. Figure 1A shows the receptor potential in response to illumination steps of increasing intensity. In Fig. 1B the same responses are shown on a slower time scale. At the lowest intensities the photoreceptor response to a step of light consists of a monophasic increase of the receptor potential (Fig. 1A, B), i.e. at these intensities peak and plateau potentials coincide. Responses to higher intensities consist of an initial rapid peak followed by a plateau (Fig. 1A), which subsequently slowly decays to a steady state level (Fig. 1B; see Laughlin and Hardie 1978). Figure 1A also shows that both response latency and rise time decrease with increasing illumination intensity (e.g. Hamdorf and Kirschfeld 1980).

The intensity dependence of the peak and plateau of the receptor potential and that of the normalized reflectance increase of the pupil mechanism are shown together in Fig. 1C. The dynamic range of the peak and plateau potentials covers more than 6 log-units. In the dark-adapted photoreceptor a half-maximal receptor potential is elicited with a monochromatic irradiance of an axial point source at the most effective wavelength (500 nm) of about $4 \cdot 10^{10}$ quanta $\text{cm}^{-2} \text{s}^{-1}$ (Laughlin and Hardie 1978; Hardie 1979). This intensity is effectively about that of log intensity -3 in Fig. 1.

The threshold intensity for the reflectance increase is about 2–3 decades below the saturation intensity for the peak potential, and the range of the pupil response as measured by reflectance is about 3 log-units. The latter result is comparable with the dynamic range of the pupillary reflectance increase as reported by Franceschini (1972) for the fruitfly *Drosophila*.

Figure 2A shows the reflectance change at different test wavelengths for four adaptation intensities (white

