Distribution and Properties of Sex-Specific Photoreceptors in the Fly *Musca domestica*

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**Summary.** 1. In male houseflies (*Musca domestica*) the frontal dorsal region of the eye contains a unique class of central rhabdomere (R7/8) not found in other eye regions or in female flies (Fig. 1). The rhabdomeres may be recognised in vivo by their red autofluorescence, and are called here 7r and 8r respectively.

2. Difference spectra of 7r rhabdomeres, measured by microspectrophotometry of single rhabdomeres are indistinguishable from those of R1–6 (Fig. 2).

3. Intracellular recordings coupled with dye injections have established that: a) 7r cells are indistinguishable from the peripheral photoreceptors R1–6, at least with respect to spectral, angular and absolute sensitivities, response waveform and noise characteristics (Figs. 4, 5; Table 1); b) 8r cells however are clearly distinguishable by virtue of their spectral sensitivity (Fig. 6), noise characteristics and response waveform (Fig. 5).

4. Axonal profiles from cells stained intracellularly with the dye Lucifer yellow (Fig. 9) show that: a) 7r cells do not project to the medulla but terminate in the upper region of the lamina cartridge layer where they also project out one or more lateral branches; b) 8r cells project long axons through to the medulla.

5. Electron microscopic examinations of cells initially identified by their autofluorescence indicate that 7r cells approximate many features of R1–6 cells, including cell body, rhabdomere and axonal diameters. In these respects 8r cells differ and show the characteristic morphology of other R7 and R8 cells (Fig. 8, Table 2).

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**Abbreviations:** R1–6 and R7/8 classes of retinula cells in each fly ommatidium; R1–6 having peripherally, and R7/8 centrally located rhabdomeres; 7y, 7p, 7r and 8y, 8p, 8r subclasses of retinula cells; R7 and R8; MSP microspectrophotometry; PS polarisation sensitivity; PDA prolonged depolarising afterpotential; APS 50 axial peak sensitivity at 50% peak response level

**Introduction**

A general feature of compound eye organisation is the subdivision of the photoreceptors into those with short visual fibres (s.v. f's) which only reach the first optic neuropile (lamina) and those with long visual fibres (l.v. f's) which project through to the second optic neuropile (medulla) (Cajal and Sanchez 1915). In flies the s.v.f's from each ommatidium are represented by the 'peripheral' photoreceptors R1–6 and the l.v.f's by photoreceptors R7 and R8. The rhabdomeres of cells R7 and R8 form a single light-guiding cylinder, the distal portion being formed by R7 and the proximal portion by R8, so that with axial illumination the light reaching R8 is first filtered by the rhabdomere of R7 (Trujillo-Cenoz and Melamed 1966; Boschek 1971). The axons of R7 and R8 have generally been considered to project directly to the medulla without forming any synapses in the lamina. This is in direct contrast to the axons of cells R1–6 which synapse on second order cells in the lamina according to the well known neural superposition pattern (Braitenberg 1967).

In this and previous papers (Hardie 1977, 1979; Hardie et al. 1979; Kirschfeld et al. 1978) we have addressed ourselves to the problem of elucidating the properties of cells R7 and R8, so that – with the already well documented properties of cells R1–6 – we can complete a functional description of the fly retina. This work has been complicated by the discovery that receptors R7 and R8 do not form an homogeneous population. Initially this was thought to be restricted to a division into 7 yellow (7y) and 7 pale (7p) rhabdomeres (nomenclature based upon appearance in transmitted light) which have been shown to contain different pigment systems (Kirschfeld et al. 1978; Kirschfeld 1979). The properties of these cells have already been elucidated electrophysiologically (Hardie et al. 1979; Smola and Meffert 1979). The
newly developed technique of in vivo observation of rhabdomere autofluorescence (Franceschini et al. 1981) allowed rapid observation of large eye regions, and with it the discovery that apart from the 7y and 7p rhabdomeres (which can easily be recognised by their green and non-autofluorescence respectively) there also existed a third, red fluorescent class of central rhabdomere. Furthermore this class of central rhabomere appeared to be restricted to a specific region of the male eye only (Franceschini et al. 1981). In this paper we describe, in detail, the properties of these classes of R7 and R8, not only in terms of electrophysiology, but also in terms of distribution, microspectrophotometry and both axonal projection and ultrastructure. A brief report of some of the properties of the sex-specific R7 cells has already been published (Franceschini et al. 1981).

Materials and Methods

Preliminary observations revealed that males of not only Musca, but also Calliphora and Drosophila all possess red fluorescing central rhabdomeres in their frontal dorsal retina. However, only in Musca is the local representation so high (>90%), furthermore the eye of Musca is more suitable for obtaining a clear image of rhabdomere autofluorescence. For these two reasons all experiments were performed upon laboratory stocks of the housefly Musca domestica. Both wild type and white-eyed mutants fed on standard medium diets were used.

