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Ultraviolet Sensitivity of Fly Photoreceptors R7 and R8: Evidence for a Sensitising Function

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Abstract. Responses to continuous spectral scans in the ultraviolet (uv) have been measured intracellularly from the central retinula cells R7 and R8 in the fly (*Musca,* female). The spectral sensitivities thus obtained have a resolution limited by the bandwidth of the light supplied by the monochromator (0.3–1.5 nm).

One class of R7 cells, classified as 7y (Kirschfeld et al. 1978) shows three conspicuous peaks of sensitivity at 337, 355 and 373 nm (Fig. 3a). The underlying R8 cells (8y) also show three peaks but at slightly shorter wavelengths – 334, 350, and 369 nm (Fig. 3c), coinciding with those seen in the peripheral photoreceptors R1–6 (Gemperlein et al. 1980; Kirschfeld et al. 1982). Another class of R7 cells (7p) showed a spectral sensitivity function with a single peak at 330 nm. The underlying R8 cells (8p) also show a single-peaked function with maximum at 460 nm (Fig. 3b and d).

The results are interpreted as providing evidence for the hypothesis that uv sensitivity in 7y and 8y cells is conferred by a uv absorbing sensitising pigment similar to that demonstrated in R1–6 cells. The spectra of both 7p and 8p cells can be simply interpreted as deriving directly from the absorption of a rhodopsin with the appropriate λ max.

Key words: Musca-photoreceptors R7 and R8 – uv sensitivity – Sensitising pigment

Introduction

In recent years several lines of evidence have indicated the presence of a 'sensitising pigment' in a certain class of fly photoreceptor (cells R1–6). This pigment absorbs mainly in the ultraviolet (uv) and is thought to transfer the energy of absorbed quanta to the rhodopsin molecule by means of a Förster (1951) type resonance transfer (Kirschfeld et al. 1977; Minke and Kirschfeld 1979; Stark et al. 1977; Kuo 1980). Despite its obvious adaptive advantage of

significantly increasing the overall absorption in photoreceptors and although well documented in photosynthetic systems, this phenomenon of sensitisation has, to date, only been demonstrated in this one type of animal photoreceptor.

Using a novel and technically demanding technique of 'Fourier Interferometry' Gemperlein et al. (1980) recently measured the spectral sensitivity of fly photoreceptors from the ERG with a refined resolution (of ca. 1 nm). Unexpectedly the result showed a fine structure in the uv with three distinct peaks (332, 350, and 369 nm). This spectrum has now been confirmed using more conventional illumination, both by intracellular recording and from absorption measurements, using microspectrophotometry, of single R1–6 cells (Kirschfeld et al. 1982). Apart from providing supporting evidence for the sensitising hypothesis (as no rhodopsin is known to show such an absorption spectrum) this result also provides us with a 'fingerprint spectrum' with which to search for similar sensitising pigments in other photoreceptors, and thus to extend the, as yet, isolated example of this novel phenomenon.

The cells chosen for the present investigation were the remaining classes of fly photoreceptors, R7 and R8, in particular because an earlier study (Hardie et al. 1979) had already suggested the possibility of a uv sensitising pigment in one of these cells. R7 and R8 are both small photoreceptors and in cross-section their common rhabdomere lies in the centre of the ommatidium surrounded by the rhabdomeres of the R1–6 cells. The rhabdomere of R7 lies on top off (distal to) that of R8 forming a single light guiding cylinder. Studies using microspectrophotometry (Kirschfeld et al. 1978), fluorescence studies (Franceschini et al. 1981a) and intracellular recordings (Hardie et al. 1979) indicate that these cells fall into two classes according to the pigments in their rhabdomeres. These have been termed 7 yellow (7y) and 7 pale (7p) on the basis of their appearance in transmitted white light. For simplicity R8 cells have also been classified according to the type of overlying R7, hence 8y and 8p (Hardie et al. 1981). There exists in addition a third class limited to the foveal region of male flies only, but these are not considered in the present work (but see Franceschini et al. 1981b; Hardie et al. 1981).

The rationale of this study has been to reexamine the spectral sensitivity of these cells with high resolution measurements in the uv in an attempt to provide evidence for, or against, a sensitising function in these receptors too.