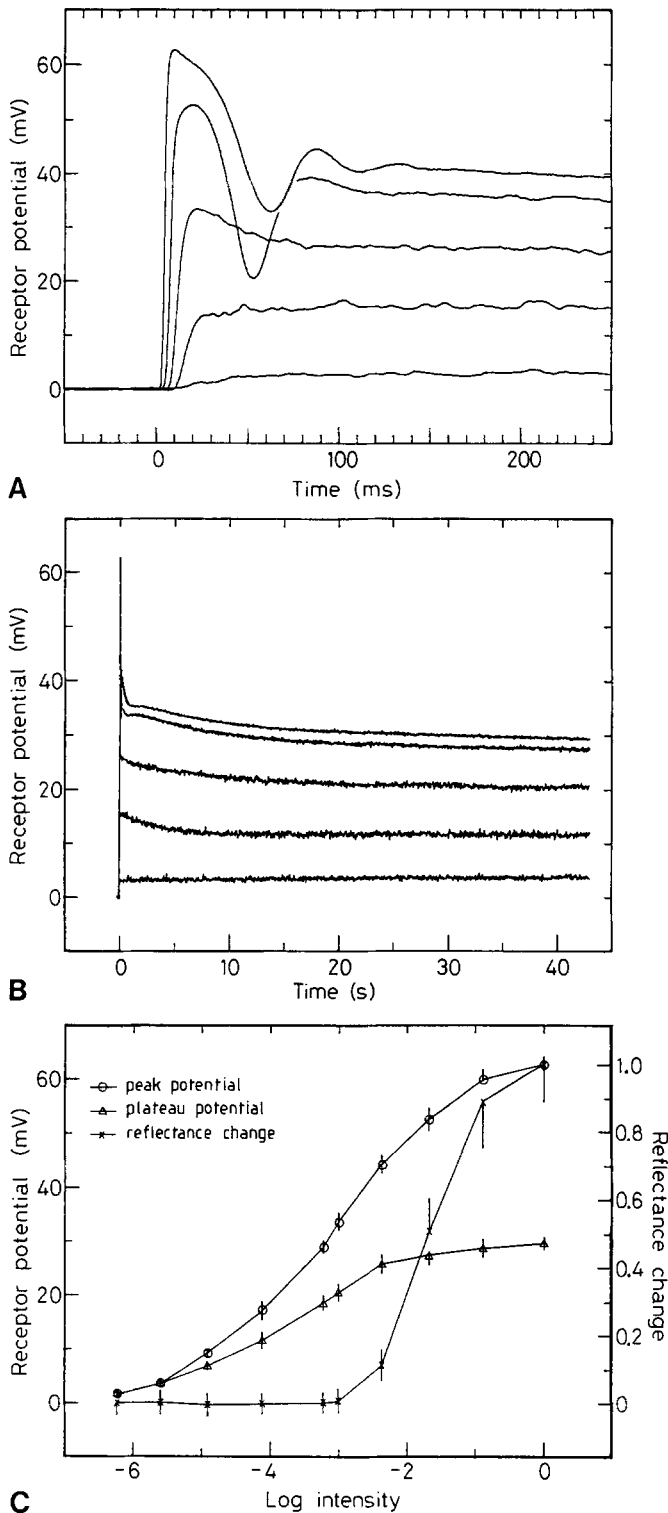


Fig. 1. **A** Receptor potential as function of time relative to the onset of the adaptation illumination (log intensity: -5.6 , -4.1 , -3.0 , -1.7 , 0 ; see **C**). At the lowest intensities the response consists of a monophasic increase of the receptor potential. Responses to higher intensities show a rapid peak followed by plateau. **B** Same responses as in **A** but on a slower time scale showing the decrease of the initial plateau to a stable level. At the lowest intensity the receptor potential readily reaches the stable level and thus peak and plateau potential coincide (see also **C**). **C** Intensity response curve of peak potential (\circ) and plateau potential (Δ)

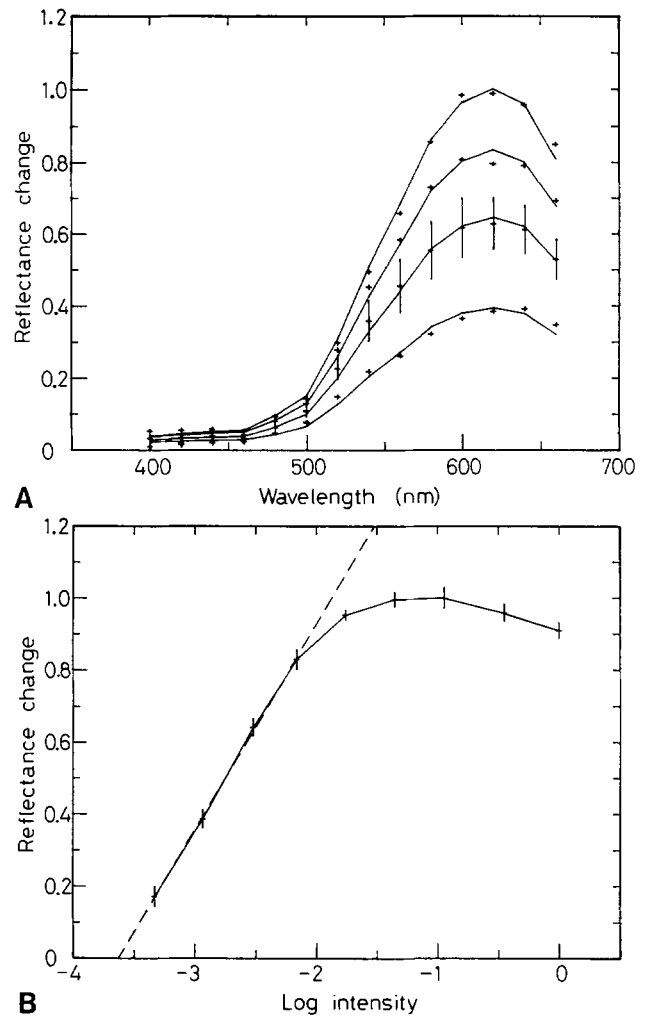


Fig. 2. **A** Averaged reflectance change ($n=9$) at different test wavelengths for four adaptation intensities (white light) measured in one fly (log intensity: -2.94 , -2.52 , -2.16 , -0.95 ; see **B**). For one adaptation intensity the standard deviation, which is comparable for all adaptation intensities, is given. The reflectance change spectra are proportional to an averaged reflectance change spectrum obtained from different flies and adaptation intensities, as seen from the fits (continuous lines). **B** Intensity response curve of the reflectance change. The continuous curve gives the proportionality factor \pm SE resulting from the fit of the reflectance spectra **A**. For the four lowest intensities the reflectance change can be well approximated by a linear function (dashed line). At higher intensities the reflectance change first saturates, followed by a small decrease at the highest intensities

and reflectance change (\times). Mean \pm SD of 4 measurements of the receptor potential in one cell are shown together with the reflectance change from the deep pseudopupil of the same eye. The plateau potential was determined between 40 and 43 s after the onset of the adaptation illumination (OG570, Schott) with a total duration of 45 s. Immediately after the adaptation illumination, the reflectance was tested at test wavelength $\lambda_t=580$ nm

