Electrophysiological Properties of R7 and R8 in Dipteran Retina

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Intracellular recording and dye injection have directly demonstrated the absolute and spectral sensitivities of R7 and R8 in the eye of *Calliphora*. R8 has major peak of sensitivity at 547 nm with less than half the bandwidth of a theoretical rhodopsin, and a subsidiary peak at 358 nm. R7 is confirmed as being an ultraviolet receptor. Both have absolute sensitivities similar to those of R1–6. These results are inconsistent with the scotopic (R1–6), photopic (R7 and R8) theory of fly vision.

The photoreceptors in the dipteran retina fall into two anatomical classes: receptors R1–6 which have peripheral rhabdomeres and send short axons which terminate on postsynaptic elements in the lamina; and receptors R7 and R8 which have central rhabdomeres and send axons straight through the lamina to the medulla [1]. Several authors have proposed that this anatomical subdivision is the basis of two visual subsystems which can be detected at the behavioural level [2], or the level of units in the optic lobes [3]. In particular, a scotopic subsystem with low acuity and a spectral sensitivity peaking at 360 nm and 490 nm, has been presumed to be driven by R1–6, and a photopic subsystem with high acuity, and a spectral sensitivity peaking in the blue at 460 nm has been presumed to be driven by R7 and R8 [2, 3]. Although the spectral properties of R1–6 are reasonably well known [3, 4] and approximate those of the scotopic subsystem, the spectral properties of R7 and R8 are inadequately known, largely because of the technical difficulties of recording from the very small cells. However recent data from ERG's of *Drosophila* mutants [5], intracellular recordings from *Calliphora* [6]; and microspectrophotometry in *Musca* [7] now clearly indicate that R7 is an ultraviolet (UV) sensitive receptor with little or no sensitivity in the green. Our knowledge of R8 however is much less complete and at present is only available from the ERG of *Drosophila* mutants lacking R1–7, which shows a single blue peak [5], and intracellular recording in *Calliphora* [8], where measurements were made at only two wavelengths. The major aim of the present work was to record from R8 directly and to stain it by intracellular dye injection for subsequent identification.

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Female wild type *Calliphora stygia* or *Lucilia serricata* were stimulated with a point source using a 900 W Xenon arc lamp and quartz optics described elsewhere [9]. Intracellular recordings were made using ultrafine micropipettes having resistances of 500–1000 MΩ when filled with an aqueous solution of Procion yellow M4RAN. The electrode was advanced through a small hole in the eye and on into the lamina and chiasma where most of the successful recordings were made. Dye injection was achieved with only 10 nA minutes of iontophoresis and standard wax histology was used to recover stained cells.

Once in the lamina or chiasma, apart from frequent penetrations of large monopolar cells, L1 and L2 (identified by their hyperpolarising waveforms [10]), cells were occasionally encountered with depolarising waveforms typical of receptors but with certain distinctive features (Fig. 1). Firstly at low light levels the responses were very noisy with large discrete waves presumed to represent photon noise; secondly the maximum response (V_{max}) was unusually small (18–30 mV) and characterised by a long rounded transient; thirdly the penetrations were short lived (max 20 minutes), finally none of these cells (twenty three in all) had a spectral sensitivity resembling the 360 nm and 490 nm peaked profile of R1–6 [3].

The spectral sensitivities fell into three distinct classes. Eleven had 100% sensitivity at 358 nm and less than 10% sensitivity beyond 464 nm. Some of these were pure UV cells with less than 10% sensitivity beyond 420 nm and some had a secondary peak

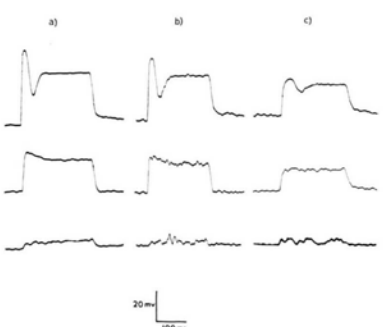

Fig. 1. Typical responses of a) R1–6, b) R8 in the retina, and c) R8 in the chiasma. Responses are chosen at approximately 10%, 50% and 95% V_{max} in each case. The response of R8 is noisier than that of R1–6 at low light levels and is considerably smoothed and attenuated in the chiasma.