Methods

Recording and Stimulation

Experiments were performed on *Musca domestica* white-eyed mutants. Only females were used and recordings were taken from the frontal-dorsal and frontal-ventral quadrants of the eye. The flies were raised on a vitamin A rich medium and used between 5 and 14 days post emergence. Intracellular recordings were made with 2M KCl filled micropipettes (resistances greater than 120 megohms) lowered into the eye through a small hole cut dorsally in the cornea as previously described (Hardie 1979).

To achieve the necessary high spectral resolution spectral sensitivities were measured using light from a 75W Xenon arc lamp passed through a double prism monochromator (Zeiss MM 12). The double prism construction has the advantage of extremely low levels of broad- or sideband stray light. Light was subsequently delivered to the eye via a quartz fibre optics light guide focussed onto the eye with a quartz lens. In the white-eyed fly this situation represents effectively non-axial illumination, and thus minimises potential waveguide and self-screening effects, or, in the case of R8, screening by the R7 rhabdomere.

Typically, scans were made in the range 310–400 nm using a continuous low intensity light regime which elicited, maximally, a receptor potential of only 5–10 mV. Because of the very noisy response of the central retinula cells (Hardie 1979) the responses to between five and 40 scans were signal averaged to obtain an acceptable signal to noise ratio. The raw data thus obtained were transformed through the cells' response intensity functions and corrected according to the spectral output of the illumination system in order to obtain spectral sensitivity functions. The response intensity functions were obtained by presenting a continuous stimulus which was increased in intensity by discrete steps. The responses to repeated intensity scans were averaged in a similar manner. The resolution of this method is limited by the bandwidth of the monochromatic light (set by the slit width of the monochromator) which can still produce measurable responses in the cell. In these experiments the bandwidth (at 50% level) varied between 0.3 and 1.5 nm. Digitalisation and subsequent computation and correction of the raw spectra were accordingly performed at 1 nm intervals using a graphic tablet and PDP 11 computer. The light source was calibrated using an EG and G radiometer (550-1) with the detector placed at the position of the fly. The possibility of broad band stray light or side bands was excluded by demonstrating that there was no measurable transmission with (nominally) uv wavelength settings of the monochromator when a 435 nm cut-off filter (GG 435, Schott) was inserted in the beam. The wavelength scale of the monochromator was calibrated to within 0.1 nm using the α and β lines of a deuterium lamp.

Cell Identification

The cells studied in these experiments were not marked by intracellular dye injection, but were identified according to criteria developed from previous dye injection experiments (Hardie et al. 1979; Hardie unpubl.). Firstly both R7 and R8 can be distinguished from the much more commonly encountered R1–6 cells by their noisy responses (resulting from larger quantum bumps). The different classes of R7 and R8 are further distinguished by the gross features of their spectral sensitivity functions (Fig. 1). In addition the different functional classes of R7 and R8 may be confidently identified with the rhabdomere type 7y or 7p. By association with their overlying R7 rhabdomere, R8 cells are classified as 8y or 8p (Hardie et al. 1981).

The two classes of R7 are both single peaked ultraviolet receptors. 7p is distinguished by having less than 10% sensitivity at wavelengths beyond 400 nm,