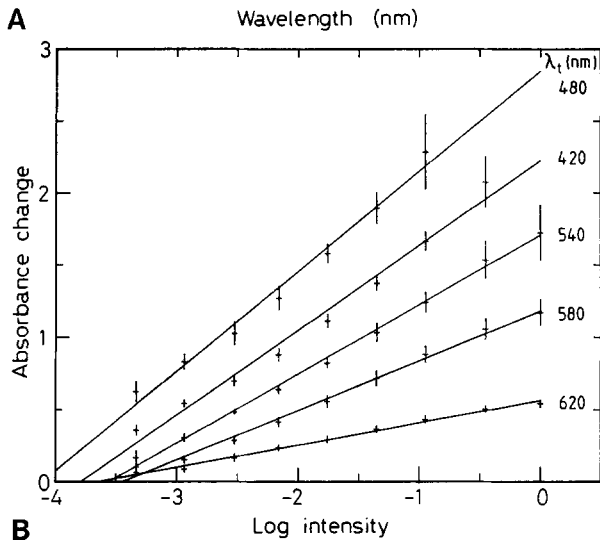
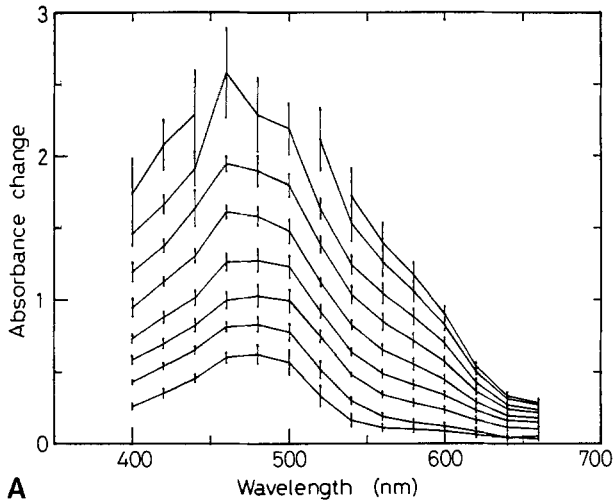


Fig. 3. **A** Absorbance change spectra at different adaptation intensities (white light; mean \pm SD, $n=9$; see **B** for the steps in intensity). At the two highest adaptation intensities the spectra are not complete in the wavelength region in which the pupil absorbance is high (see text). The absorbance change spectra peak at about 470 nm. **B** Intensity response curve of the absorbance change at 5 test wavelengths (same data as in **A**). The intensity response curves at different test wavelengths are fitted with a linear function (solid lines). The fits intersect the intensity axis at different intensities, i.e. the threshold intensity is wavelength dependent (see Fig. 4)

light). We constructed a spectral template by averaging well over 200 reflectance spectra obtained from a total of 6 flies. The peak of the template reflectance spectrum is at about 620 nm. The reflectance spectra measured at different adaptation intensities could all be well fitted with this template, and thus they appear to be proportional to each other. The proportionality factor of the reflectance spectra is a measure for the intensity dependence of the pupil mechanism (Fig. 2B). At the lowest adaptation intensities used here the reflectance change increases linearly with the logarithm of the adaptation intensity. The linear fit through the first four data points (*dashed line*, Fig. 2B) yields a threshold intensity of -3.6 log-units. At higher adaptation intensities the reflectance change levels off towards a saturation level and even

