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PERIPHERAL VISUAL FUNCTION IN THE FLY

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DECLARATION

Parts of Chapter I, namely the wavelength dependence of angular sensitivity functions and some of the theoretical analysis, were performed in collaboration with Professors G.A. Horridge and K. Mimura (Horridge, Mimura and Hardie, 1976). The work described in Chapter IV was performed in collaboration with Dr. S.B. Laughlin, who performed similar experiments in dragonfly retina and lamina (Laughlin and Hardie, In press). The work described in Chapter V has been submitted, essentially unchanged, to the Journal of Comparative Physiology as a joint paper: "Hardie, R.C., Franceschini, N., McIntyre, P.: Electrophysiological analysis of the fly retina II: spectral mechanisms in R7 and R8". Dr. N. Franceschini collaborated with some of the experiments, and in particular, conceived of the 'in vivo recovery' technique for identifying stained cells (Appendix B). The theoretical analysis described in the appendices of Chapter V was performed by Dr. P. McIntyre. Otherwise, I declare all the original work presented in this thesis to be my own.

R. C. HARDIE
The following papers and abstracts have also been published, or accepted for publication during the course of this thesis:


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Receptors and peripheral neurons of the dipteran visual system have been studied in the flies, *Calliphora stygia* (wild type), *Musca domestica* (white-eyed mutant) and *Calliphora erythrocephala* (chalky mutant), principally by means of intracellular recording. Recordings were made from: the large peripheral retinula cells, Rl-6; the small, central, retinula cells, R7 and R8; and the large monopolar cells (LMC's), L1, L2 and L4. Representatives of each cell type were identified by intracellular dye injection with Procion yellow.

The angular sensitivity functions of the receptors are shown to be at, or near the limit set by lens diffraction and rhabdomere diameter. Receptors R7 and R8, whose rhabdomeres are narrower than those of Rl-6, also have narrower angular sensitivity functions.

Many of the basic response properties of photoreceptors are described in detail for the first time. For R7 and R8, spectral sensitivities, polarisation sensitivities, absolute sensitivities, and dark and light-adapted intensity/response functions have been determined. In Rl-6 cells, light-adaptation shifts the peak of spectral sensitivity, and narrows the angular sensitivity function, but had no effect upon the polarisation sensitivity. These new results from identified receptors lead to a reappraisal of receptor involvement in optomotor behaviour.
A new functional class of photoreceptor (Rl-6) is described in which the spectral and polarisation sensitivity are both dependent upon the intensity of the test flash used to measure them. In addition, the polarisation sensitivity is strongly dependent upon the stimulus wavelength. The experimental analysis suggests that these anomalous properties derive from the events occurring in a single cell, and this finding may have important implications with respect to the process of transduction in photoreceptors.

A detailed study is made of the processes of light-adaptation in receptors Rl-6 and their postsynaptic elements, the LMC's. Consecutive stages of light-adaptation are isolated by comparing the performance of receptors and LMC's when subjected to identical regimes of dark- and light-adaptation over a 5 log unit range of intensities, and possible mechanisms of light-adaptation are considered. A comparison with the performance of higher order visual interneurons indicates that the majority of adaptation is completed at the level of the LMC. A comparison of the results with those of similar studies in other arthropods and, also the lower vertebrates reveals striking analogies, which, it is argued, represent an optimal strategy for light-adaptation.

The receptors R7 and R8 are intensively investigated to elucidate their function in terms of the pigments in their rhabdomeres. There are two spectral classes of R7 which appear to have completely different pigments in their
rhabdomeres. The properties of R8 are profoundly influenced by the absorption of light in the overlying rhabdomere of R7.
GENERAL INTRODUCTION

The faculty of seeing is the most striking of all our senses, and it is probably the amazing richness of visual perception, more than any other factor, which has fascinated so many scientists, and attracted them to the task of unravelling the mysteries of visual processing.

The reason why vision is such a striking modality is that, for man at least, this sensory channel can transmit more information than any other sense. In the object world, this information is contained in modulations of intensity as a function of: space, time, wavelength and the E-vector direction of polarised light. The job of the visual system is to process this vast amount of information and, so extract the features of biological importance to the animal concerned, whilst the task of the scientist is to work out how this is achieved.

Because of the complexity of the systems confronting him, the vision researcher may increase the odds in his favour by selecting a preparation (and techniques) most suitable for the particular problem with which he is concerned.

Thus, those interested in the biophysics of transduction have often chosen preparations, such as the barnacle or *Limulus* (review: Meech and Brown, 1976), which have comparatively huge photoreceptor cells that may be simultaneously penetrated with at least two microelectrodes, and may be readily perfused with experimental solutions.
Many of those interested in the operation of the peripheral visual system have recorded from eyecup preparations of lower vertebrates, such as *Necturus* (Werblin, 1973), skate (Green et al., 1975) or tiger salamander (Werblin, 1978). The advantage of these preparations is that all the major cell classes may be relatively easily impaled (including receptor, bipolar, amacrine, horizontal and ganglion cells) thus allowing analysis of function at all levels.

The cortical areas, of cats and monkeys in particular, have been extensively studied by those interested in complex information processing at higher levels (reviews: Henry, 1977; Hubel and Wiesel, 1977). This choice however has been prompted, more by a desire to come as close as possible to the human situation, than for the experimental amenability of the preparation itself.

If one is interested in all aspects of visual processing, and how these combine to produce visually mediated behaviour, then one is tackling a very large problem, that encompasses all aspects of peripheral and central nervous function.

In this case the preparation chosen must be amenable to study at every level in the nervous system, with as many techniques as possible.

In many respects the fly can be considered such a preparation (reviews: Reichardt, 1969; Kirschfeld, 1972; Hausen, 1977). In general the choice of an insect is favourable since there are perhaps $10^3 \times$ less visual
interneurons than in man or the higher vertebrates (Kirschfeld, 1976). This immediately reduces the complexity of the task enormously, but not at the expense of making the study irrelevant to proponents of vertebrate and, in particular, human, visual processing. It is quite clear that insects are capable of many complex visual feats. Thus sophisticated and accurate motion detection abilities are shown by many flying insects including flies (Collett and Land, 1975), pattern recognition (review: Wehner, 1975) and colour vision (v.Helversen, 1972) are well established in bees, and evidence has recently been given for the latter faculty in flies (Menne and Spatz, 1977). Contrast detection and light-adaptation are also common features of both insect and vertebrate visual systems. Where parallel studies have been performed in insects and vertebrates, the basic physiology underlying the visual processes is seen to be remarkably similar (Laughlin, 1976b) - and a particular example of this is discussed in Chapter IV with respect to light-adaptation. Unlike the situation in vertebrates, however, where the visual signal is effectively lost to the physiologist in the visual cortex, and does not reappear until the behavioural output, there is the potential in insects to describe visual behaviour in terms of neural correlates at every level from the photoreceptor to the muscle.
What then, are the special features of the fly that make it one of the most widely used insect visual preparations?

a) Anatomy

Firstly, as in many insects, the anatomical layout of the fly's optic lobes is beautifully ordered. A point to point projection of the visual world is maintained through the lamina, medulla, lobula plate and lobula (Strausfeld, 1976a; 1976b). Extensive anatomical studies using electron microscopy (EM) and Golgi techniques have mapped out the fly retina and optic lobes in great detail (Cajal and Sanchez, 1915; Boschek, 1971; Strausfeld, 1976b) and there is now a firm basis to give an anatomical correlate for practically any neuron in the fly visual system. Many of the cell types, particularly those of the retina and lamina have virtually identical geometry repeated right across the lobe (Strausfeld, 1976a), and thus, with caution, physiological results from only a few cells of one type, can be used to generalise about that cell type in each of the many thousands of retinotopic columns.

b) Electrophysiology

Physiologically the fly's visual system may be sampled at many levels, including the receptors (Burkhardt, 1962); lamina (Zettler and Järvelä, 1971; Arnett, 1972); medulla (Bishop et al., 1968; Mimura, 1971; DeVoe and Ockleford, 1976), lobula plate (Dvorak, et al., 1975; Hausen, 1976) and ventral nerve chord (Hardie, 1977a). At some of these levels it is
possible to maintain stable intracellular recordings, however, it must be confessed, that at present there are still many small neurons (e.g. in the lamina and medulla) that are at, or beyond, the limits of present intracellular recording techniques.

a) Behaviour

A physiological study of a nervous system is incomplete without behavioural correlates. It is, after all, visual behaviour we are trying to explain in terms of the units of the visual system. Fortunately, the fly has a number of behavioural responses that may be studied quantitatively. The most important of these has been the optomotor turning reaction (Fermi and Reichardt, 1963; review: Reichardt, 1969) which has been used to assess the output of the visual system with respect to all the major visual parameters (spatial, spectral, intensity, polarisation etc.). Other behavioural responses that have proved useful, include: phototaxis (with which conditioning experiments may be performed - Menne and Spatz, 1977); optomotor thrust and roll responses (Götz, 1968; Srinivasan, 1977); pattern induced flight orientation (review: Reichardt and Poggio, 1976) and landing behaviour (review: Taddei-Feretti and Talens, 1975). 

d) Optics

The optical qualities of the fly's eye have also proved rather useful in the analysis of visual function in the fly. The fly, has rather an unusual optical system and accompanying anatomical projection, first described by Kirschfeld (1967) and Braitenberg (1967) and 'christened' the neural
superposition eye. In most insects, with apposition eyes, the photoreceptors of a single ommatidium form a common fused rhabdom, and thus all have identical visual axes. In the fly however, each ommatidium contains seven separate rhabdomeres with divergent visual axes. Six of the rhabdomeres are derived from single photoreceptors (R1-6), and these surround a narrower central, tiered rhabdom formed from two photoreceptors (R7 and R8). The projection of the visual axes may be judged from the pattern of the rhabdomere tips which are seen to conform to a strict geometric arrangement that is repeated over all the eye (except that the patterns in the dorsal and ventral halves of the eye are mirror images of each other). The divergence angles of adjacent rhabdomeres' visual axes are more or less identical to the angles between specific adjacent ommatidia. As a result, any point in space is simultaneously monitored by a single rhabdomere in each of seven separate ommatidia. Further, the geometric arrangement of these seven ommatidia is the same as that of the rhabdomere tips in one ommatidium.

The axons of rhabdomeres having the same field of view leave their individual ommatidia beneath the basement membrane and come together in the same cartridge in the lamina. Six of these (from receptors R1-6) synapse on the same second order cells (the large monopolar cells (LMC's), L1 and L2) in the lamina (Braitenberg, 1967), whilst the remaining two, which derive from the common, tiered central rhabdom R7/8, pass straight through to the second optic neuropile (the medulla).
This projection, initially worked out optically (Kirschfeld, 1967) and anatomically (Braitenberg, 1967), has been confirmed electrophysiologically by Scholes (1969) who recorded depolarising responses in the lamina, whilst stimulating single facets, and found that the facets contributing to the response conformed to the predicted geometric pattern.

The characteristic pattern of rhabdomere tips can also be seen in the phenomenon of the deep-pseudopupil (Franceschini and Kirschfeld, 1971b). By focussing a low power objective at the level of the centre of curvature of the eye the images of rhabdomeres in a number of adjacent ommatidia are superimposed, and an intensified and magnified image of the pattern of rhabdomere endings within a single ommatidium is obtained.

The unique optical qualities of the fly's eye have had several applications in the study of the fly's visual system. 1. Individual, identified rhabdomeres may be stimulated by illuminating the eye via a system of apertures. One aperture is imaged at the centre of the eye, and is placed so as to restrict stimulation to one rhabdomere type of the deep-pseudopupil pattern. Another aperture is then imaged at the cornea and restricts stimulation to one facet. The result is that only one, identified rhabdomere in a single ommatidium is stimulated. This technique has been used to elicit behavioural optomotor responses to sequential stimulation of only two rhabdomeres in the same ommatidium (Kirschfeld, 1972; Kirschfeld and Lutz, 1974), and also to demonstrate that
the activation of the longitudinal pupil mechanism (Kirschfeld and Franceschini, 1969) is a function of individual retinula cells (Franceschini, 1972).

2. Because of the rigid geometrical demands of a neural superposition eye, various optical parameters, such as receptor spacing and focal lengths must be related by simple formulae (Stavenga, 1975). This enables focal lengths, for example, to be calculated without reference to thick lens formulae.

3. In general, because the optics have been so well studied, the fly's eye is an ideal preparation in which to analyse questions about the optimisation of optical design (Snyder et al., 1977) and the physical basis of resolution (Kuiper, 1966; Horridge et al., 1976; and see Chapter I).