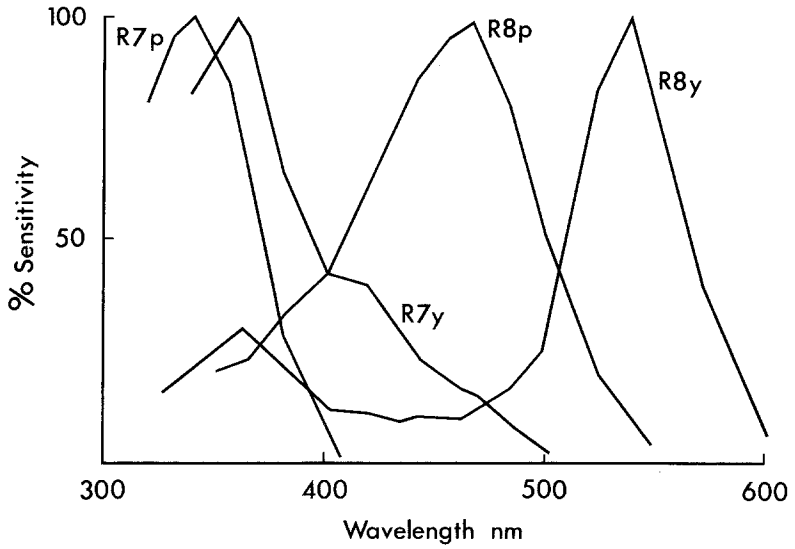

Fig. 1. Summary diagram showing spectral sensitivity functions of the different classes of R7 and R8 as previously measured using isoquantal flashes of axial monochromatic light at 10–20 nm intervals. These 'gross' spectral sensitivities have been shown to be characteristic of the various cell classes (7y, 7p, 8y and 8p) by extensive recordings coupled with dye-injection (Hardie 1979; Hardie et al. 1979; Hardie unpubl.; Smola and Meffert 1979) and in the present experiments were used as a basis for identifying the R7 and R8 cells encountered

7y has a long tail of sensitivity (> 10%) extending to ca. 500 nm. 8y is distinguished by a major peak at ca. 530 nm and very low sensitivity in the blue (400–480 nm), 8p shows a single blue peak of sensitivity (460 nm). The curves are summarised in Fig. 1. These gross features of the spectral sensitivity functions could be readily recognised from a single spectral scan covering the range 350–550 nm, on-, or off-axis[1], which was routinely performed before proceeding to measure the detailed uv spectrum.

Because female flies were used in these experiments we did not encounter the third, sex-specific, class of R7 and R8 (7r and 8r) however previous experiments (Franceschini et al. 1981; Hardie et al. 1981) indicate that they contain a pigment system similar to that found in R1–6 cells.

Results

Spectral sensitivities were measured in 22 central retinula cells. According to the criteria outlined in the preceding section these included: six 7y; six 8y; five 7p and five 8p cells. Examples of raw, uncorrected, signal averaged spectra from single cells are shown in Fig. 2. As described in the Methods section these curves

1 The curves in Fig. 1 were measured using axial illumination in which case screening effects play a significant role in shaping the spectral sensitivity functions, particularly for 7y and 8y. With diffuse (off-axis) illumination both these cell types show a significant relative increase in sensitivity in the blue (400–500 nm) region (Hardie et al. 1979)

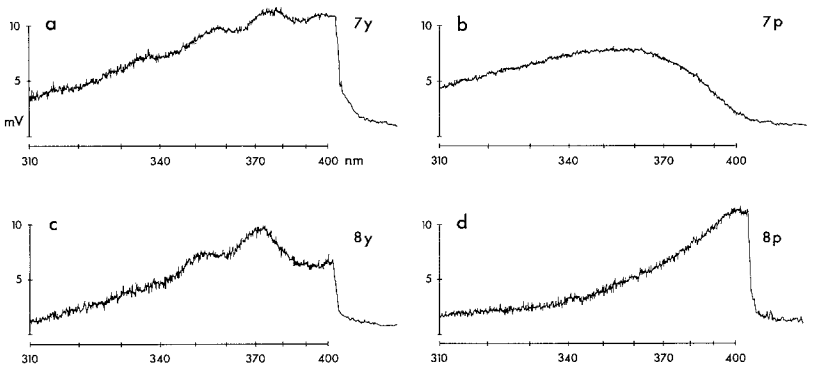

Fig. 2a–d. Representative examples of 'raw' data – responses of single cells to continuous scans in the range 310–400 nm. The signal/noise ratio has been enhanced by averaging the responses from n repeated scans. **a** 7y, $n = 8$; **b** 7p, $n = 10$; **c** 8y, $n = 5$; **d** 8p, $n = 7$. Each scan lasts 16 seconds. The non-linear wavelength scale reflects the non-linear relationship between wavelength and prism rotation in the monochromator

were then digitalised at 1 nm intervals, transformed through the cells' response intensity functions and corrected for the spectral output of the illumination system. Figure 3 shows the averaged data (± standard error) for all cells of each class. An R1–6 spectrum (Kirschfeld et al. 1982) is included for comparison.