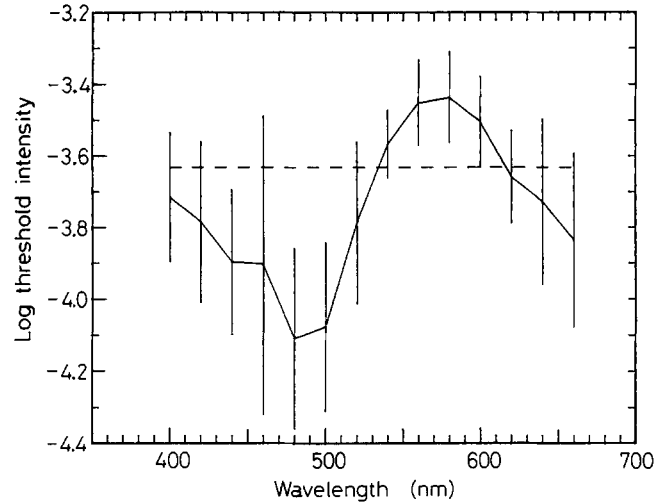


Fig. 4. Threshold intensity of the absorbance change as function of test wavelength (solid line). Threshold intensity and SE are calculated from the linear fits in Fig. 3B. Threshold intensity for the reflectance change (*dashed line*, from Fig. 2B) is not wavelength dependent and falls within the range of threshold intensities of the absorbance change

decreases at the highest intensities. Although this decrease is rather small, it was found in all flies investigated at these intensities. The intensity response curve of the reflectance change in Fig. 1C shows no decrease at the highest intensities, because in this experiment the adapting light was red instead of the white light used in Fig. 2B. The pupil was driven to half maximum reflectance at $\log I = -1.6$ with red light (Fig. 1C) and at $\log I = -2.8$ with white light (Fig. 2B). The white light hence is effectively 1.2 log-units more intense than the red light. We note here that the reflectance changes caused by the light-driven pupil were measured after the 3-min adaptation illumination.

We also measured the transmission changes caused by the pupil mechanism after a 3-min adaptation time at the same test wavelengths and under the same adaptation light intensities as used before. Figure 3A shows the calculated changes in absorbance for nine adaptation intensities. The absorbance change peaks in the blue, at about 470 nm. At low and medium adaptation intensities complete absorbance spectra could be obtained. However, at the highest adaptation intensities it was impossible to obtain reliable values, because the difference between the transmission signal of the background and that in the light-adapted state became less than the measurement error.

At first notice, the absorbance spectra seemed to be proportional, but this appears not to be the case. The proportionality was tested with the same method as used in testing the proportionality of the reflectance spectra; a template giving acceptable fits for all absorbance spectra could not be constructed, however. The intensity response curve of the absorbance change thus cannot be described with one curve for all test wavelengths. Figure 3B shows the intensity response curve of the absorbance changes for 5 test wavelengths together with a least

squares linear fit. The non-proportionality of the spectra (Fig. 3A) is reflected by the fact that the linear fits intersect the intensity axis at different points (Fig. 3B), i.e. the threshold intensity of the pupillary mechanism depends on the test wavelength (Fig. 4). Not surprisingly, the threshold intensity derived for the reflectance change (Fig. 2), given in Fig. 4 by the dashed line, falls within the range of threshold intensities of the absorbance change.

Extrapolation of the linear fits of Fig. 3B for the test wavelengths 460 and 480 nm to the highest adaptation intensity yields an absorbance change of no less than 2.8 ± 0.3 log-units.

Discussion

We have simultaneously measured the receptor potential and the reflectance change due to pupillary pigment migration occurring upon light adaptation in the blowfly eye (Fig. 1). The threshold for pupillary reflectance increase is found at an intensity at which the peak of the receptor potential is about 50% of its maximal value and the plateau potential levels off towards saturation. From intracellular measurement of the receptor sensitivity at 500 nm relative to that at 359 nm in *Musca*, Vogt et al. (1982) concluded that the intensity threshold for the pupillary response is 1 log-unit below the intensity needed to elicit a half-maximal receptor potential. The difference in the threshold values of the pupil mechanisms in *Calliphora* and *Musca* may be partly due to the fact that the threshold intensity depends upon test wavelength, as we have found from our absorbance measurements, but is possibly also due to the usage of another method. We note that Franceschini (1972), using one and the same method, found a difference of 1 log-unit in the threshold intensities of the pupil mechanisms of *Musca* and *Drosophila*. Nevertheless, we clearly find that in *Calliphora* receptor potential and pupil mechanism have distinctly different light dependencies. The same conclusion was drawn for *Musca* by Franceschini and Wilcox (pers. comm.).