4. Microspectrophotometry (MSP) of the visual pigments can be more readily applied in fly than in insects with fused rhabdons, since in flies, the open rhabdomere situation allows one to readily examine single cells in isolation (Langer and Thorell, 1966; Kirschfeld et al., 1977). This analysis can even be performed, in vivo, using the phenomenon of the deep-pseudopupil, on the assumption that cells of the same anatomical class have similar pigments (Stavenga et al., 1973; Stavenga, 1976). In Chapter V, measurements of the spectral properties of cells R7 and R8 are considered in light of recent MSP data (Kirschfeld et al., 1978) and theories suggested for the origins of the cells' spectral properties.
5. Under conditions of optical neutralization of the cornea (which may be achieved by use of a water immersion objective) individual rhabdomere tips may be recognised by focussing down through the lenses which now act effectively as clear windows (Franceschini and Kirschfeld, 1971a). This technique has found a special application in the 'in vivo recovery' of dye injected retinula cells (see Appendix B and Chapter V).

e) Genetics

A further bonus has come from a novel source, namely genetics. As well as being an important visual preparation, dipterans (Drosophila in particular) are also widely used in genetics, and a most fruitful merger between the two fields has occurred with the exploitation of visually deficient Drosophila mutants. These include transduction mutants (Pak et al., 1976; Minke, 1977), and behavioural mutants (Heisenberg and Götz, 1975), but to date, the most useful have been those lacking specific receptor types (Benzer, 1967). These have been exploited to investigate the relative roles of receptor types in behaviour (Heisenberg and Buchner, 1977; Hu and Stark, 1977) and also to analyse the spectral properties of the different cell classes (Harris et al., 1976). As discussed in Chapter V, however, the latter approach necessarily misses some important aspects of spectral mechanisms in the central retinula cells, R7 and R8. In general, though, since the genetic lesion acts at a level that is inaccessible to the surgeon's knife it has a unique potential in the analysis of visual function.
The problem of analysing a nervous system may be broken down to the problem of analysing the transfer characteristics at successive stages in the system. To analyse the transfer characteristics at any one level, one measures the output (a unit's response) whilst controlling the input, i.e. the activity of presynaptic elements, or, in the case of receptors, the physical stimulus. For higher order interneurons there must be considerable uncertainty as to the activity of the input, and for precise information one must work from the periphery inwards. Thus at the receptor level, the input (physical stimulus) may be accurately controlled to the limits of calibration, and shot noise, and the output may be accurately analysed from the amplitudes of the graded potentials. Having determined how the visual stimulus is transferred through the receptor's spectral and polarisation sensitivities, intensity/response function and spatial and temporal frequency responses, one can then address oneself to the problems of analysing transfer at successive synaptic levels. In general terms a firm knowledge of receptor properties must be considered a basic requisite for interpreting data from other levels in the visual system. The change from the language of the physical world to that of the receptors represents one of the most drastic of all the transfer functions, and information retained by the receptors represents all the information available to the whole visual system.

Although the receptors in the fly had been studied by numerous authors over almost 20 years (Kuwabara and Naka, 1959; Burkhardt, 1962; Scholes, 1969; Järvi-lehto and Zettler, 1973) several basic questions had still not been answered, and
therefore, the receptors in particular, and also their immediate postsynaptic elements, the large monopolar cells, were made the subject of this study.

Two basic sorts of question may be asked at this level, and both have been considered in the present study. The first concerns the fundamental basis of a unit's properties in terms, e.g. of pigments, optics, transduction, membrane events and synaptic phenomena. The second concerns the roles of the units studied in the visual system as a whole.

Because of the detailed information available about optics (Kirschfeld, 1972; Stavenga, 1975) and photopigments (Hamdorf et al., 1973; Kirschfeld et al., 1977; 1978), the fly is an excellent preparation in which to determine the influence of these factors upon the receptor's properties. Two particular questions, the origin of the angular sensitivity function, and the origin of the spectral properties of R7 and R8 are considered in Chapters I and V respectively.

In general, the photoreceptors of the fly cannot be considered a good preparation for analysing transduction and membrane phenomena in comparison for example, to Limulus and the barnacle. However, the discovery of a class of receptors with some unusual properties, that might reflect some features of the transduction mechanism, prompted a detailed analysis of their properties, and this is described in Chapter III.
The second class of question (the *roles* of receptors and interneurons in vision), however, provided the initial motivation for this study. One specific question considered was: what are the relative roles of the different receptors in visual behaviour? At the start of this thesis, information on this appeared contradictory. The studies of Eckert (1971) and McCann and Arnett (1972) led to the conclusion that receptors R7 and R8 dominated optomotor behaviour at high light intensities, when patterns of high spatial frequency were used to stimulate the animal. Their results suggested that the receptors R7 and R8 had a single blue-peaked spectral sensitivity, and a degree of polarisation sensitivity. In support of this, the spectral sensitivity correlated quite well with the absorption spectrum measured in the rhabdomere of R7/8 by Langer and Thorell (1966), however, direct data on R7 and R8's properties, from a few intracellular recordings, suggested there was no polarisation sensitivity, and that R7, at least, was an ultraviolet receptor (Järvi-lehto and Moring, 1974; Smola and Meffert, 1975). Other data, from *Drosophila*, *e.r.g*s, did, however, indicate that R8 was a blue receptor, (Harris *et al.*, 1976) and thus perhaps the behavioural studies were right after all. Obviously what was needed, before detailed speculation could be made about receptor involvement in behaviour, was a detailed study of the properties of the receptors themselves, recorded directly using intracellular micro-pipettes. Such a study is described in Chapter II. Some of the conclusions reached in that study were also reached, quite independently, from a behavioural study in *Drosophila* mutants (Heisenberg and Buchner, 1977).
The second major question about the role of specific elements in behaviour, concerned the limiting roles played by LMC's and photoreceptors in light-adaptation. This was investigated by examining the responses of both cell classes to identical regimes of dark and light-adaptation, thus isolating consecutive stages of adaptation, and then comparing these to behavioural or higher order correlates. This study also highlighted some questions regarding the mechanisms and the functional significance of light-adaptation, and all these topics are considered together in Chapter IV.

To gain the maximum useful information from the sort of electrophysiological analysis presented in this thesis, it is essential to be able to identify one's recordings with an anatomical cell type. Ideally every recording should be followed by intracellular dye injection and subsequent histological recovery. However, in achieving this ideal one severely restricts the number of cells from which data can be collected, and therefore a compromise was reached. In this study, examples of every physiological class encountered were stained with fluorescent dyes on several occasions (see Appendix B). Because of the consistency of the results, and the lack of overlap between physiological classes, it was subsequently decided that physiological criteria alone, were sufficient to attribute anatomical identity to any recording.
CHAPTER I

ANGULAR SENSITIVITY OF PHOTORECEPTORS
1. Angular sensitivity functions of individual retinula cells were determined from intracellular recordings of photoreceptors identified as R1-6, R7 and R8 in the flies Calliphora stygia (wild type) and Musca domestica (white).

2. Values for $\Delta \rho$ (the width of the angular sensitivity function at the 50% level) are given: a) for receptors in different eye regions (Fig. 1.4); b) for receptors with different rhabdomere diameters (Table 1.4); and c) in the same cell, using different wavelengths of monochromatic light (Fig. 1.6).

3. The results are compared with the predictions of a simple theory (Kuiper, 1966; Horridge et al., 1976; Snyder, 1977) which assumes that the angular sensitivity function may be generated by convolving a function representing the Airy disc with the rhabdomere acceptance function. Two approximations for the rhabdomere acceptance function were tested, a circular step function (top hat profile) and a Gaussian function (after Snyder, 1977).

4. The comparison reveals, that in general terms, fly photoreceptors have $\Delta \rho$ values at, or about the lower limit set by the combined physical constraints of diffraction and rhabdomere diameter (Table 1.6). The circular step function seems the most reasonable approximation for the rhabdomere acceptance function, at least in cells R1-6.
5. Whilst the influence of rhabdomere diameter in determining $\Delta \rho$ was clearly established, the influence of the Airy disc could not be detected, and therefore the exact physical basis of image formation in compound eyes is still open to question.

6. Angular sensitivities were also determined under conditions of light-adaptation (Fig. 1.7 and Table 1.5). A consistent reduction of $\Delta \rho$, compared to that measured in the dark, was observed, and possible mechanisms for this phenomenon are discussed.
INTRODUCTION

Without spatial information, visual perception would be reduced to an overall impression of ambient intensity and colour. A primary function of the visual system, therefore, must be to extract as much information as possible from the spatial contrasts in the real world. Therefore, a description of the performance of the eye in terms of resolution and acuity is an essential part of a description of the whole visual system. In this respect the performance of the receptors must be considered limiting, as any information that is lost between the real world and the receptor's response cannot be regained at a later stage.

It has long been recognised that two factors are important in determining the ability of an eye to detect high spatial frequencies (e.g. Barlow, 1952; Snyder et al., 1977). Firstly there is the inter-receptor spacing, or (in compound eyes) the interommatidial angle. Thus for two points to be distinguished as such there must be a receptor to detect each point, and one in between to monitor their separation. For a hexagonal lattice, simple geometry tells us that the finest pattern that may be sampled by the receptor array has a period of $\sqrt{3}\Delta \phi$, (where $\Delta \phi$ is the interommatidial angle) (Snyder, 1977). $\Delta \phi$ has been measured in flies previously, (Burkardt et al., 1966; Beersma et al., 1975) and is not considered further here. The second factor, a description of which is the subject of this chapter, is the angular sensitivity function. This factor, which may be characterised by the acceptance angle $\Delta \rho$ (the width of the angular
sensitivity function at half height), determines the ability of the receptors to resolve contrasts. In practice, virtually no contrast can be resolved from a pattern that has a period of less than $\Delta \rho$ (e.g. Snyder, 1977).

The angular sensitivity of fly photoreceptors has also been studied by several authors previously (e.g. Washizu et al., 1964; Scholes, 1969; Streck, 1972; Horridge et al., 1976) however, an accurate description of this parameter, and how it varies with eye region and the state of adaptation is still not available. Also, some of the early data on this topic may be inaccurate owing to damage caused to the optics by the, sometimes, gross surgery employed. The only study comparing angular sensitivity in different regions of the fly's eye (Washizu et al., 1964) sampled only two general regions. The only previous study investigating the effect of light-adaptation (Vowles, 1966) described receptive fields of Musca photoreceptors in terms of responses only (sensitivity was not calculated), and the wide fields and small responses reported, in the light of more recent data (e.g. Scholes, 1969), are indicative of a damaged preparation. A definitive comparison of angular sensitivities in the two receptor subclasses (R1-6 and R7/8) has not previously been presented from identified photoreceptors. One aim of this chapter, therefore, has been to provide reliable data on angular sensitivity in different receptors, in different eye regions, and in different states of adaptation, since this information is essential for the interpretation of spatial transfer characteristics at other levels in the visual system. The present results are discussed in this context in the next
chapter. Secondly, there are several theoretical accounts for the origin of the angular sensitivity function (e.g. Kuiper, 1966; Pask and Snyder, 1975; Horridge et al., 1976; Snyder, 1977; In press), and in this chapter, the applicability of such theories is tested by making a comparison between theoretical and experimentally derived acceptance angles.
METHODS

The majority of experiments were performed upon Calliphora stygia (wild type), but a few experiments were also performed upon pupilless white-eyed mutants of Musca domestica (white). Females, only were used. General details of the preparation recording and stimulating apparatus are found in Appendix A, but the following points are of particular relevance to the present series of experiments.

All angular sensitivity measurements were made using a small source subtending 0.4° at the fly's eye. For light-adapted determinations, a second, adapting beam was delivered through a separate light-guide (Dual beam method, Appendix A). This light guide was also mounted on the Cardan arm, but light from it was back-reflected (by means of a front surface mirror) onto a diffuse reflecting screen (20° in visual angle) which had the test spot situated at its centre. During a light-adapted angular sensitivity determination, both centre and surround were moved, however the movement required for the determination was only ca. 3-5°, and this was found to cause virtually no modulation in response to the surround (Fig. 1.7). Sensitivities were calculated from the peak response amplitudes, by referring them to the appropriate dark or light-adapted V/log I curve determined in the same cell. For horizontal angular sensitivity determinations, angles were corrected for the vertical elevation (Burkhardt and Streck, 1965).
The history of angular sensitivity measurements indicates that great care must be taken in preparing the animal to avoid spurious results caused by damage to the optics. To ensure the best results, surgery was minimal, consisting of a small hole (less than 5 facets in diameter) carefully cut in the dorsal cornea. The optics were checked after each experiment by examining the deep pseudopupil pattern (Franceschini and Kirschfeld, 1971b) which was normal in appearance except in the immediate vicinity of the hole. In addition, measurements were only made from cells some distance (at least 10 ommatidia) from the hole.

The criteria used for identification of different cell classes are explained in Appendix B.
The angular sensitivity function describes the amount of light effectively absorbed by a photoreceptor as a function of the angular position of a point source. Theories accounting for the generation of this function have been considered by several authors including Kuiper (1966) Horridge et al., (1976) and Snyder (1977), and are reviewed briefly here.