7y Cells

The spectral sensitivity of 7y cells shows three clear peaks in the uv. Close examination of Fig. 3 however shows that the peaks are shifted ca. 4–5 nm relative to the 'fingerprint' spectrum obtained from R1–6 cells (Gemperlein et al. 1980; Kirschfeld et al. 1982). Reference to individual spectra shows that this is consistent from cell to cell. Comparison with our own single cell data from R1–6 cells (Kirschfeld et al. 1982) shows no overlap. For example, with respect to the major (central) peak, *all* R1–6 cells showed a peak at 350 nm, whereas in 7y cells, although there was a slight variation (probably attributable to the greater noise levels in the responses), all the peaks fell between 353 and 356 nm with average λ max 355.3 ± 0.76 nm (SD).

8y Cells

The spectral sensitivity of 8y cells also shows three clear peaks in the uv. However, in contrast with 7y cells, the peaks coincide *exactly* with those seen in R1–6 cells – namely 334 nm, 350 nm and 369 nm (Fig. 3). Although the stimulus configuration can be considered largely nonaxial there is a certain axial component. To ensure that this result was not due to a screening effect by the overlying R7 rhabdomere, in two cells the spectral sensitivity was also measured with the light guide aligned 30° away from the cell's visual axis. In this case the spectral sensitivity in the uv still showed the three peaks.