The light-induced migration of the pupillary pigment granules in the photoreceptor cells requires Ca^{2+} (Kirschfeld and Vogt 1980). Recently, Sandler and Kirschfeld (1988) simultaneously measured the light dependence of the receptor potential and the extracellular Ca^{2+} concentration. Comparing these results with those in Fig. 1A, we see that the extracellular Ca^{2+} concentration decreases dramatically in the intensity region in which the pupil is activated. Apparently, the light-induced increase in intracellular Ca^{2+} concentration activates the pupil (see Kirschfeld and Vogt 1980).

As is shown in Fig. 2A, the shape of the reflectance spectrum is independent of adaptation intensity, and the reflectance spectrum at different adaptation intensities is proportional to an average template reflectance spectrum, peaking near 620 nm. Kirschfeld and Franceschini (1969; Franceschini and Kirschfeld 1976) measured reflectance spectra for the pupil mechanism in *Musca* and

Drosophila. Calculating the reflectance change from $\Delta R(\lambda) = R_1(\lambda)/R_d(\lambda)$, with $R_1(\lambda)$ the reflection signal of the light-adapted eye and $R_d(\lambda)$ that of the dark-adapted eye [cf. Eqn. (1), Material and methods], they obtained spectra peaking at 580 and 540 nm, respectively. Applying this method of calculation to our *Calliphora* data yields a reflectance spectrum quite similar to that obtained by Kirschfeld and Franceschini (1969) for *Musca*. This calculation method incorporates a background due to reflections from other pigments and ocular structures, however, thus leading to deformed spectra. We favour the calculation procedure as done in this paper, because in this way one calculates the reflectance change caused by the pupillary pigment granules alone, independent of background reflectance (see Material and methods). All the same, we conclude that the pupillary granules of *Calliphora* and *Musca* have very similar reflectance spectra and thus contain very similar pigments (see also Langer 1975; Stavenga 1989).

The absorbance change caused by the pupil mechanism (Fig. 3) was calculated after subtraction of the background from the transmission signal. We used the transmission signal measured from a position next to the deep pseudopupil, which is not necessarily identical to the actual background signal in the deep pseudopupil. At low adaptation intensities, a small error in the estimation of the background has only a little effect on the calculated absorbance change. However, at the highest adaptation intensities the effect becomes dramatic at those test wavelengths at which the pupil absorbance is large, i.e. in the wavelength range 400–560 nm. For example, when the transmission signal drops from an initial 100% to 3% and the real background contribution is 2% of the initial transmission signal, the real pupil absorbance is 2.0. However, if 2.6% of the initial transmission is estimated as the background contribution, then the absorbance is overestimated, i.e. calculated to be 2.4. We presume, however, that the background estimation is very reasonable, because the calculated pupillary absorbance spectrum compares quite satisfactorily with that of Stavenga et al. (1973), taken from measurements on the superimposed image of rhabdomeres of photoreceptor cells R3; in those measurements contamination of the transmission signal by a background contribution was negligible. The effect of the background correction on the intensity response curves of the absorbance change (Fig. 3B) appears to be negligible at test wavelengths above 580 nm. With zero background correction the absorbance continues to increase there even at the highest adaptation intensities, whilst saturation takes place at the shorter wavelengths. With the applied background correction a very similar, monotonic increase of pupillary absorbance with light intensity occurs at all test wavelengths (Fig. 3B), indicating that the background signal in the deep pseudopupil is indeed very similar to that adjacent to the pseudopupil.

From Figs. 2B and 3B it is clear that the dynamic range of reflectance and absorbance change do not coincide. This result seems to be in disagreement with earlier results (Franceschini 1972). At test wavelengths at which

pupil absorbance is substantial, the dynamic range is very easily underestimated, however. When the pupil absorbance is considerable, i.e. when the transmission signal has dropped to a few percent of the dark-adapted value, further transmission decreases are hard to detect, especially when background contributes to the transmission signal. Therefore, in order to determine the dynamic range of the pupil by absorbance measurements, the test wavelength has to be chosen so that pupil absorbance remains rather small.