The basic concept underlying these theories is that the point source generates an image at the level of the rhabdomere tips, and that this sweeps across the rhabdomere's cross section as the point source is moved (Fig. 1.1). The light absorbed by the rhabdomere is then predicted by convolving a function describing the intensity distribution in the image of the point source, with the rhabdomere acceptance function, which describes the proportion of incident light captured by the rhabdomere as a function of position on its cross-section. Intuitively, this sort of theory is applicable to single lens eyes, where all the receptors must be served by the same lens, but in many insects, with fused rhabdom, apposition eyes (e.g. locusts and mantids), it is possible that the dioptrics act as a light funnel, and it may be incorrect to think of an image moving across the rhabdomere tips. In the case of the fly, though, because the rhabdomeres are separate, and because of the neural superposition design (Kirschfeld, 1967), one can in fact be confident that an image of a point source can physically move across the rhabdomere tips.
Figure 1.1 The origin of the angular sensitivity function. Rays are focused through a lens of diameter, D, upon the end of a rhabdomere (R) of diameter, d. As the distant point source is moved in front of the lens, the Airy disc sweeps across the rhabdomere tip, thus generating the angular sensitivity curve. Because the focal length, f, is measured from the posterior nodal point, (PNP) the refractive index of the medium does not enter into the calculation. (Figure reproduced from Horridge et al., 1976)
Figure 1.2. A series of theoretical angular sensitivity functions generated by convolving a circular step function with a Gaussian approximation for the Airy disc. Each curve is that predicted for an Rl-6 cell in frontal eye regions of Calliphora as the wavelength increases from 330 nm to 550 nm. $\Delta \rho$ values are indicated alongside each curve. Since the curves are symmetrical only half of each angular sensitivity function has been plotted. Numerical assumptions used for computing the curves are those found in Table 1.2.
In a diffraction limited lens system, a point source generates a blurred image known as the Airy disc. The intensity distribution across the disc can be approximated very well by a Gaussian function with angular width\(^1\), at half height, of:

\[
\Delta \alpha = \frac{\lambda}{D} \text{ radians} \quad \ldots \quad (1.1)
\]

where \(\lambda\) = the wavelength of light \textit{in vacuo}, and \(D\) = diameter of the lens aperture (i.e., the facet diameter in the present situation).

If the rhabdomere accepts all the light that impinges upon its cross section, then the rhabdomere's acceptance function can be considered as a simple step function, which shall be assumed to be circular in section ('top hat' profile) and of width equal to the rhabdomere diameter. Unfortunately, the convolution of a Gaussian and such a circular function has no analytical solution, and to generate theoretical angular sensitivity functions on this model, reference was made to tables of the "offset circle probabilities for the circular normal distribution" (also known as the circular coverage function) (Owen, 1962). Theoretical functions were plotted for all the experimental situations, and \(\Delta \rho\) values measured from these plots, some examples of which are shown in Figure 1.2.

---

\(^1\) All angles inside the eye (such as \(\Delta \alpha\)) are measured from the posterior nodal point as then these are comparable with external angles (such as \(\Delta \rho\)).
Snyder (1977) suggests a simpler alternative to this somewhat tedious method of generating $\Delta \rho$ values. The rhabdomere acceptance function is also assumed to be Gaussian, with a width at half height equal to the rhabdomere diameter. This approximation was chosen for its convenience, since the convolution of two Gaussian functions has a simple analytical solution, and in terms of angular widths at half height,

$$\Delta \rho^2 = (d/f)^2 + \Delta \alpha^2 \quad \ldots \ldots \quad (1.2)$$

where $d =$ the rhabdomere diameter, $f =$ the focal length (from the posterior nodal point) - and therefore $d/f$ represents the angular width of the rhabdomere in radians. For very narrow rhabdomeres, where waveguide effects become important a derivative of $d$ should be used. This is given by Snyder (In press) as:

$$d' = 0.47 \ d \ \text{exp} \ 1/V \quad \ldots \ldots \quad (1.3)$$

where $V =$ the waveguide parameter, which is inversely proportional to wavelength. According to Snyder (In press) this derivative should be used when $V < 2.0$, which is probably the case for rhabdomeres R7/8 at wavelengths longer than 500nm (Kirschfeld and Snyder, 1975).

---

1 The waveguide parameter is given by:

$$V = \frac{2 \ \pi \ d \ \sqrt{n_1^2 - n_2^2}}{\lambda} \quad \ldots \ldots \quad (1.4)$$

where $n_1$ and $n_2$ are the refractive indices of the rhabdomere and its surrounding, respectively.
To generate theoretical angular sensitivities with either of the above formulations a knowledge of the following parameters is required:

- \( f \) ... the focal length
- \( d \) ... the rhabdomere diameter
- \( D \) ... the facet diameter
- \( V \) ... the waveguide parameter.

With the exception of \( d \) and \( V \) in *Calliphora*, values of all these parameters, in both *Musca* and *Calliphora*, have been measured or estimated previously, and the numerical assumptions used in the calculation of \( \Delta \rho \) values are listed in Table 1.2, along with the source of the data. Rhabdomere diameter (\( d \)) in *Calliphora stygia* was measured directly from cross sections of eyes. Since the rhabdomeres taper along their length, these measurements were made at the distal ends of the rhabdomeres, just below the rhabdomere caps - as indicated in Figure 1.3. Estimates of diameters from both light and electronmicroscopy (EM) are listed in Table 1.1. \( V \), in *Calliphora* was assumed to be the same as in *Musca*.

In the results that follow \( \Delta \rho \) values of R1-6 cells in different eye regions and using different wavelengths are described, and a comparison made between \( \Delta \rho \) values of R1-6 and R7/8 in frontal eye regions. As a test of the usefulness and/or validity of the simple theory described, these experimental results are compared with theoretical values generated by both the above formulations. Finally an account of the effect of light-adaptation upon angular sensitivity is presented.
Figure 1.3  Cross-sections of the ommatidia in *Calliphora stygia*, at the level of the rhabdomere tips. Sections are 1µm thick, stained in toluidine blue, and fixed and embedded as for electron-microscopy. Rhabdomere diameters were determined at the level indicated (arrow).
Diameters of rhabdomere tips in *Calliphora stygia*. Fixation and embedding was by standard EM techniques; each value is the average based on 12 measurements, values in μm.

**BY ELECTRON MICROSCOPY:**

<table>
<thead>
<tr>
<th>Rhabdomere</th>
<th>Major Axis</th>
<th>Minor Axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1-6</td>
<td>1.93</td>
<td>1.71</td>
</tr>
<tr>
<td>R7</td>
<td>0.9 × 0.7</td>
<td></td>
</tr>
</tbody>
</table>

**BY LIGHT MICROSCOPY:**

<table>
<thead>
<tr>
<th>Rhabdomere</th>
<th>Major Axis</th>
<th>Minor Axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1-6</td>
<td>1.98</td>
<td>1.59</td>
</tr>
<tr>
<td>R7</td>
<td>1.2 × 1.0</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 1.2
Optical parameters assumed for theoretical derivations of angular sensitivity functions.

<table>
<thead>
<tr>
<th>Source</th>
<th>CALLIPHORA</th>
<th></th>
<th></th>
<th>MUSCA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rl-6 (frontal)</td>
<td>Rl-6 (lateral)</td>
<td>R7/8 (frontal)</td>
<td>Rl-6 (frontal)</td>
<td>Rl-6 (lateral)</td>
<td>R7/8 (frontal)</td>
</tr>
<tr>
<td>D (µm)</td>
<td>24</td>
<td>20</td>
<td>24</td>
<td>22</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>d (µm)</td>
<td>1.9</td>
<td>1.9</td>
<td>0.9</td>
<td>2.0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>f (µm)</td>
<td>70</td>
<td>40</td>
<td>70</td>
<td>55</td>
<td>35</td>
<td>55</td>
</tr>
<tr>
<td>V (at 500 nm)</td>
<td>2.6</td>
<td>2.6</td>
<td>1.6</td>
<td>2.6</td>
<td>2.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Sources:
- D, effective facet diameter, (Calliphora), from measurements by Ribi (unpublished)
- D (Musca), from Stavenga (1975)\(^1\)
- f, focal length (Calliphora), from Seitz (1968)
- f (Musca), from Stavenga (1975)\(^1\)
- d, rhabdomere diameter (Calliphora), the present study (Table 1.1)
- d (Musca), from Boschek (1971)
- V, the waveguide parameter determined in Musca (Kirschfeld and Snyder, 1975) and assumed to be similar in Calliphora.

\(^1\)These measurements are for male specimens, however values in female specimens of Musca are not very different (McIntyre, personal communication).
RESULTS

1. Angular sensitivity as a function of eye region

For this survey, recordings were all made during a one week period, using cultured flies of the same generation, so as to minimise any sources of variation related to age or genetic background. Different eye regions were sampled by simply varying the direction and starting point of the electrode track. For the greatest convenience, eye region is defined by the coordinates of each cell's visual axis, which may be read off from the coordinates on the Cardan arm, when the point source is accurately centred on the cell's visual axis. Coordinates are given relative to the longitudinal axis of the fly's head (anterior-posterior axis). Measurements were made by recording the peak responses to 100 ms flashes of monochromatic light of 541 nm, as the point source was moved through the centre of the cell's visual field in $1/4^\circ$ or $1/2^\circ$ steps.

A total of 45 cells of good stability, deep resting potential, high maximum response ($V_{\text{max}} > 50 \text{ mV}$) and similar high absolute sensitivity, were sampled during this period. In each cell, 2 - 4 repeated determinations of angular sensitivity were made in the horizontal plane. All cells included in the sample represented cells R1-6, as judged by their spectral sensitivity and relatively noise free responses (see Appendix B).

The majority of cells (33) had the vertical coordinate of their visual axes within $\pm 20^\circ$ of the horizontal, and in Figure 1.4 the acceptance angle, $\Delta \rho$, of this sample is
Figure 1.4  A plot of $\Delta \rho$ values in *Calliphora* against eye position. Each point represents the $\Delta \rho$ of one cell (Rl-6) averaged from 2-4 repeated determinations. Eye position ($H^0$) is expressed in terms of the horizontal coordinate of the cells visual axis relative to the anterior-posterior axis of the fly. For the sample shown here (33 cells), the vertical coordinates of the cells' visual fields all fell within $\pm 20^\circ$ of the horizontal plane. The stars represent the theoretical $\Delta \rho$ values calculated for frontal and lateral eye regions using the circular coverage model.
Δρ values not included in Figure 1.4 because the vertical visual axes of the cells did not fall within ± 20° of the horizontal. Each Δρ value is the average of 2-4 determinations. The coordinates express the position of each cell's visual axis with respect to the longitudinal (anterior - posterior) axis of the fly's head. H° = degrees left of the longitudinal axis; V° = degrees above or below (±) the axis. Cells are listed in a series: starting with the most dorsal unit and progressing to the most ventral unit.

<table>
<thead>
<tr>
<th>Δρ</th>
<th>H°</th>
<th>V°</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.36</td>
<td>110</td>
<td>+30</td>
</tr>
<tr>
<td>2.36</td>
<td>102</td>
<td>+25</td>
</tr>
<tr>
<td>1.23</td>
<td>15</td>
<td>+24</td>
</tr>
<tr>
<td>1.69</td>
<td>18</td>
<td>+23</td>
</tr>
<tr>
<td>2.09</td>
<td>35</td>
<td>+23</td>
</tr>
<tr>
<td>2.20</td>
<td>35</td>
<td>-28</td>
</tr>
<tr>
<td>2.49</td>
<td>34</td>
<td>-28</td>
</tr>
<tr>
<td>2.33</td>
<td>28</td>
<td>-38</td>
</tr>
<tr>
<td>2.28</td>
<td>20</td>
<td>-58</td>
</tr>
<tr>
<td>2.64</td>
<td>20</td>
<td>-60</td>
</tr>
<tr>
<td>3.31</td>
<td>10</td>
<td>-68</td>
</tr>
<tr>
<td>2.46</td>
<td>18</td>
<td>-73</td>
</tr>
</tbody>
</table>
plotted as a function of the cells' horizontal coordinate, revealing a consistent increase in $\Delta \rho$ values from frontal, through to lateral eye regions. The remaining results are listed in Table 1.3.

2. Angular sensitivity as a function of rhabdomere diameter.

Each ommatidium in the fly contains 6 peripheral photoreceptors (R1-6) with rhabdomeres of approximately similar size, and two central photoreceptors (R7 and R8) which share a common tiered rhabdom with a significantly smaller diameter (Fig. 1.3). By recording from both R7 (or R8) and an R1-6 cell in the same (or nearby) ommatidium, it was hoped to reveal directly the effect of rhabdomere diameter upon the acceptance angle.

Since R7 and R8 may only be recorded rarely, this comparison was initiated after first penetrating either an R7 or R8 (criteria for identification are given in Appendix B). After first determining the angular sensitivity in one of the central retinula cells (using monochromatic light of wavelength 358 nm for an R7, or 541 nm for an R8 cell), the electrode was carefully manoeuvred in an attempt to penetrate an R1-6 cell in the same ommatidium. The angular sensitivity of the R1-6 cell was then determined, with the same wavelength, for a direct comparison (Fig. 1.5). A knowledge of the juxtaposition of the receptors in the region recorded from is useful in this context. In the frontal eye regions recorded from in these experiments, in the ventral sector (below the equator) R7 is the first cell of an ommatidium to be encountered (Kirschfeld, 1967), and simply advancing the electrode by up to 10\mu m should
Figure 1.5  Original records from Calliphora showing angular sensitivity runs: first in an R7 cell, and then in an Rl-6 cell in a nearby ommatidium. Each response is to a 100 ms flash from a 0.4° point source as it is moved through the centre of the cell's visual field in 1/2° steps. Following the angular run in the Rl-6 cell, an intensity/response function was determined with 100 ms flashes incremented in 0.25 log unit steps.
result in a good chance of penetrating another cell (Rl-6) in the same ommatidium. Above the equator, however, (dorsal sector), R7 is the last cell to be encountered in an ommatidium, and now one should withdraw the electrode by ca.15\(\mu\)m and then advance again in order to penetrate an Rl-6 cell in the same ommatidium. In actual fact, in only 4 cases was it possible to be confident that an Rl-6 cell had been penetrated in the same ommatidium, and in the other cases recordings were made from Rl-6 cells up to 6 ommatidia distant. However, since the optical parameters of ommatidia change little over such a short distance, (e.g. Stavenga, 1975), the comparison of \(\Delta\rho\) values is still believed to reflect the effect of rhabdomere diameter. The 'in vivo recovery' technique for identification of intracellularly stained photoreceptors (Appendix. B) was employed to demonstrate directly that it is indeed possible to judge the proximity of sequentially recorded cells, purely by reference to the relative depths of the penetrations (monitored by a micrometer gauge on the micromanipulator).