or shoulder at around 440 nm. Two were positively identified as R7 by recovery of Procion filled cells, on the anotomical criteria of the presence of an axon in the chiasma and a cell body terminating in the most distal retina. These results confirm the existing data on R7's spectral properties [5–7]. Two, only, had a single blue peak of spectral sensitivity, but were not marked. The other ten cells showed a spectral sensitivity profile previously unreported in Diptera (Fig. 2). All ten showed a major peak of sensitivity at 547 nm and a subsidiary peak at 358 nm. The major peak was very sharp, having less than half the bandwidth (at the 50% sensitivity level) of the theoretical rhodopsin absorbance calculated from the Dartnall nomogram. Three of these cells were identified as R8 by Procion injection, one being shown in Fig. 3. The necessary anatomical criteria for identification were, an axon projecting through the chiasma into the medulla and a cell body terminating in the proximal half of the retina. These ten cells were recorded with widely divergent visual axes and there is thus no reason to suspect a specific retinal localisation of this colour type. Although the majority of recordings were made in *Calliphora stygia*, a receptor cell with indistinguishable spectral sensitivity was recorded from in the chiasma of another species of fly, *Lucilia serricata*.

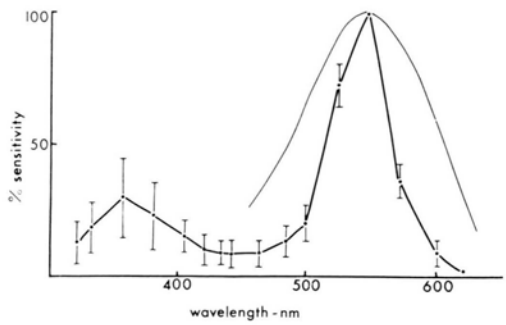

Fig. 2. Spectral sensitivity of R8; average of eight units (11 determinations). Error bars represent ±1. OSD. The theoretical absorbance of a rhodopsin of the same peak absorbance is included for comparison.

Occasionally R7 and R8 could be recorded from in the retina thus allowing a more direct comparison of the waveform with R1 – 6 (Fig. 1). In the retina both R7 and R8 showed a V_{max} of ca. 50 mV and a rapid transient, both similar to R1 – 6, suggesting that the low amplitude, smoothed waveform seen in the chiasma is a result of signal attenuation following passive propagation along the axon [11]. The noise level in the response to low light levels is even greater, with fluctuations of up to 5 mV (compared with 1 – 2 mV in R1 – 6). This result implies a higher voltage gain per photon in R7 and R8, as