The reflectance change originates from pigment granules in the distal part of the photoreceptor, whilst pigment granules aggregating along the whole length of the rhabdomere can contribute to the absorbance change. This argument, together with the fact that activation of the pupil is a local effect (Beersma 1979), explains why the intensity dependence of reflectance and absorbance change can rather differ. Further experimental results and an interpretation of these differences are presented by Roebroek and Stavenga (unpublished).

Even at the highest adaptation intensity no saturation state seems to be approached (Fig. 3B, $\lambda_t = 540\text{--}620$ nm). Extrapolation of the intensity response curve at 460 and 480 nm results in an absorbance change of 2.8 ± 0.3 log-units. Muijser (1980) found in *Calliphora*, with blue test light, a pupil factor of 1.7 log-units at adaptation intensities at which the reflectance of the pupil saturates. This value is quite comparable with our results (see Figs. 2B, 3B). Howard et al. (1987), by comparing the signal-to-noise ratio of the photoreceptors in a normal and a pupilless mutant of the fly *Lucilia cuprina*, found that at extreme light intensities the pupil effectively attenuates by 2 log-units. These experiments were, however, performed with white light (420–750 nm). The pupil effectiveness at wavelengths at which the pupil absorbance is maximal hence must be larger. In a separate paper we further discuss optical and electrophysiological measurements of the pupil effectiveness (Roebroek and Stavenga, in press).

The wavelength dependency of the threshold intensity of the absorbance change (Fig. 4) can be understood from the notion that fly rhabdomeres act as optical waveguides (Snyder 1975). Van Hateren (1984) determined that in *Calliphora* two waveguide modes can exist in the wavelength region of 400–500 nm. Because the boundary wave of the second order mode extends further from the waveguide than the first order mode (see Nilsson et al. 1988), the pupillary pigment granules preferentially absorb light from the second order mode. On one hand, this causes a narrowing from the angular sensitivity curve (Smakman et al. 1984), but it will furthermore cause a lower threshold intensity in the blue than at the longer wavelengths. Van Hateren (1984) also demonstrated that in the longer wavelength range only one mode can exist. Generally, it holds for waveguide modes that the longer the wavelength the further the boundary wave extends outside the waveguide. Or, the longer the wavelength the lower the pupil threshold intensity will be. The tendency of decreasing threshold intensity with increasing test wavelength is shown in Fig. 4 in the re-

gion of 400–500 nm and above 580 nm. The steep increase in threshold intensity between 500 and 580 nm coincides with the transition region of two to one allowed waveguide modes (see also van Hateren 1985).

Intracellular measurements indicate that the spectral sensitivity shifts 30–40 nm to the shorter wavelengths as a result of the pupil mechanism (Hardie 1979). Vogt et al. (1982) showed that this shift can be well explained from the absorbance spectrum of the pupil as found by Stavenga et al. (1973), which is, as noted above, similar to the spectra in Fig. 3A. The derived absorbance spectra are clearly only characteristic for the pupil, i.e. the assembly of photoreceptor pigment granules. The absorption spectrum of the pigment contained in the granules will be at least slightly different, because of the involvement of waveguide effects (see Vogt et al. 1982).

The function of the pupil mechanism in fly photoreceptors appears to be manifold. In this paper we have concentrated on the control of the light flux in the photoreceptors, which can be diminished a few orders of magnitude. The second function is the control of photoreceptor angular sensitivity (Smakman et al. 1984). Furthermore, the pupil favours the photochemical regeneration of converted visual pigment molecules (Stavenga et al. 1973; Stavenga 1980). Finally, Hardie (1979) and Vogt et al. (1982) established that the pupil shifts the sensitivity spectrum of the receptor potential towards the blue.

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References

- Beersma (1979) Spatial characteristics of the visual field of flies. PhD thesis, Groningen
- Bernard GD, Stavenga DG (1979) Spectral sensitivities of reticular cells measured in intact, living flies by an optical method. *J Comp Physiol A* 134:95–107
- Boschek CB (1971) On the fine structure of the peripheral retina and lamina ganglionaris of the fly, *Musca domestica*. *Z Zellforsch* 118:369–409
- Franceschini N (1972) Pupil and pseudopupil in the compound eye of *Drosophila*. In: Wehner R (ed) Information processing in the visual systems of arthropods. Springer, Berlin Heidelberg New York, pp 75–82
- Franceschini K, Kirschfeld K (1976) Le contrôle automatique du flux lumineux dans l'oeil composé des Diptères. Propriétés spectrales, statiques et dynamiques du mécanisme. *Biol Cybern* 21:181–203
- Hamdorf K, Kirschfeld K (1980) "Prebumps": Evidence for double-hits at functional subunits in a rhabdomeric photoreceptor. *Z Naturforsch* 35c:173–174
- Hardie RC (1979) Electrophysiological analysis of fly retina. I. Comparative properties of R1–6 and R7 and 8. *J Comp Physiol A* 129:19–33
- Hateren JH van (1984) Waveguide theory applied to optically measured angular sensitivities of fly photoreceptors. *J Comp Physiol A* 154:761–771