\(\Delta\rho\) values in R7/8 and nearby Rl-6 cells, along with the estimated proximity of the cells compared, are shown in Table 1.4. With one exception the \(\Delta\rho\) value of the central retinula cell is significantly smaller than that of the Rl-6 cell, as predicted by Kirschfeld or Franceschini (1968). Since all the cells were in the same general eye region (visual axes \(\pm 30^\circ\), both vertically and horizontally of the longitudinal axis), where Figure 1.4 reveals that there is little variation of \(\Delta\rho\) (at least in Rl-6), the results may be usefully averaged
to give what are considered to be reliable estimates of $\Delta \rho$ for both R1-6 and R7/8 in frontal eye regions.

So far, all the data presented have been derived from measurements in *Calliphora stygia*. Whilst few angular measurements were made in other species, it is worth reporting here the few values determined in *Musca domestica* (white). From 4 R1-6 cells in frontal regions, the average $\Delta \rho$ value was $2.3^\circ$ (cf. $2.5^\circ$ measured by Scholes (1969)); and from 3 R7 cells in a similar region the average $\Delta \rho$ value was $1.6^\circ$.

3. Angular sensitivity as a function of wavelength

According to Eq. (1.1), the size of the Airy disc can be varied, whilst recording from the same cell, simply by the use of different wavelengths. With the appropriate numerical assumptions (Table 1.2) and assuming a circular step function for the rhabdomere acceptance function, the theory predicts that $\Delta \rho$ should in fact be relatively insensitive to variations in the Airy disc diameter over the range of wavelengths visible to the fly (Fig. 1.2). To test this prediction, the angular sensitivities of 10 R1-6 cells were measured at different wavelengths. All cells were from the frontal region of the eye, and measurements were made routinely in the green (523 or 541 nm) and the ultraviolet (333 nm). In one cell, the angular sensitivity was determined at 11 different wavelengths. The results are plotted in Figure 1.6, compared with the predictions from the theory described. As predicted there is very little indication of any wavelength dependence of $\Delta \rho$ (see also, Horridge et al., 1976).
TABLE 1.4

Angular sensitivities of a) R7 and b) R8 compared to R1-6 in Calliphora. $\Delta \rho$ values are the average of two or more determinations. In each case the R7/8 axis is given relative to the anterior-posterior axis of the fly. $^0_L \pm ^0 = \text{degrees left of the anterior-posterior axis}$, and $^0_+ \text{ or } ^0_- \text{ = degrees above or below the anterior-posterior axis.}$ Proximity indicates how many ommatidia distant was the R1-6 with which the $\Delta \rho$ value is compared.

a)

<table>
<thead>
<tr>
<th>$\Delta \rho \text{ R7}$</th>
<th>Axes</th>
<th>$\Delta \rho \text{ R1-6}$</th>
<th>Proximity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.94°</td>
<td>6°$L$,0°</td>
<td>1.22°</td>
<td>3 omma.</td>
</tr>
<tr>
<td>1.14°</td>
<td>12°$L$,8° +</td>
<td>1.57°</td>
<td>2 omma.</td>
</tr>
<tr>
<td>1.60°</td>
<td>25°$L$,8° +</td>
<td>1.70°</td>
<td>ca. 5 omma.</td>
</tr>
<tr>
<td>1.60°</td>
<td>0°$L$,30° -</td>
<td>1.85°</td>
<td>same omma.</td>
</tr>
<tr>
<td>1.03°</td>
<td>20°$L$,5° +</td>
<td>1.56°</td>
<td>ca. 6 omma.</td>
</tr>
<tr>
<td>1.04°</td>
<td>10°$L$,3° +</td>
<td>1.68°</td>
<td>2 omma.</td>
</tr>
<tr>
<td>1.32°</td>
<td>20°$L$,10° +</td>
<td>1.57°</td>
<td>same omma.</td>
</tr>
</tbody>
</table>

Mean $\Delta \rho = 1.26° \pm 0.59° (1.0 \text{ S.D.})$ $\quad 1.61° \pm 0.13° (1.0 \text{ S.D.})$

b)

<table>
<thead>
<tr>
<th>$\Delta \rho \text{ R8}$</th>
<th>Axes</th>
<th>$\Delta \rho \text{ R1-6}$</th>
<th>Proximity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.32°</td>
<td>26°$L$,7°</td>
<td>1.20°</td>
<td>same omma.</td>
</tr>
<tr>
<td>1.11°</td>
<td>22°$L$,0°</td>
<td>1.88°</td>
<td>same omma.</td>
</tr>
<tr>
<td>1.15°</td>
<td>13°$L$,6° +</td>
<td>1.4°</td>
<td>ca. 5 omma.</td>
</tr>
</tbody>
</table>

Mean $\Delta \rho = 1.19°$ $\quad 1.46°$
Figure 1.6  Angular sensitivity as a function of wavelength in *Calliphora*. $\Delta \rho$ values come from 10 cells in frontal eye regions. ○, Measurements in vertical plane. ●, Measurements in horizontal plane. ▲, Measurements from one cell in which angular sensitivity was determined at 11 wavelengths (in the vertical plane). Line A (broken) is the theoretical prediction assuming a Gaussian approximation for the rhabdomere acceptance function. Line B (solid) is the theoretical prediction assuming a circular step function for the rhabdomere acceptance function.
4. Spectral sensitivity

The comparison between $\Delta \rho$ values in Rl-6 with those in R7/8, demonstrates that the rhabdomere diameter is an important factor in determining the angular sensitivity function. Whilst the lack of wavelength dependence, just described, is consistent with there being an Airy disc formed at the level of the rhabdomere tips, such a negative result cannot be considered proof of this. What was hoped to be a more sensitive test for the influence of the Airy disc was thus devised. The amount of light captured by a rhabdomere from an axial point source is predicted to depend upon the relationship between the rhabdomere and Airy disc diameters. Thus if the Airy disc falls completely within the rhabdomere's cross section, all the light may be captured, but if the Airy disc is larger, then some of the light will be lost. The predicted effect is shown in Figure 1.2 by the absolute heights of the angular sensitivity functions at $0^\circ$. The effect should be to enhance sensitivity to shorter wavelengths. However, no such effect should be observed when measuring sensitivity with an extended source. To test this predicted effect of the Airy disc diameter, spectral sensitivity, determined with a point source ($0.1^\circ$), was compared to that measured using an extended source ($3^\circ$). Following these tests, the angular sensitivity was determined to ensure that it was as narrow as expected for the eye region in question. Two cells were tested in this way. Both were located in frontal eye regions, and had $\Delta \rho$ values of $1.6^\circ$ and $1.7^\circ$ respectively. Repeated determinations, however, failed to show any significant differences between spectral sensitivities
measured with either the point or the extended source.

5. Angular sensitivity as a function of adaptation

The data so far presented has come from dark-adapted animals. Whilst this is the very information required for a comparison with theoretical predictions of angular sensitivity (which make no assumptions regarding adaptation), a complete account of the angular properties of photoreceptors should include a description of light-adapted angular sensitivity, as this information may be of value in interpreting results from studies of behavioural acuity, which are often made in a light-adapted situation.

In 14 Rl-6 cells in Calliphora stygia, angular sensitivity measured in the dark was compared to that measured in the presence of an adapting light (white) of sufficient intensity to generate a peak response of 75% $V_{\text{max}}$. The adapting light (consisting of a large field, 20° in visual angle) was turned on, and following the one or two minutes required for the plateau depolarisation to reach a steady level, angular sensitivity was determined, as in the dark, by recording responses to incremental test flashes as the point source (at the centre of the adapting surround) was moved through the cell's visual field in $1/2^\circ$ or $1/4^\circ$ steps (Fig. 1.7). Finally a light-adapted $V/\log I$ curve was determined so that the responses could be converted to sensitivities. The results, shown in Table 1.5 reveal that in every case there is a slight reduction in the acceptance angle, and overall, the light-adapted $\Delta \rho$ values averaged 80% ± 14% of the dark-adapted values.
Figure 1.7  Original records showing angular sensitivity runs in an Rl-6 cell in *Calliphora*. Following two runs (using 1/2° steps) in the dark-adapted state (1), a white adapting surround is turned on (2), and following equilibration of the plateau depolarisation, repeated determinations are made in the light-adapted state, first using 1/4° steps (3), and then 1/2° steps (4). Finally a light-adapted intensity/response function is determined (5) so that the responses may be converted to sensitivities. The unit in question is unit 19.S.6 of Table 1.5.
TABLE 1.5

Acceptance angles (Δρ) determined in Rl-6 cells under conditions of dark-(DA) and light-adaptation (LA). Each value is the average of 2-4 determinations. All data from Calliphora. Also tabulated is the ratio between the dark- and light-adapted values (DA/LA).

<table>
<thead>
<tr>
<th>UNIT</th>
<th>Δρ LA(°)</th>
<th>Δρ LA(°)</th>
<th>DA/LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.A.1</td>
<td>2.08</td>
<td>1.52</td>
<td>1.37</td>
</tr>
<tr>
<td>19.A.3</td>
<td>1.58</td>
<td>1.48</td>
<td>1.07</td>
</tr>
<tr>
<td>19.A.4</td>
<td>1.31</td>
<td>1.01</td>
<td>1.30</td>
</tr>
<tr>
<td>23.A.1</td>
<td>1.90</td>
<td>1.75</td>
<td>1.09</td>
</tr>
<tr>
<td>23.A.2</td>
<td>1.75</td>
<td>0.93</td>
<td>1.88</td>
</tr>
<tr>
<td>23.A.3</td>
<td>1.69</td>
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<td>1.39</td>
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<td>23.A.4</td>
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</tr>
<tr>
<td>23.A.5</td>
<td>2.38</td>
<td>2.31</td>
<td>1.03</td>
</tr>
<tr>
<td>20.J.1</td>
<td>1.50</td>
<td>1.34</td>
<td>1.12</td>
</tr>
<tr>
<td>20.J.2</td>
<td>1.48</td>
<td>1.37</td>
<td>1.08</td>
</tr>
<tr>
<td>19.S.4</td>
<td>1.69</td>
<td>1.25</td>
<td>1.35</td>
</tr>
<tr>
<td>19.S.5</td>
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<td>1.76</td>
<td>1.31</td>
</tr>
<tr>
<td>19.S.6</td>
<td>2.65</td>
<td>2.14</td>
<td>1.24</td>
</tr>
<tr>
<td>19.S.7</td>
<td>2.03</td>
<td>1.96</td>
<td>1.04</td>
</tr>
<tr>
<td>Average (± 1.0 S.D)</td>
<td>1.92 ± 0.41</td>
<td>1.58 ± 0.43</td>
<td>1.25 ± 0.22</td>
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</tbody>
</table>
DISCUSSION

1. Applicability of the theory

The results summarise the acceptance angles of photoreceptors in situations where the angular widths of the Airy disc and the rhabdomere vary in a predictable fashion. The experimental results are now compared with the $\Delta \rho$ values predicted from these two factors using the simple theory described earlier.

This comparison is shown in Table 1.6, and Figures 1.4 and 1.6. Values for $\Delta \rho$ were calculated using both approximations for the rhabdomere acceptance function. Those marked 'Prediction A' assumed a Gaussian rhabdomere acceptance function, and were generated analytically from Eqs. (1.1) and (1.2) (and Eq. (1.3), when $V < 2.0$).
Prediction B assumes a circular step function, and $\Delta \rho$ values were measured from plots such as those shown in Figure 1.2. The susceptibility of the resulting $\Delta \rho$ values to small errors in the estimates of $d$, $D$, and $f$ is shown in Figure 1.8, where $\Delta \rho$ values are plotted against the percentage error in all these parameters.

The following points emerge from the comparison of measured and predicted $\Delta \rho$ values.