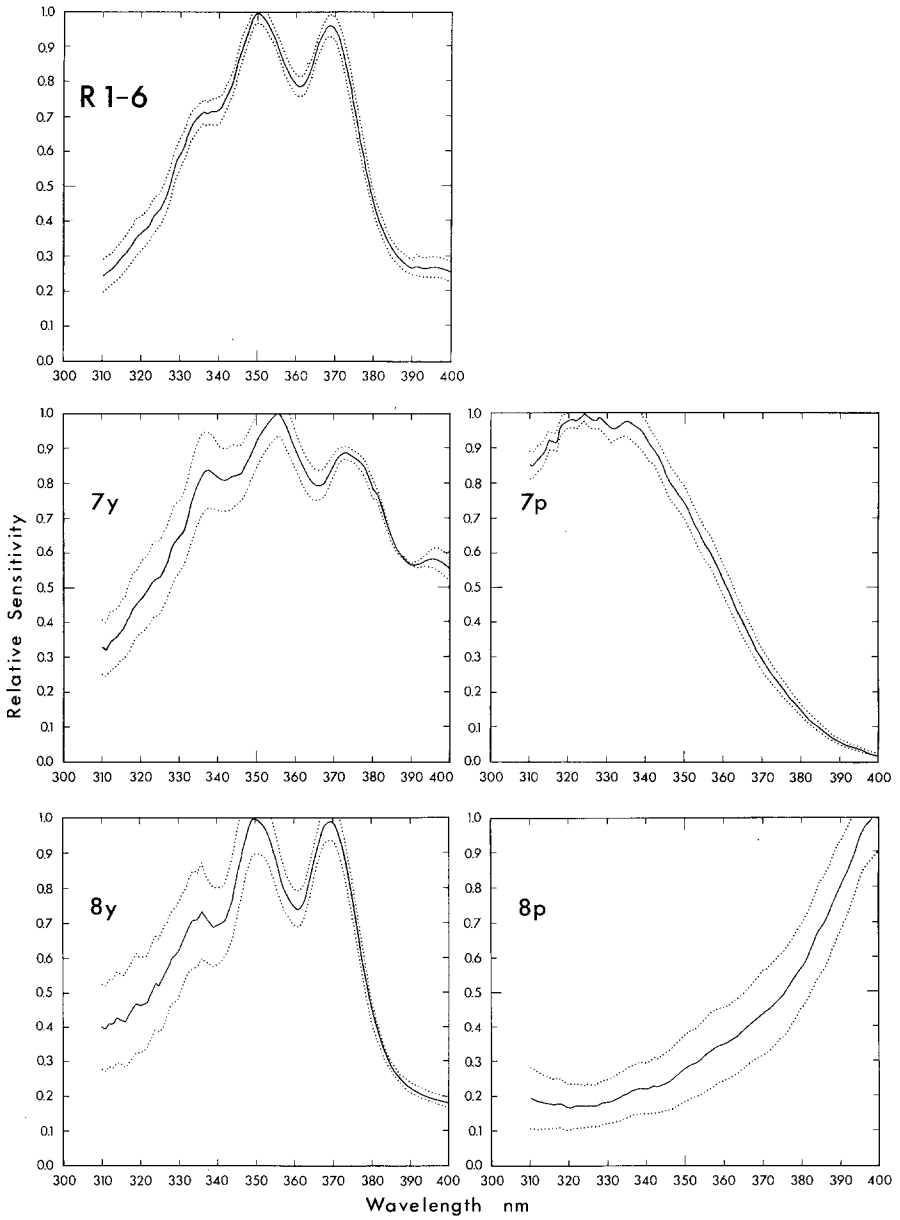

Fig. 3. Averaged data from all cells (± SE): R1–6, five cells, total of $n = 49$ scans; 7y, six cells, $n = 77$ scans; 7p, five cells, $n = 99$; 8y, six cells, $n = 41$; 8p, five cells, $n = 60$. Notice that both 7y and 8y show three clear peaks, those of 7y however being shifted by ca. 4 nm, to longer wavelengths. Neither 7p or 8p show any obvious fine structure

7p Cells and 8p Cells

The spectral sensitivity of all the 7p cells studied was uniformly smooth without any obvious fine structure. Peak sensitivity is at the very short wavelength of ca. 330 nm (Fig. 3).

8p cells also showed no fine structure in the uv range. Instead there is a smooth monotonically rising flank which peaks at ca. 460 nm (Figs. 1 and 3).

Discussion

1. 7p and 8p

The characteristic three-peaked uv sensitivity observed in cells R1–6 was taken as further strong evidence for an accessory (sensitising) pigment because it was not expected that a rhodopsin spectrum could have such a curve (Gemperlein et al. 1980; Kirschfeld et al. 1982). However at that time there existed no detailed spectra of uv rhodopsins. In this respect the smooth single-peaked spectrum obtained from 7p cells is significant as a control, demonstrating that such a fine structure does not exist in a (presumed) normal rhodopsin. That uv sensitivity in 7p cells is conferred by a normal invertebrate rhodopsin is supported by the following observations: 1. the difference spectrum, with isosbestic point at ca. 370 nm, is consistent with a uv absorbing rhodopsin photointerconvertible with a blue absorbing metarhodopsin (Kirschfeld 1979; Kirschfeld et al. in prep.); 2. this interpretation of the difference spectrum is in accord with the finding that intense uv light produces a prolonged depolarising afterpotential in 7p cells, which may be depressed by subsequent blue illumination (Hardie et al. 1979); 3. 7p cells show a clear polarisation sensitivity (PS) in the uv aligned with the microvillar direction (characteristic of rhodopsins in microvillar membranes) (Hardie et al. 1979). Note that PS and dichroism in the uv are more or less lacking in R1–6 cells where the sensitising pigment is believed to be mainly responsible for uv sensitivity (Hardie 1978; Kuo 1981a; Vogt and Kirschfeld in press).

We did not attempt to fit the 7p spectrum to a nomogram since there are no reliable nomograms in this spectral range. The data fit very closely, however, to the uv rhodopsin (R345) absorption spectrum determined in *Ascalaphus* (from Hamdorf 1979) shifted ca. 15 nm to shorter wavelengths, thus putting the peak of the 7p spectrum at 330 nm.

8p also lacks any multi-peaked fine structure and can be similarly interpreted as a normal rhodopsin spectrum although confirmatory data such as MSP are lacking.

2. 7y and 8y

Both these cell types show a pronounced three-peaked spectral sensitivity in the uv. In the case of 8y the shape corresponds very closely to the 'fingerprint' R1–6 spectrum and there would seem to be little doubt in attributing uv sensitivity in 8y cells to the same (sensitising) pigment as in R1–6 cells.