- Hateren JH van (1985) The Stiles-Crawford effect in the eye of the blowfly, *Calliphora erythrocephala*. *Vision Res* 25:1305–1315
- Howard J, Blakeslee B, Laughlin SB (1987) The intracellular pupil mechanism and photoreceptor signal:noise ratios in the fly *Lucilia cuprina*. *Proc R Soc Lond B* 231:415–435
- Kirschfeld K, Franceschini K (1969) Ein Mechanismus zur Steuerung des Lichtflusses in den Rhabdomeren des Komplexauges von *Musca*. *Kybernetik* 6:13–22
- Kirschfeld K, Vogt K (1980) Calcium ions and pigment migration in fly photoreceptors. *Naturwissenschaften* 67:516–517
- Langer H (1975) Properties and functions of screening pigments in insect eyes. In: Snyder AW, Menzel R (eds) *Photoreceptor optics*. Springer, Berlin Heidelberg New York, pp 429–455
- Laughlin SB (1975) Receptor and interneuron light-adaptation in the dragonfly visual system. *Z Naturforsch* 30c:306–308
- Laughlin SB, Hardie RC (1978) Common strategies for light adaptation in the peripheral visual systems of fly and dragonfly. *J Comp Physiol* 128:319–340
- Leutscher-Hazelhoff JT, Barneveld HH van (1983) The *Calliphora* pupil and phototransduction modelling. *Rev Can Biol Expte* 42:263–270
- Muijser H (1980) Investigations into the phototransduction in fly visual sense cells. PhD thesis, Groningen
- Nilsson D-E, Land MF, Howard J (1988) Optics of the butterfly eye. *J Comp Physiol A* 162:341–366
- Roebroek JGH, Stavenga DG (in press) On the effective optical density of the pupil in fly photoreceptors. *Vision Res*
- Sandler C, Kirschfeld K (1988) Light intensity controls extracellular Ca^{2+} concentration in the blowfly retina. *Naturwissenschaften* 75:256–258
- Smakman JGJ, Hateren JH van, Stavenga DG (1984) Angular sensitivity of blowfly photoreceptors: intracellular measurements and wave-optical predictions. *J Comp Physiol A* 155:239–247
- Snyder AW (1975) Optical properties of invertebrate photoreceptors. In: Horridge GA (ed) *The compound eye and vision in insects*. Clarendon Press, Oxford, pp 179–235
- Stavenga DG (1979) Pseudopupils of compound eyes. In: Autrum H (ed) *Vision in invertebrates (Handbook of sensory physiology, vol VII/6A)*. Springer, Berlin Heidelberg New York, pp 357–439
- Stavenga DG (1980) Short wavelength light in invertebrate visual sense cells – Pigments, potential and problems. In: Senger H (ed) *The blue light syndrome*. Springer, Berlin Heidelberg, pp 5–23
- Stavenga DG (1989) Pigments in compound eyes. In: Stavenga DG, Hardie RC (eds) *Facets of vision*. Springer, Berlin Heidelberg New York London Paris Tokyo, pp 152–172
- Stavenga DG, Zantema A, Kuiper JW (1973) Rhodopsin processes and the function of the pupil mechanism in flies. In: Langer H (ed) *Biochemistry and physiology of visual pigments*. Springer, Berlin Heidelberg New York, pp 175–180
- Vogt K, Kirschfeld K, Stavenga DG (1982) Spectral effects of the pupil in fly photoreceptors. *J Comp Physiol* 146:145–152
- Wilcox M, Franceschini N (1984) Stimulated drug uptake in a photoreceptor cell. *Neurosci Lett* 50:187–192