1) In general terms, the measured $\Delta \rho$ values are very close to the predicted values. Since the predicted values represent the theoretical lower limit set by the physical constraints of diffraction and rhabdomere diameter, it appears that in terms of acuity, the fly's ommatidium is as near perfect as its physical dimensions allow.
Figure 1.8  The susceptibility of theoretical $\Delta \varphi$ values to small changes in the parameters, $d$ the rhabdomere diameter, $D$ the facet diameter, and $f$ the focal length. A: curves generated assuming a Gaussian approximation for the rhabdomere acceptance function (Eq. 1.2); B: curves generated assuming a circular step function. The curve for $d$ (rhabdomere diameter) falls between the curves for $D$ and $f$, and has been omitted for clarity. At 0% error the parameter values are those assumed for frontal R1-6 cells in *Calliphora*, namely: $d = 1.9 \mu m$; $D = 24 \mu m$; $f = 70 \mu m$. Wavelength was 541 nm for all curves. Negative errors represent $D$ and $f$ decreasing, and $d$ increasing in magnitude, all of which result in larger estimates of $\Delta \varphi$. 
2) The two-Gaussian approximation of Snyder (1977) generates values that are consistently 20-25% higher than those predicted by the circular coverage model. This is in contrast to Snyder's claim that his approximation generates values that are insignificantly different from those generated by this model.

3) Of the $\Delta \rho$ values in Table 1.6, those considered most reliable are those for R1-6 cells in Calliphora, particularly those in frontal eye regions, where $\Delta \rho$ has been determined on numerous occasions, and consistently found to average ca.1.6°. Using the parameters of Table 1.2, these values are accurately predicted by the circular coverage model. Snyder's approximation seems inappropriate for generating these values, as all the parameters ($d$, $D$ and $f$) would have to be in error by 15% in the same direction in order for the measured value to be generated according to Eq. (1.2) (See Fig. 1.8). Notice also that Snyder's approximation predicts a greater influence of the Airy disc, and hence wavelength, than the circular coverage model, and this is not realised in the results (Fig. 1.6).

4) The most significant results that can be attributed to a single parameter are the narrower angular sensitivity functions demonstrated in cells R7/8. Since these share the same dioptric apparatus as R1-6, the narrower rhabdomere diameter of R7/8 must be responsible for this observation. This result has been predicted previously by Kirschfeld and Franceschini (1968) and small values of $\Delta \rho$ in Eristalis have been interpreted as being derived from central retinula cells (R7/8) on this basis (Horridge et al., 1976). However, the
present result is believed to be the only definitive comparison of acceptance angles in cells identified as R7/8 with those in Rl-6 cells in the same or nearby ommatidia.

5) The correspondence of the remaining Δρ values with the predictions is not as close as that demonstrated for Calliphora Rl-6 cells. However, whether this is due to the inapplicability of the theory in these situations, errors in the experimental Δρ values, or errors in the optical parameters d, D and f, it is not possible to say.

2. The physical basis of image formation

From the above, the simple theory described can be considered useful, because it is successful in generating Δρ values close to those actually measured. Whether or not this is due to the validity of the underlying physical assumptions is another question that is considered in the present section.

It is clear, that in the theory formulated, two factors are important in influencing Δρ, namely the Airy disc diameter (λ/D) and the rhabdomere acceptance function (which depends upon the rhabdomere diameter, d). As has been recognised previously (Kuiper, 1966), which ever of these is the largest will have the dominant influence upon Δρ. Kuiper (1966) assumed that λ/D (the 'diffraction limit') was the determinant factor in fly, however, as pointed out by Kirschfeld and Franceschini (1968), the fact that the different size of the rhabdomeres Rl-6 and R7/8 can be visualised in antidromic transmitted light from the rhabdomere tips, indicates that the rhabdomere diameter has a major influence on the angular
TABLE 1.6

Measured values of $\Delta \rho$ compared with theoretical predictions in *Calliphora* and *Musca*. The predicted values were calculated using the numerical assumptions found in Table 1.2. Both assumptions for the rhabdomere acceptance function were tested. Prediction A assumes a Gaussian function (Snyder, 1977) whilst Prediction B assumes circular step function.

<table>
<thead>
<tr>
<th></th>
<th>CALLIPHORA</th>
<th>MUSCA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Rl-6</td>
<td>Rl-6</td>
</tr>
<tr>
<td>(frontal)</td>
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<td>(frontal)</td>
</tr>
<tr>
<td>Measured</td>
<td>1.6$^1$</td>
<td>2.6$^2$</td>
</tr>
<tr>
<td>Prediction A</td>
<td>2.03$^\circ$</td>
<td>3.14$^\circ$</td>
</tr>
<tr>
<td>Prediction B</td>
<td>1.66$^\circ$</td>
<td>2.56$^\circ$</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>541</td>
<td>541</td>
</tr>
</tbody>
</table>

$^1$Average $\Delta \rho$ values from cells in Table 1.4

$^2$Average $\Delta \rho$ values from Rl-6 cells with horizontal visual axes greater than 100° left of the fly's anterior-posterior axis (Table 1.3 and Fig. 1.4).
sensitivity. The present quantitative theoretical calculations demonstrate that (if the theory is correct) rhabdomere diameter, is indeed the major factor determining $\Delta \rho^1$, and its influence is clearly demonstrated in the comparison of $\Delta \rho$ values in Rl-6 and R7/8 (Table 1.4 and Fig. 1.5).

The theoretical calculations predict that the influence of the Airy disc diameter should be rather small in Rl-6\(^1\). For example, varying the Airy disc by the use of different wavelengths is only expected to change $\Delta \rho$ by ca. 0.2° in the range 350-550 nm (Fig. 1.6). In cells R7/8, where $d$ is smaller, the effect of the Airy disc is expected to be more pronounced (Horridge et al., 1976). In the present results, no significant difference was detected between $\Delta \rho$ measured at 541 nm in R8, and that measured in R7 at 358 nm, however, the number of determinations in R8 (3 cells) was too small to allow a firm conclusion from this result.

Because of the difficulty of detecting the influence of the Airy disc from angular sensitivity determinations, what was hoped to be more sensitive test, was devised - namely the comparison of spectral sensitivity determined with point and extended sources. This too, however, failed to reveal any significant differences that might be attributed to the

\(^1\) At least when the circular step function is taken as an approximation for the rhabdomere acceptance function.
Airy disc. A possible explanation for the failure to see the enhancement of sensitivity towards shorter wavelengths predicted when using the point source (see Fig. 1.2) is that there is a degree of chromatic aberration that compensates for the variation in Airy disc diameter with wavelength. Chromatic aberration, that is probably sufficient to affect this compensation has in fact been measured in the ommatidium of Musca (McIntyre and Kirschfeld in preparation). However, the wavelength at which the system was best in focus was not determined, and therefore it is not possible, a priori, to say whether the aberration would be expected to compensate for, or enhance the wavelength dependence of the Airy disc diameter.

In conclusion, the exact physical basis of spatial transfer in the fly's ommatidium cannot be verified from these results. However, the rhabdomere diameter is clearly implicated as a major factor in determining $\Delta \rho$, and whilst no evidence was found to show that an Airy disc is formed at the level of the rhabdomere tips, the narrow $\Delta \rho$ values measured, indicate that the distribution of light imaged from a point source cannot be much larger than the theoretical Airy disc.

Finally it should be mentioned that Pask and Snyder (1975) have presented an alternative analytical approach to the simple theory described here, in which electro-magnetic wave theory is used to predict light capture by rhabdomeres considered as dielectric waveguides. The results of their analysis predict that there is an even greater dependence of $\Delta \rho$ upon wavelength than Prediction A (Fig. 1.6). Since this is not realised in the present results (Fig. 1.6), and because
of the greater complexity of the waveguide analysis, this theory is not considered further.

3. Light-adapted angular sensitivity

The foregoing account has compared theoretical predictions with \( \Delta \phi \) values measured under dark-adapted conditions. This is valid, because the theory considered only the lens/dioptric system and the rhabdomere tip, and no account was taken of the possible influence of accessory pigments that may move during light-adaptation. The assumption that accessory pigments do not influence \( \Delta \phi \) values in the dark is supported by the finding that \( \Delta \phi \) measured in white-eyed mutants is virtually the same as that measured in wild type flies of the same species (Washizu et al., 1964; Streck, 1972; and compare the present data from Musca (white) - \( \Delta \phi \) in frontal regions = 2.3° - with the value of 2.5° determined by Scholes (1969) in the wild type of the same species). The only significant difference appears to be that in the white-eyed mutants a higher sensitivity is maintained when the point source is some degrees off the cell's visual axis (Streck, 1972).

During light-adaptation a consistent decrease in \( \Delta \phi \) was observed (Table 1.5). To explain this on a similar theory to that used in the dark-adapted situation, it must be assumed that the rhabdomere acceptance function is modified as a result of light-adaptation. The pupil pigment granules that move to surround the rhabdomere during light-adaptation (Kirschfeld and Franceschini, 1969) might be expected to accomplish this. By effectively increasing
the refractive index surrounding the rhabdomere, both the waveguide parameter, V, and the critical angle for internal reflection will be reduced (Kirschfeld and Franceschini, 1969). Both of these factors could modify the acceptance properties of the rhabdomere. In addition, much of the incident light will be absorbed by the pupil pigment itself rather than the visual pigment, and if this 'division' of absorption has a strong angular dependence, again the effective rhabdomere acceptance function will be modified. At present however, a satisfactory theory of the angular dependence of effective absorption in such a system is not available.

Vowles (1966) suggested that the movement of secondary pigment cells during light-adaptation was responsible for the narrowing of retinula cell receptive fields. However, since the pigment cells lie in between ommatidia, it is not obvious how they could influence $\Delta \rho$ values, though they might affect the passage of stray light between ommatidia. Finally, other mechanisms, such as a physical change in the rhabdomere diameter or the distance between the lens and the rhabdomere tips, can be suggested, however these are considered unlikely and are not discussed further.

4. Summary of conclusions

The results include a more or less comprehensive account of acceptance angles in different eye regions, different receptor classes, and at different wave-lengths. The effect of light-adaptation on $\Delta \rho$ is also described. These data are hoped to be of value in the interpretation of spatial transfer characteristics at other levels in the visual system and an
example of this interpretative value is to be found in Chapter II.

The comparison with theoretical predictions, reveals that a simple theory is successful in generating $\Delta \rho$ values close to those observed, and also that the acuity of individual receptors approaches the lower limit set by the physical constraints of diffraction and rhabdomere diameter.

The comparison of angular sensitivity in R1-6 cells and R7/8 cells demonstrates that the rhabdomere diameter is an important factor in determining the angular sensitivity function, however, no evidence could be found for the existence of an Airy disc, imaged from a point source at the level of the rhabdomere tips. Because of this failure, it has to be admitted that the exact physical basis of image formation in the fly's eye (or indeed in any eye) is still open to question.
CHAPTER II

FUNCTIONAL COMPARISON OF R1-6, R7 AND R8.
SUMMARY

1. Intracellular recordings were made from the photoreceptor classes R1-6, R7 and R8 in the flies Calliphora stygia (wild type) and Musca domestica (white), with the aim of revealing properties relevant to an understanding of receptor involvement in visual behaviour.

2. An adapting light, of sufficient intensity to activate the pupil mechanism, shifts the 490 nm peak of spectral sensitivity in R1-6 towards shorter wavelengths by 40-50 nm in Calliphora stygia (wild type) but not in the pupilless, white-eyed mutant of Musca domestica (white) (Fig. 2.1). This spectral shift is independent of photopigment equilibria and has a time course similar to that of the pupil closure mechanism (Fig. 2.2).

3. There are two spectral classes of R7. Both have a single peak of sensitivity in the ultraviolet (uv), but one has the peak at ca. 340 nm and has less than 10% sensitivity beyond 400 nm, whilst the other has the peak at ca. 360 nm and also a long tail of sensitivity (> 10%) extending to 500 nm. The majority of R8 cells have a major peak of sensitivity at around 540 nm (Fig. 2.3).

4. Polarisation sensitivity (PS) in Calliphora R1-6 averaged 2.0 when measured in the green, and was not significantly affected by light-adaptation. In Musca R1-6, PS averaged 1.9. In R7, PS was similar in Calliphora (average 2.2), but reached values of up to 6 in Musca. PS could not always be detected in R8, but when present, averaged 1.7, in Calliphora.
5. Absolute sensitivities, defined as the reciprocal of the quantal flux required to generate a 50% maximum response using axial light of peak wavelength ($AP_{50}$) are higher in R7 and R8 due to the higher voltage gain per quantum (Table 2.1).

6. All receptor classes light-adapt in a similar manner and continue responding to increments of intensity under the brightest adapting regimes used (Fig. 2.5).

7. These new results from identified receptors lead to a reappraisal of the possible roles of R1-6 and R7 and R8 in optomotor responses elicited under different regimes of test pattern wavelength and intensity.
INTRODUCTION

The study of photoreceptors can be considered to have two major applications. The first has to do with an understanding of the origins of receptor function, itself, e.g. in terms of optics, pigments and transduction - problems that may be loosely termed biophysical. Secondly, and the main concern of this Chapter, a detailed knowledge of receptor output is essential for a complete appreciation of the functioning of the visual system as a whole. The fly is a useful preparation for the study of the former (as discussed in Chapters I, III and V); but because it has been studied extensively at a variety of levels and with numerous techniques (reviews: Reichardt, 1969; Kirschfeld, 1972; Hausen, 1977) it is in the latter context that the fly has become a preparation, par excellence, for the analysis of visual processing.