In 7y cells the presence of a uv sensitising pigment has already been suggested (Hardie et al. 1979; Kuo 1981a). Based on a synthesis of the available MSP data (Kirschfeld et al. 1978; Kirschfeld 1979) with intracellular measurements, Hardie et al. (1979) concluded that the unusually skewed spectral sensitivity (see Fig. 1) of these cells was best explained by a blue absorbing rhodopsin (photointerconvertible with a green absorbing metarhodopsin) which was however screened by a dense blue absorbing photostable pigment-tentatively identified as a carotenoid (Kirschfeld et al. 1978; McIntyre and Kirschfeld 1981). The high uv sensitivity was then tentatively attributed to a uv sensitising pigment. This interpretation was supported by Kuo (1981a) who found that the relative sensitivity in the uv and the blue was dependent upon age in much the same way as uv vs. green sensitivity in R1–6 cells.

Kuo also resolved the discrepancy between the results of Hardie et al. (1979) and those of Smola and Meffert (1979) who found, in contrast to a single-peaked function, a twin-peaked (uv and blue) spectral sensitivity. According to Kuo (1981b) the results of Smola and Meffert were due to the use of flies relatively deficient in vitamin A. Such a dietary explanation for the discrepancy had in fact already been suggested by Hardie et al. (1979).

Specifically the evidence suggesting that 7y cells also contain a uv sensitising pigment similar to that found in R1–6 cells was the following:

1. The spectral sensitivity of the cells shows a major peak in the uv (previous 'gross' determinations putting the peak at ca. 360 nm) (Hardie et al. 1979), whereas the difference spectrum measured microspectrophotometrically indicates a rhodoposin with peak absorption at 430 nm (Kirschfeld 1979; McIntyre and Kirschfeld 1981);

2. As in R1–6 cells (Hardie 1978) no polarisation sensitivity in the uv can be reliably determined in 7y cells – in contrast to 7p cells which as argued are presumed to contain a uv rhodopsin (Hardie et al. 1979).

A possible objection to the suggestion of a uv sensitising pigment in 7y cells is raised by the failure to observe a uv induced fluorescence in these cells (Franceschini et al. 1981a), whereas the uv sensitising pigment in R1–6 cells is thought to be responsible for the observed broad band bluish/white fluorescence peaking at ca. 470 nm (Franceschini et al. 1981a; Franceschini 1982). However this discrepancy may be explained by the high concentration of a blue absorbing photostable pigment in 7y cells (presumed to be a carotenoid) which may be expected to effectively mask any fluorescence.

The demonstration here of the characteristic three-peaked vibrational infrastructure in the spectral sensitivity is strong evidence further supporting the presence of a sensitising pigment in 7y cells. The ca. 4 nm bathochromic shift in the position of the peaks with respect to the 'fingerprint spectrum' from R1–6 cells presents no serious difficulties in interpretation and could be explained either by interactions of the sensitising molecule with other membrane components or slight chemical modification. For example as discussed in detail elsewhere (Franceschini 1982; Kirschfeld et al. 1982) amongst the most likely candidates for the sensitising pigment is retinol. This normally absorbs maximally at 325 nm but on binding to proteins the spectrum is red shifted and can develop a vibrational infrastructure very similar to that in fly photoreceptors

(Hemley et al. 1979; Fugate and Song 1980). The exact position of the three peaks can be expected to depend upon the nature of the binding protein and assuming that the protein in question is the rhodopsin itself, the different rhodopsins in 7y c.f. R1–6 cells may be sufficient to explain the shift. If retinol is indeed the sensitising molecule a further explanation is also possible as it is known that under certain conditions a 'retro' derivative (in which the conjugated system is displaced one carbon atom back into the ring) may be readily formed, the absorption spectrum of which (again in a protein complex) shows the three peaks appropriately red shifted (Hemley et al. 1979; Fugate and Song 1980).

In conclusion the search for the 'fingerprint spectrum' in the remaining photoreceptor classes in the fly *Musca* proved successful in two of the four classes thus significantly extending the previously isolated example of sensitisation in animal photoreceptors. The result further indicates that the same sensitising pigment molecule is capable of transferring energy to three different rhodopsins with λ max's at ca. 420 nm (7y); 490 nm (R1–6) and 520 nm (8y), a range further extended by Minke and Kirschfeld's (1979) finding that the metarhodopsin in R1–6 cells (λ max = ca. 570 nm) is also sensitised by the sensitising pigment.

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