Auto fluorescence Mapping

The distribution maps of the different classes of central rhabdomeres (Fig. 1) were made from white-eyed flies. Although it is possible to see that the situation is qualitatively similar in wildtype flies, the various screening pigments greatly reduce the clarity of the rhabdomere auto fluorescence, thus making mapping impractical. Intact, living flies were mounted at the centre of a precision goniometer (Leitz Universal drehstisch) and the retinae viewed under conditions of optical neutralisation of the cornea (Franceschini and Kirschfeld 1971). This was achieved using a 25× water immersion objective (Leitz 25/0.65) in combination with a Leitz Orthoplan microscope with epillumination fluorescence (using broadband blue excitation, 420-490 nm, from a Xe arc lamp). Maps were constructed from a series of colour slides (Ektachrome 400) taken of neighouring fields of ommatidia with an Orthomat viewing system described above for the maps. Certain cells were selected for histological examination, the eyes were then embedded in either paraffin or araldite and sectioned at 10-15 μm.

Microspectrophotometry

The procedure and equipment for measuring difference spectra (Fig. 2) were essentially the same as described by Kirschfeld et al. (1978). For white-eyed flies it was possible to identify individual red-fluorescing rhabdomeres in the microspectrophotometer by first drawing a map of the distribution of different R7 classes under the fluorescence microscope (see above). Subsequently an eyecup preparation of the same eye (cut at such a level that R8 rhabdomeres were excluded) was mounted in the microspectrophotometer (Leitz MPV 2). By comparing the distribution of 7y rhabdomeres (which could easily be detected in the microspectrophotometer from their dichroism) with the map, individual red-fluorescing rhabdomeres could be identified. In wildtype flies, where a better signal-noise ratio could be obtained (due to the reduction of stray light by screening pigment), R7 cells were sampled at random in the frontal-dorsal part of the male eye, identity as red-fluorescing R7's being assumed from the similarity of the results to those from white-eyed flies.

Transmitted intensities, I, were measured at specific wavelengths λ after adapting the eye with light of either 600 nm or 460 nm and the extinction difference

\[ \Delta E = \log \frac{I(\lambda, \text{ad. 600 nm})}{I(\lambda, \text{ad. 460 nm})} \]

was calculated. Alternatively continuous scans through the whole spectrum were measured after adapting the eye first to 600 nm, and then to 460 nm. \( \Delta E(\lambda) \) was then computed as above. This procedure was also used for measuring the difference spectrum of rhabdomeres R1-6.

Electrophysiology

a) Recording and Dye-Injection. All experiments were performed on white-eyed flies with dye-filled glass microelectrodes having resistances of 400-1,800 megohms when filled with Lucifer yellow CH (Stewart 1978). These were lowered into the retina via a small hole cut in the extreme dorsal cornea. All recordings were made from the retinal side of the basement membrane. Contamination from the electroretinogram was minimised by also placing the indifferent electrode (platinum wire) through a small hole in the extreme ventral part of the same retina. Before recording, the eye was preadapted with red light (Schott, RG 630 filter).

Injection of dye was achieved by passing negative DC current from a constant current source. Identification of stained cells was performed in vivo (Franceschini and Hardie 1980) using the same viewing system described above for the maps. Certain cells were also selected for histological examination, the eyes were then embedded in either paraffin or araldite and sectioned at 10-15 μm.

b) Illumination. Stimulation was essentially as described by Hardie (1979) and Hardie et al. (1979), however a new set-up was used. The two stimulating beams, which were mixed via a pellicle, originated from a 150 W Xenon arc lamp and a 12 V quartz halogen lamp respectively. The tip of the quartz light guide, which delivered the light to the eye subtended 2.4° at the fly’s eye and was mounted on a Cardan arm device. For angular sensitivity measurements the size of the light source was reduced to 0.1° by means of an aperture placed in front of the light guide’s tip. Calibrations were made using an EG and G radiometer/photometer (550-1) with the sensor (a silicon photovoltaic detector) placed at the position of the fly.

Anatomy

In order to study the anatomy of identified types of central rhabdomeres, initially the different classes of central rhabdomeres from a small area in a white-eyed male were mapped under the fluorescence microscope. So that specific rhabdomeres could later be identified at the level of the electron-microscope, an area was chosen with several landmarks: namely – the margin of the eye, the equator (about which the rhabdomere patterns show mirror symmetry), and finally a ‘marker ommatidium’ which contained a ‘mistake’ such as an inverted/rotated pattern or additional/missing rhabdomere(s). The area was also chosen so as to contain all three classes of central rhabdomeres. Subsequently the flies were fixed and embedded in araldite according to standard procedures (see Ribi 1976). For light microscopy 1 μm sections were stained with Toluidine blue. For electron microscopy serial, ultrathin sections were cut at approximately 2 μm intervals.