A particular case of importance concerns a comparison of the properties of the different receptor classes, R1-6, R7 and R8. Anatomical and optical considerations have led to the concept that the photoreceptors, R1-6 on the one hand, and R7/8 on the other, represent two functional subsystems (Kirschfeld and Franceschini, 1968). Receptors R1-6 have been designated as a high sensitivity system (HSS) by virtue of their large rhabdomeres and the fact that six cells with the same field of view project short axons that converge on the same second order cells in the first optic neuropile (the lamina) (Kirschfeld, 1967; Braitenberg, 1967). The other two receptors (R7 and R8) share a common tiered
rhabdom and have been considered as functionally separate from R1-6 since they project long axons through to the second optic neuropile (the medulla). R7 and R8 have been designated as a high acuity system (HAS) with low absolute sensitivity, because of their narrower rhabdomeres (Kirschfeld and Franceschini, 1968).

In the past it has been assumed that one or other receptor system may be selected to drive the optomotor pathway by appropriate choice of the intensity and spatial wavelength of the stimulating pattern. Based upon this assumption several properties have been attributed to the receptors from purely behavioural data. Thus R7 and R8 have been attributed a blue-peaked spectral sensitivity, and a degree of polarisation sensitivity, and estimates of acceptance angles have been derived for both receptor classes (Eckert, 1971, 1973; McCann and Arnett, 1972). A recent behavioural study in visually deficient Drosophila mutants (Heisenberg and Buchner, 1977) shows, however, that in this species at least, R7/8 never appear to dominate the optomotor pathway (a necessary assumption of the earlier authors).

It is clear, that despite extensive electrophysiological (e.g. Burkhardt, 1962; Scholes, 1969; Järvilehto and Moring, 1974; Smola and Meffert, 1975) and microspectrophotometrical (Langer and Thorell, 1966; Hamdorf et al., 1973; Stavenga, 1976; Kirschfeld and Franceschini, 1977) investigation, our knowledge of receptor physiology is insufficient to confirm, either the basic concept of a functional division of receptor properties (Kirschfeld and Franceschini, 1968), or the
interpretation of receptor involvement in behaviour (Eckert, 1971). The major aim here, has been to use intracellular recordings from identified receptors, to supply reliable data which allow a clearer interpretation of results (both past and future) from other levels in the visual system.

The particular areas considered important for study included accurate determinations of spectral, polarisation and angular\(^1\) sensitivities in receptors R7 and R8 for comparison with similar properties in R1-6, as the available data (Smola and Meffert, 1975; Järvillehto and Moring, 1974; 1976) is, at best, scanty. Moreover data upon how such properties may be affected by light-adaptation in R1-6 was collected, and a comparison of the operating curves of R1-6 and R7/8 at different adapting intensities made, because many behavioural studies are performed in light-adapted situations, whereas most previous receptor physiology in fly concerns dark-adapted properties (but see, Dörrscheidt-Küfer, 1972, and Chapter IV).

\(^1\) Angular sensitivities in both R1-6 and R7/8 were described in the last Chapter. Since these results approached the theoretical limit set by diffraction and rhabdomere diameter, it is felt that they are probably the most reliable data available. Therefore, they are used, alongside the data collected in this chapter, in the discussion of receptor subsystems and their involvement in behaviour.
The results essentially confirm (with some qualification) the predicted differences between R1-6 and R7/8 with respect to sensitivity and spatial resolution (Kirschfeld and Franceschini, 1968) but call for a reappraisal of the roles of the two receptor systems in determining optomotor behaviour.
METHODS

Experiments were performed on *Calliphora stygia* (wild type) and white-eyed mutants of *Musca domestica* (white). Females, only, were used. Details of the cultures, preparation, recording and stimulation via a 900W Xenon arc lamp with quartz optics are described in Appendix A. The criteria used for identification of cells are explained in Appendix B.

Intensity and sensitivity measurements

For light-adapted sensitivity determinations, one or other of two methods was employed. For light-adapted spectral sensitivities, and light-adapted intensity/response functions, both test and adapting beams were "mixed" via a half-silvered quartz mirror, and the stimulus presented to the fly by means of a common light-guide (mixed beam method, Appendix A). For light-adapted polarisation sensitivity measurements, the dual beam method (Appendix A) was used, whereby the adapting beam was delivered by a separate light guide, and projected (by reflection off a front surface mirror) onto a diffuse, reflecting screen, with the test spot situated at its centre. Only the test beam was polarised in this configuration.

Intensities throughout this chapter, are expressed either in terms of monochromatic quantal flux, or by the effect upon the receptor potential, which can, itself, be defined in terms of equivalent quantal flux by the average $V/\log I$ curve (values of the reciprocals of quantal flux
required to generate a $50\% V_{\text{max}}$ response in dark-adapted receptors are found in Table 2.1). Details of methods used for calibrations are found in Appendix A.

A major hindrance to precise correlation of existing behavioural data with the results presented here, is that the units used in behavioural work are usually luminance values (apostilbs or cd/m$^2$). These units are determined by apparent (subjective) brightness to human subjects, and thus depend upon the relation between the spectral output of the light source used, and the spectral sensitivity of Man. When different light sources are used to stimulate a preparation with a spectral sensitivity dissimilar to Man's, luminance values are no longer directly comparable, and for insects, where ultraviolet sensitivity is often high, they may be in error by orders of magnitude under some circumstances.

At present the only study that equates luminance values with equivalent monochromatic quantal flux in fly is that of Scholes and Reichardt (1969) who used the receptor cells (R1-6) of *Musca* to compare the effects of monochromatic light (expressed in quanta.s$^{-1}$.cm$^{-2}$) and a broad spectral source (expressed in apostilbs). The conversion factor for the source (the phosphor of a Tektronix P5 CRO) was $1 \text{ apostilb} = 2 \times 10^9 \text{ quanta.s}^{-1}$.cm$^{-2}$ (at 476 nm).
RESULTS


As reported by many authors the majority of receptors R1-6 have similar spectral sensitivities, characterised by two major peaks at ca. 360 nm and 490 nm in Calliphora (Burkhardt, 1962; McCann and Arnett, 1972; Horridge and Mimura, 1975). It has also been established that pre-illumination with intense monochromatic light can affect this spectral sensitivity by altering the rhodopsin/metarhodopsin ratio (Rosner, 1975; Tsukahara and Horridge, 1977a). In the present experiment, however, the aim was to see if there was any other effect that could be observed during a less extreme adapting light. For this purpose a monochromatic light of a rhodopsin generating wavelength (591 nm) was chosen, and the spectral sensitivity measured in the presence of this adapting light was compared to that determined following subsequent dark-adaptation. The adapting intensity used was sufficient to generate a 75% V_max peak response. As rhodopsin is not converted into metarhodopsin in the dark, this method ensures that the rhodopsin/metarhodopsin equilibrium (in this case greater than 95% rhodopsin) was the same for both dark- and light-adapted determinations. The dark- and light-adapted spectral sensitivities in Calliphora (wild type), shown in Figure 2.1, reveal a pronounced shift of peak sensitivity towards shorter wavelengths during light-adaptation. The major effect is a selective reduction of sensitivity in the green, and there appears to be negligible wavelength specific attenuation in
the region below 420 nm (the apparent enhancement in this region is an artifact of normalisation). At present the longitudinal pupil mechanism of the receptor cells themselves is considered the most likely candidate for causing this shift in spectral sensitivity. Supporting evidence for this view comes from a control experiment in which it was attempted to duplicate the result in white-eyed mutants of *Musca* (white) which lack all accessory eye pigments including the pupil granules. The result shows that, in contrast to wild type flies, long wavelength adaptation causes no significant change in spectral sensitivity (Fig. 2.1).

If this shift in spectral sensitivity is indeed due to the action of the pupil, it is predicted that it should follow the same time course. To measure the time course of the shift, the sensitivity at 499 nm was determined as a function of the time following the onset of the adapting light. The wavelength 499 nm was chosen, as it is here that sensitivity is most attenuated by light-adaptation (Fig. 2.1). In order to control against sensitivity changes not related to the spectral shift (e.g. membrane adaptation) sensitivity at 499 nm was compared to that at 358 nm, since there appears to be insignificant wavelength specific attenuation at this wavelength (see above). The experimental protocol actually used was as follows. First the cell was pre-adapted with a rhodopsin generating wavelength (591 nm) and allowed to dark-adapt again. Then the adapting light (again 591 nm) was turned on and immediately afterwards, short test flashes of wavelength 499 nm were superimposed at rapid intervals (Fig. 2.2). Following equilibration of the responses, the cell was allowed to dark-
Figure 2.1  The effect of light-adaptation upon spectral sensitivity: above, in Calliphora (wild type); and below, in the pupilless, white-eyed mutant of Musca. All curves are normalised to 100%. Each point is the average of two or more repeated determinations in 6 R1-6 cells in Calliphora, or 3 R1-6 cells in Musca.

- - - - , dark-adapted spectral sensitivities.

- - - - , light-adapted spectral sensitivities.
Figure 2.2  Time course of the light-induced shift in spectral sensitivity demonstrated in Fig. 2.1. Sensitivity to light of wavelength 499 nm (relative to that at 358 nm) is plotted against t, the time measured from the onset of the adapting light. Triangles and circles are results from two independent determinations in the same cell. The solid line (open circles) is the measured time course of pupil closure (Franceschini, 1972) normalised to the overall change in sensitivity. The inset shows two of the records used to calculate the curve, with incremental responses to short, rapidly repeated test flashes of either 358 nm or 499 nm, riding on top of the slowly decaying plateau response to the adapting light (591 nm).
adapt again, and the procedure repeated, but now using test flashes of wavelength 358 nm. Finally a light-adapted V/log I function was determined so that responses could be converted to sensitivities. Responses at 499 nm were compared to those at 358 nm at identical time intervals, and sensitivity at 499 nm (relative to that at 358 nm) calculated by referring these responses to the V/log I curve determined at the same adapting intensity. The resulting time course of the spectral shift was then compared to that of the pupil closure mechanism, as determined by Franceschini (1972), and found to correlate very closely (Fig. 2.2).

2. Spectral sensitivities of R7 and R8

All R7 cells investigated had a major peak of sensitivity in the uv in the range 340-360 nm, as previously reported (Smola and Meffert, 1975; Hardie, 1977b). However, two subclasses could be clearly distinguished from the position of the peak and the degree of sensitivity remaining at wavelengths beyond 400 nm. Both have been identified as R7 by intracellular dye injection in Musca and Calliphora (see Appendix B). One class (25/91 cells) has a peak of sensitivity at ca. 340 nm and less than 10% sensitivity at wavelengths longer than 400 nm. The other class (66/91 cells) has a peak at ca. 360 nm and a long tail of sensitivity (> 10%) extending into the green (Fig. 2.3).

The majority of R8 cells in Calliphora (wild type) have a major peak of sensitivity at ca. 540 nm and a secondary, much smaller peak at ca. 360 nm, but only ca. 10% sensitivity in the range 400-500 nm (Fig. 2.3). The existence
of this class of R8 was also demonstrated on one occasion each, in *Musca* (white), and *Lucilia sericata* (wild type) (Hardie, 1977b). There are good reasons for suspecting the existence of an additional class of R8 with a peak of sensitivity at around 520 nm (see Chapter V), but these cells were not detected in the present study. A more detailed analysis of spectral sensitivities in both R7 and R8 is described in Chapter V.

3. Polarisation sensitivity in R1-6

Polarisation sensitivity (PS), has previously been measured in R1-6 cells by several authors (e.g. Kuwabara and Naka, 1959; Autrum and v. Zwehl, 1962; Scholes, 1969; Horridge and Mimura, 1975). However, measurements of PS in R1-6 were repeated in the present study for two reasons. Firstly for a comparison with results from R7 and R8 in the same populations of flies (see next section) and secondly to see if there was any effect of light-adaptation upon this parameter. Monochromatic light of wavelengths 499 nm, 541 nm or 572 nm was chosen for determinations as it is known that PS in fly R1-6 cells can depend upon the wavelength employed but is relatively constant at wavelengths longer than 430 nm (Horridge and Mimura, 1975). A detailed account of PS as a function of wavelength in different functional classes of R1-6 is given in Chapter III. Dark-adapted PS values, determined from the responses to flashes of light as a polaroid is rotated in 10° steps through 180° and back again, averaged 2.0 ± 0.5 S.D. (20 determinations on 6 cells) in *Calliphora* (wild type). In 3 cells repeated PS measurements were made during the presence
A minority (ca. 30%) of R7 cells are purely ultraviolet (uv) sensitive, and have a peak at ca. 340 nm (o-o-o). The remainder (ca. 70%) (●-●-●) also have a major peak in the uv, but shifted towards longer wavelengths (ca. 360 nm). In addition they have a long tail of sensitivity extending into the green. The majority of R8 cells have the spectral sensitivity previously described (Hardie, 1977b). Curves are averaged, normalised results from many cells in both Musca (white) and Calliphora (wild type) - as no difference was observed between the two species. Error bars represent ± 1.0 S.D.
of an adapting surround (white light) of sufficient intensity to generate a 75% peak response. Under these conditions PS averaged $1.9 \pm 0.1$ S.D. but was not significantly different from the dark-adapted values in the same cells.