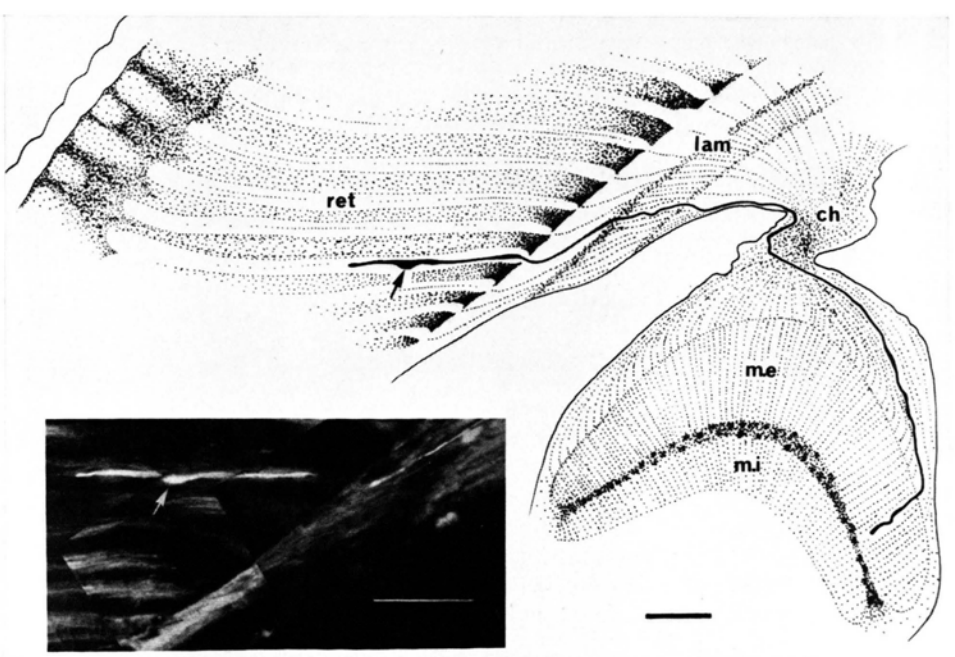

Fig. 3. Camera lucida drawing of an R8 cell stained by Procion yellow. The inset shows a photograph of the diagnostic feature, namely, the cell body terminating in the proximal half of the retina. The cell's nucleus is marked by the arrows. Scale bars: 50 μm. ret. retina; lam. lamina ganglionaris; ch. chiasma; m.e. medulla externa; m.i. medulla interna.

has been shown in UV sensitive cells in dragonfly retina [12]. This conclusion is borne out by measurements of absolute sensitivity (defined as the reciprocal of the number of quanta incident on the cornea, of peak wavelength, on axis, required to generate a 50% V_{max} response: *i. e.* the Axial Peak Sensitivity at 50% level, of Laughlin [12]) calculated from the response intensity functions. Despite their smaller rhabdomere (1/2 the length and 2/3 the diameter) R7 and R8 have a sensitivity equal to or greater than R1 – 6. Mean values ($n = 7$ for each class) were: R7, $2.6 \pm 1.3 \times 10^{-11}$ $q^{-1} \cdot cm^2 \cdot s$; R8, $3.4 \pm 2.2 \times 10^{-11}$ $q^{-1} \cdot cm^2 \cdot s$; and R1 – 6, $2.4 \pm 0.5 \times 10^{-11}$ $q^{-1} \cdot cm^2 \cdot s$.

The inescapable conclusion from these results is that the majority of R8 cells in *Calliphora stygia* have the spectral sensitivity shown in Fig. 2. This colour type has also been found in *Lucilia serricata*. In addition a cell type has been reported in the dronefly, *Eristalis* with a sharp peak at 540 nm (though with no sensitivity in the UV) and suggested to represent R8, though on less conclusive grounds [13]. Taken together these results argue for the widespread occurrence of an R8 with this or similar spectral sensitivity in dipteran retinae.

These findings have serious implications for the scotopic (R1 – 6), photopic (R7 and R8) subsystem theory. Neither R7 nor R8 as shown here, has the blue spectral sensitivity which characterises the photopic subsystem [2, 3]. In addition the absolute sensitivities of R7 and R8 are equal to or greater than R1 – 6. Further recent studies on visually deficient *Drosophila* mutants do not support the scotopic/photopic subsystem hypothesis [14], and my own recent results show that light adaptation of R1 – 6 narrows their angular sensitivity function and also results in a spectral shift towards the blue.

The severe deviation of R8's spectral sensitivity from a nomogram (Fig. 2) suggests the possibility of screening effects. An obvious candidate is R7's rhabdomere, the majority of which have recently been found to contain a blue absorbing carotenoid [7]. The screening effect of this pigment might account for R8's exceptionally low sensitivity in the blue. In addition, it is suggested the two blue sensitive cells recorded from represent R8 cells lying beneath R7 rhabdomeres which lack the carotenoid [7]. Note that, in *Drosophila* mutants lacking R1 – 7, where a blue spectral sensitivity is observed [5], this screening effect would be absent. There would also be no possibility of interaction with other cell types [13], or, as white-eyed mutants were used, screening by other retinal pigments – both possible causes of deviation from a nomogram.

Whatever the basis of R8's spectral sensitivity, it is clearly established that the population of central rhabdomeres in *Calliphora* include at least two (and probably three) distinct colour types spanning a wide spectrum, which, it is tentatively suggested, could allow trichromatic colour vision.

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