In Musca (white) R1-6 only dark-adapted values were determined, and in this species, PS averaged $1.9 \pm 0.4$ S.D. (15 determinations in 5 cells).

4. Polarisation sensitivity in R7 and R8

Behavioural experiments have revealed a strongly polarisation sensitive response elicited by single rhabdomere stimulation of R7 and R8 (Kirschfeld and Lutz, 1974). However, the available data on PS from intracellular recordings in R7 and R8, though scanty, suggest there is very little or none (Järvilehto and Moring, 1974; Smola and Meffert, 1975). In this study the experiments have been repeated using a variety of wavelengths in case a significant PS was previously not detected owing, perhaps, to an inappropriate choice of wavelength. In fact almost every R7 investigated showed significant PS when measured using blue light (442 nm) though very little, or no PS in the uv. Values in Calliphora (wild type) were close to those found in R1-6 - average = $2.2 \pm 0.6$ S.D. from a sample of 9 cells. In Musca (white) R7, however, values as high as 6 were found - average $4.7 \pm 1.3$ S.D. from a sample of 5 cells. Data from R8 are less consistent, and in several cases no PS could be demonstrated. In three cells in Calliphora (wild type) however, PS was detected, the average value being $1.7 \pm 0.6$ S.D. (at 541 or 572 nm). Limited recording time did not allow the
determination of light-adapted PS values in either R7 or R8. Again, a more detailed account of PS, and its wavelength dependence in R7 and R8 is deferred to Chapter V.

5. **Absolute sensitivities and operating ranges of R1-8**

Of obvious importance to those interested in identifying relative receptor contribution at higher levels is a knowledge of the relative quantum capture and voltage outputs of the different cell types as a function of intensity. Voltage output is defined by the V/log I curve, and, for cells with similarly shaped V/log I curves, can be described by the measure of Axial Peak Sensitivity at the 50% level (APS$_{50}$) which is defined as the reciprocal of the number of quanta.s$^{-1}$.cm$^{-2}$ of peak wavelength required to generate a 50% $V_{max}$ response when using an axial point source (Laughlin, 1976a). On this measure, preliminary results indicated that voltage output as a function of intensity in R7/8 (recorded in the chiasm) was at least as high as that in R1-6 (Hardie, 1977b). These measurements have now been repeated more accurately in the retina on a larger sample of cells, and the results are shown in Table 2.1.

These results essentially confirm the earlier data (Hardie, 1977b), but now clearly indicate that R7, in particular, is significantly more sensitive than R1-6 by a factor of about 3x. Notice that in terms of absolute voltage in the retina R7 and R8 produce similar signals to R1-6 (Table 2.1) but it should be recalled that when recording in the chiasm, potentials in R7/8 are much smaller ($V_{max}$ $\simeq$ 30 mv), indicating
Maximum responses ($= V_{\text{max}}$) and axial peak sensitivities ($= APS_{50}$, the reciprocal of the number of quanta of peak wavelength required to generate a 50% $V_{\text{max}}$ response when using an axial point source) in *Calliphora* (wild type) photoreceptors. The point source used subtended 0.4° at the fly's eye. Cells are from the same general eye region (approximately foveal) and chosen for the quality of recording.

<table>
<thead>
<tr>
<th>V_{\text{max}} \pm 1.0 \text{ S.D. mV}</th>
<th>APS_{50} \pm 1.0 \text{ S.D. quanta}^{-1}\text{cm}^2\text{s}</th>
<th>\text{Highest APS}_{50} \text{ quanta}^{-1}\text{cm}^2\text{s}</th>
<th>\text{Lowest APS}_{50} \text{ quanta}^{-1}\text{cm}^2\text{s}</th>
<th>\text{Peak Wavelength nm}</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1-6 (n = 14)</td>
<td>55 \pm 3.8</td>
<td>2.8 \pm 1.2 \times 10^{-11}</td>
<td>5.8 \times 10^{-11}</td>
<td>8.2 \times 10^{-12}</td>
</tr>
<tr>
<td>R7 (n = 19)</td>
<td>52 \pm 14.5</td>
<td>8.6 \pm 4.1 \times 10^{-11}</td>
<td>1.6 \times 10^{-10}</td>
<td>3.6 \times 10^{-11}</td>
</tr>
<tr>
<td>R8 (n = 7)</td>
<td>54 \pm 11.0</td>
<td>5.8 \pm 4.0 \times 10^{-11}</td>
<td>9.2 \times 10^{-11}</td>
<td>9.0 \times 10^{-12}</td>
</tr>
</tbody>
</table>
attenuation of the signal (and presumably the noise) via passive propagation (Zettler and Järvilehto, 1973; Hardie, 1977b).

This measure of sensitivity (the $\text{APS}_{50}$) is a function of both the number of quanta effectively absorbed and the voltage gain per quantum. The responses to light at low intensities indicate that R7 and R8 in fact produce a much larger response to each quantum, as judged by the size of the discrete potentials presumed to represent 'quantum bumps' (Fig. 2.4). Unfortunately the gradation of the bumps into the membrane and recording noise did not allow accurate counting of bumps, except for the case of Musca (white) Rl-6 cells, and thus it was not possible to determine directly the relative quantum capture efficiencies (Q.C.E.) of the different cell types. In one Rl-6 cell in Musca (white), however, it was possible to gain a good estimate of Q.C.E. by counting the number of bumps generated in response to accurately calibrated low levels of illumination. The bump rate at five different intensities was compared to the estimated quantal flux per facet (assuming a facet area of 500 $\mu$m$^2$) and a mean Q.C.E. of $26 \pm 4\%$ was obtained (with reference to an axial point source and peak wavelength).

The outputs of Rl-6 and R7 were also compared at different states of light-adaptation by determining $V$/log I curves in the presence of steady adapting lights. Figure 2.5 shows families of such curves in both Rl-6 and R7 obtained by plotting total intensity ($\log I_a + I_c$) against total response measured from the dark-adapted resting potential (see Chapter IV
Figure 2.4  Left: responses of R1-6 and R7 to very low light intensities in *Calliphora* (wild type). In both cases clear shot noise is observed - presumably representing response to single quanta - however, in the case of R7 the amplitude is considerably greater. On the right the response of R1-6 to low light levels in *Musca* (white) is shown. In this species bump amplitude is considerably larger. Figures indicate the absolute quantal flux (peak, axial quanta. cm$^{-2}$.s$^{-1}$).
for the rationale behind this convention). Both cell classes shift their V/log I curves along the log intensity axis to a similar degree and continue responding to intensity increments with the brightest adapting intensities tested. The only significant difference between cell types appears to be that the adapted plateau response level in R7 is consistently higher than in Rl-6 (Fig. 2.5).
Figure 2.5  Dark and light-adapted V/log I curves in R1-6 and R7. The total intensity, log I = log (I_a + I_t), has been plotted against the normalised total response (measured from the dark-adapting resting potential). The dotted lines represent the height of the steady state plateau responses to the adapting light. Arrows on the abscissa indicate the intensity of the adapting lights. Both types of receptor shift their V/log I curves along the intensity axis as the adapting intensity is increased. In addition both cell types continue responding at the brightest adapting intensities used.

- o-o-o dark-adapted V/log I curves
- •-•-• series of light-adapted V/log I curves

Intensities are expressed in terms of log units above PAQ_{50} for R1-6 in both graphs. Data from Calliphora (wild type).
DISCUSSION

1. Effects of light-adaptation in R1-6

The 40-50 nm shift in the peak of spectral sensitivity in R1-6 under the influence of light-adaptation is a striking phenomenon that deserves special mention here before discussing the major point of the Chapter - namely the functional comparison of R1-6 and R7 and R8.

Two lines of evidence strongly indicate that the shift in spectral sensitivity is due to the influence of the pupil pigment granules which move to surround the rhabdomeres under the influence of light (Kirschfeld and Franceschini, 1969). Firstly, the effect is not observed in white-eyed mutants of Musca which lack the pupil pigment (Fig. 2.1). This mutant also lacks all other accessory pigments in the retina, but the pupil pigment is the only one known to migrate rapidly in response to light. Secondly, the time course of the shift correlates very well with the time course of pupil closure (Fig. 2.2). To ensure that the adapting intensity used in these experiments was sufficient to activate the pupil, a fly (Calliphora) was mounted so as to observe the deep pseudopupil (Franceschini and Kirschfeld, 1971b) and illuminated with intensity and wavelength identical to those used in the physiological measurements. It was then possible to see that the pupil was in fact more or less fully activated - as witnessed by the development of the characteristic yellow glow (Franceschini and Kirschfeld, 1976).
In the last Chapter, it was demonstrated that light-adaptation at a similar intensity, causes a reduction in the acceptance angle of R1-6 cells (Table 1.5) and it was suggested that the longitudinal pupil mechanism could be responsible for this phenomenon also. It should be acknowledged that effects of the pupil mechanism upon both spectral and angular properties of fly photoreceptors have been predicted, upon a priori grounds, previously (Kirschfeld and Franceschini, 1969; Stavenga et al., 1973; Langer, 1975), however, the present data is believed to represent the first electrophysiological evidence for such functions.

2. Receptor classes and the optomotor pathway.

The optomotor turning response has been studied as a function of intensity, wavelength of light, plane of polarisation and also the spatial wavelength of the stimulating pattern. In this section use is made of the acceptance angles described in Chapter I, together with the results of the present Chapter, in an attempt to gain a better understanding of receptor involvement in the functioning of the visual system as a whole.

Studies, in Musca, of both the optomotor response (Eckert, 1971) and the activity of units in the optic lobes presumed to be involved in this behaviour (McCann and Arnett, 1972) have shown a "Purkinje" shift - in that a single blue peaked spectral sensitivity is found when the test pattern is of high intensity and low spatial wavelength (3°), whereas broader stripes and lower intensities generate a twin peaked spectral
sensitivity similar to that seen in receptors Rl-6. With respect to receptor involvement in the optomotor pathway, three general hypotheses may be suggested to interpret these findings.

I. The turning response generated using high intensities and low spatial wavelengths is a result of R7/8 domination of the optomotor pathway under these conditions. (This was the previous authors' interpretation).

II. R7/8 do not influence the behaviour at all and the properties of R1-6 alone determine the behaviour. (This has been suggested by Heisenberg and Buchner (1977) from their results in Drosophila).

III. The behaviour generated at high intensities and low spatial wavelengths is due to a modifying influence by R7/8 upon a response still dominated by R1-6. (This might be inferred from the result of Kirschfeld and Lutz, 1974).

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1 As pointed out by Snyder (1975), unlike the spectral sensitivity in Rl-6 cells, Eckert's results actually reveal a peak of sensitivity in the uv that is significantly higher than that in the green. However, the results of McCann and Arnett (1972) do not show this feature, whilst confirming Eckert's results in other respects. In addition the accuracy of Eckert's data in the uv has recently been questioned by Heisenberg and Buchner (1977). From these considerations, therefore, the present discussion will be based upon the assumption that at low light levels, and with broad stripes, optomotor responses have a spectral sensitivity similar to that found in R1-6 cells, as found by McCann and Arnett (1972).
The first of these hypotheses is excluded on the grounds that extensive recordings of central retinula cells (over 100 units in the present study) have failed to reveal any with a single blue-peaked spectral sensitivity. In fact the major common feature of R7/8 spectral sensitivity is the low sensitivity in the blue (Fig. 2.3).

If hypothesis I is excluded, then it is predicted that R1-6 should still be dominating the optomotor response at spatial wavelengths of 3°. To check that this is possible, use is made of the measured Δρ values (Table 1.6, Chapter I) to calculate the modulation transfer functions (M.T.F.'s for the different receptor classes. The M.T.F. characterises the transfer of contrast modulations in the object world as a function of spatial wavelength (λ₀), and at the receptor level is given by:

\[ M.T.F. = \exp \left[ -3.56 \left( \frac{\Delta \rho}{\lambda_0} \right)^2 \right] \]  

(Götz, 1964) Eq. (2.1)

M.T.F.'s for different receptor classes in both Musca and Calliphora are plotted in Figure 2.6. In Musca at λ₀ = 3° the modulation transfer in R1-6 cells = 0.12 which should in fact be sufficient to generate an optomotor response, as the contrasts used in the experiment were high (0.9) and behaviourally determined contrast thresholds for broader stripes are of the order of 0.01 - 0.05 in Musca (Eckert, 1973). Furthermore, dark-adapted Δρ values were used for the calculation.

As discussed in Chapter V, it is no longer possible to be confident of the existence of a class of central retinula cells with a single-peaked blue spectral sensitivity, as reported by Hardie (1977b). But even if they do exist, they only account for 2 out of 112 recordings from central retinula cells, and thus are unlikely to account for the behavioural results.
If the intensities used in the behavioural experiments were sufficient to cause a narrowing of angular sensitivity (as found in Calliphora - see Chapter I) an even larger modulation transfer would result. Finally, in order to explain the behavioural finding that acuity decreases at low luminances (Eckert, 1973), it is preferred to accept the conclusion of Dvorak and Snyder (1978), that such behaviour is best explained by neural pooling at low light levels.

Hypothesis II (i.e. that the optomotor pathway is driven only by R1-6) gains some support from the observed shift in spectral sensitivity believed to be caused by the pupil (Fig. 2.1), since this is of the same order as the "Purkinje shift" observed behaviourally. However, it is not clear if the intensities used in the behavioural experiments were sufficient to activate the pupil, and in addition the behavioural result shows a loss of the uv sensitivity peak not found at the receptor level. This discrepancy might possibly be explained, however, by a species difference in pupil absorbance spectra (between Musca and Calliphora), or inaccuracies in Eckert's uv data which have recently been questioned by Heisenberg and Buchner (1977). A second possible explanation is suggested by the discovery, in Calliphora, of a new functional class of R1-6 in which the spectral sensitivity depends upon the intensity at which it is measured (see Chapter III). Although at low intensities the spectral sensitivity resembles that seen in other R1-6 cells, at higher intensities a single blue-peaked spectral sensitivity is found. This class of cells was not, however, detected in Musca.
The third hypothesis (that R7/8 influence an Rl-6 dominated response) is the most favoured. If true, then the influence of R7 and R8 must presumably be inhibitory. Thus inhibition from R8 (with a 540 nm peak) could shift the 490 nm peak towards shorter wavelengths, whilst R7 inhibition could suppress sensitivity in the uv. This interpretation is suggested by the findings of Kirschfeld and Lutz (1974) who demonstrated inhibition of an Rl-6 mediated optomotor response by stimulation of R7/8 using an optical technique to stimulate individual identified rhabdomeres. Further support comes from the fact that the difference between the M.T.F.'s of R7/8 and Rl-6 (in Musca) when plotted against spatial wavelength, peaks very close to the 3° used in the behavioural experiments (Fig. 2.6). Since voltage modulation in light-adapted retinula cells is proportional to contrast modulation (see Chapter IV), this peak identifies the spatial wavelength at which the voltage response in R7/8 is maximal compared to that in Rl-6. Thus the spatial wavelength chosen by Eckert (1971) happens, in fact, to be that most likely to reveal any effect of R7/8 should they in fact contribute to the optomotor response.

3. Polarisation sensitivity

An extant paradox between behavioural and electro-physiological results has been the question of polarisation sensitivity in the central rhabdomeres, R7 and R8. Although

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1 This argument assumes that light-adapted V/log I slopes in Rl-6 and R7/8 are similar. Reference to Figure 2.5 shows that this is approximately true.
Figure 2.6  Modulation transfer functions (M.T.F.'s) in R1-6 and R7/8 calculated from Eq. (2.1) (see text). On the right M.T.F.'s are plotted as a function of spatial wavelength for both *Musca* and *Calliphora*, assuming the $\Delta \rho$ values measured in foveal regions electrophysiologically (values given alongside each curve). A plot is also shown for $\Delta \rho = 3.5^\circ$ (the value assumed for *Musca* R1-6 by Eckert, 1971). On the left the algebraic difference between the M.T.F.'s in R1-6 and R7/8 in both species is plotted as a function of spatial wavelength, which, as argued in the text, should reflect the relative voltage outputs of the cells in a light-adapted situation.
one might discount the evidence of PS in the behaviourally identified "HAS" (Eckert, 1971; McCann and Arnett, 1972) on the grounds of the uncertainties discussed in the last section, the elegant result of Kirschfeld and Lutz (1974) is not open to misinterpretation, because they evoked a strongly polarisation sensitive reaction using single rhabdomere stimulation of R7/8. However, the available physiology from R7 and R8 (Järvilehto and Moring, 1974; Smola and Meffert, 1975) failed to reveal PS in R7, although in one case a small PS was observed in R8 by Järvilehto and Moring (1976).

In contrast, the present results show that R7, in particular, has a rather high PS when measured with blue light, and a moderate PS was also detected in R8 on occasion. However, the measured PS (maximum of 6.0, in Musca R7) is not as great as the 100:1 measured behaviourally (Kirschfeld and Lutz, 1974), and therefore some neural enhancement must also be proposed.

4. Absolute sensitivity

An important aspect of the functional comparison of R1-6 and R7 and R8 is a description of their relative sensitivities. Sensitivity is a much used term that may be defined in several ways. For the comparison of photoreceptors, two general types of definition may be considered - each applicable to particular situations (See Appendix A, Table A.1).

(i) Detection, or quantum sensitivity - the reciprocal of the intensity required for the signal to be detected against background noise. All else being equal (e.g. voltage gain per quantum and membrane noise) this is proportional to
quantum capture efficiency (Q.C.E.) i.e. the proportion of quanta in the stimulus that are effectively absorbed by the photopigment.

(ii) Range sensitivity - the reciprocal of the intensity required to generate a criterion response, e.g. 50% $V_{\text{max}}$ - which defines the location of the intensity/response function on the intensity axis (for curves with similar $V/\log I$ slopes). This measure may refer to any adaptational state and in the dark-adapted state has been defined as the Axial Peak Sensitivity ($\text{APS}_{50}$) by Laughlin (1976a).

In addition, it should be emphasized that each definition may be qualified with respect to either a point, or an extended source, and when comparing receptors with different angular sensitivities, the relative sensitivities will be different in the two cases. In this study only sensitivity to point sources was measured, however, it is fairly simple to estimate the relative sensitivity to an extended source given the angular sensitivity functions (see Table 1.6, Chapter I). Thus, for a point source, sensitivity is proportional to the height of the angular sensitivity function. For an extended source, sensitivity is given by the volume under the angular sensitivity function, which is proportional to the height of the angular sensitivity function times the width$^2$ ($\Delta \rho^2$). Since the constant of proportionality is the same in the two cases, in order to calculate the relative sensitivity (R1-6:R7/8) for an extended source, one need only multiply the relative sensitivity for a point source
by a factor reflecting the relative $\Delta \rho$ values ($\Delta \rho^2_{Rl-6} : \Delta \rho^2_{R7/8} = 1.64$ (using values measured in Calliphora, Table 1.6, Chapter I).

Detection sensitivity is important under threshold conditions when the signal must exceed the noise. Further, at absolute threshold, behavioural (Reichardt et al., 1968) and electrophysiological (Lillywhite, 1977) evidence indicates that the visual system is capable of detecting and responding to single quanta. Unfortunately it proved impossible to compare quantum capture in different cell classes directly, and only in Musca Rl-6 (one cell only) was it possible to gain an estimate of quantum capture efficiency (26% - with respect to peak wavelength and an axial point source). The only other estimate available for fly (also Musca) is that of Kirschfeld (1966) who gives a mean Q.C.E. of 62.7%. However, there is some doubt about the origin of his recordings, and his method of derivation (analysis of root mean square noise) is questionable, since it relies upon the assumption that all noise in the response is attributable to photon shot noise - an assumption that has recently been shown to result in a significant error (Lillywhite and Laughlin, 1978).

For comparison of relative quantum capture in Rl-6 and R7/8 we have to rely upon theoretical estimates based upon angular sensitivity functions and rhabdomere lengths (c.f. Kirschfeld, 1972). For this calculation, it is assumed that quantum capture from an axial point source is proportional to the height of the angular sensitivity function, and for an extended source, proportional to the height $\times$ the width$^2$ ($\Delta \rho^2$).
Relative heights, taken from theoretical angular sensitivity functions, as in Figure 1.2 (Chapter I), are: \( R_{1-6} = 0.9; \) \( R_7 = 0.4. \) \( \Delta \beta \) values (measured), taken from Table 1.6, are: \( R_{1-6} = 1.6^\circ; R_{7/8} = 1.26^\circ. \) The effect of the rhabdomere length may be estimated from the equation for exponential absorption:

\[ A = 1 - e^{-\alpha c \ell} \] ....... Eq. (2.2)

where \( \alpha \) (the normalised absorbance coefficient) = 1.0 at peak wavelength, \( c \) (the pigment concentration per unit length) is assumed to be 1% per \( \mu \text{m} \), and \( \ell \) (the rhabdomere length) is assumed to be 200 \( \mu \text{m} \) for \( R_{1-6} \) and 130 \( \mu \text{m} \) for \( R_7 \). With these assumptions, it is calculated that individual \( R_{1-6} \) cells capture 2.7x as many quanta as \( R_7 \) from a point source, and 4.5x as many quanta from an extended source. \( R_8 \) is expected to catch slightly less than \( R_7 \) due to its shorter rhabdomere, and also because some of the incident light will already have been absorbed by the rhabdomere of \( R_7 \). This however, is for light of peak wavelength for each cell class. Quantum capture under natural conditions will depend upon the relation of the spectral sensitivity to the spectral content of the environmental light. To correct for this the spectral sensitivities of the different cell classes are convolved with the curve describing spectral content of normal daylight (Wyszecki and Stiles, 1967, Table 1.4. Colour temperature - 6500\(^\circ\)K). The correction factors thus derived, and normalised for \( R_{1-6} \), are: \( R_{1-6}, 1.0; R_7, 0.27 \) and 0.12 (for the two spectral classes); \( R_8, 0.49. \) It must also be recalled that six \( R_{1-6} \) cells with the same field of view converge onto the same second order cells (large monopolar cells = LMC's) and thus in comparison to this pathway individual \( R_7 \) and \( R_8 \) cells lose out by a factor of six.
At conditions other than absolute or incremental thresholds, one is more concerned with the voltage output as a function of intensity. This is described by the dark- and light-adapted V/log I curves, and since these are of similar shape, they can be compared (with respect to a point source) by reference to the APS\textsubscript{50}'s or the equivalent range sensitivities in the light-adapted state. Surprisingly, despite their presumed lower quantum capture, R7 and R8 are more sensitive, on the measure of APS\textsubscript{50}, than individual R1-6 cells (Table 2.1), and, as argued, this probably reflects their greater voltage gain per quantum. Interestingly, if the APS\textsubscript{50} measures are corrected for daylight (as above) then the resulting sensitivities for the different cell classes are within 0.1 log units of each other (with the exception of the pure uv class of R7 which has only 1/3 the sensitivity of the others). This situation is remarkably similar to that described in the dragonfly (Laughlin, 1976a) where it was argued that the high APS\textsubscript{50} in UV cells (also presumed to be conferred by large bump amplitudes) was to compensate for the paucity of uv quanta in the environment, and thus enable this cell type to operate at similar voltage outputs to the other cell classes. From the dark-adapted V/log I curves therefore it would seem that in the fly, as well, the different cell classes are designed to operate at similar voltage outputs. Reference to the light-adapted curves of Figure 2.5 shows that this remains true over a range of adapting intensities, as the light-adapted V/log I curves in both R1-6 and R7 shift along the log I axis to a similar degree. This is in marked contrast to the situation in vertebrate duplex retinae where
the dark-adapted rods are more sensitive (in terms of range sensitivity) than the cones, and, in addition, under light-adaptation the rods saturate completely (failing to show any further response to increments of light) at intensities where the cones are still responding and shifting their V/log I curves parallel to the log I axis (Normann and Werblin, 1974).

To summarise, it is expected that R7 and R8 inevitably capture fewer quanta than R1-6 cells, and thus have higher absolute and incremental thresholds. However, all cell classes appear designed to operate at similar voltage outputs over a wide range of intensities, and examination of the dark- and light-adapted V/log I curves show no features that would indicate a division into scotopic and photopic subsystems (cf. rod-cone eyes). The only major difference appears to be that both R7 and R8 show consistently higher levels of plateau depolarisations during light-adaptation (Fig. 2.5). This may be related to the fact that their signals must be conveyed passively to the medulla before any synaptic connections are made. If so, this could mean that an important part of their function might be to signal information regarding sustained light levels, a feature of the visual world that is lost in the R1-6, LMC pathway (See Chapter IV).

5. Comparison between different genera of flies

Caution must be exercised when comparing the properties of visual systems in different flies. In the present study only minor quantitative differences were detected between the properties of receptors of Calliphora and Musca, (apart
from those attributable to the absence of accessory pigments in the white-eyed mutant). In particular, the comparison between properties of R1-6 and R7 and R8, although performed most exhaustively in Calliphora, appears to be very similar in Musca. However, much caution must be taken when comparing these larger species with Drosophila (the other dipteran extensively used for analysis of the visual system). Thus the spectral sensitivities of the receptors have different peaks — at least with respect to R1-6 and R8 (Harris et al., 1976). In addition the pupil pigment has different spectral properties, at least with respect to the colour of back-scattered light (Franceschini and Kirschfeld, 1976).

6. General conclusions and receptor function

This study started with the aim of analysing previously unreported receptor properties relevant to the interpretation of data from other levels in the visual system. In addition, the discovery of some novel phenomena, particularly with respect to the effect of light-adaptation in R1-6 cells, has prompted the question of how these effects are generated. At present it seems likely that the pupil mechanism is responsible for the spectral shift and also, possibly, for the smaller Δp values observed during light-adaptation.

It is hoped that the functional comparison of R1-6 and R7 and R8 will aid future interpretation of visual function in the fly and help in the design of further behavioural experiments. A summary of the more important properties of R1-8 is shown in Table 2.2. The results bear out, in general terms, the predictions of absolute sensitivity and
A summary of some important properties of Rl-6, R7 and R8 in *Calliphora* and *Musca*. Values in brackets are light-adapted values (Rl-6 only). $S(\lambda)$ indicates the major peaks of spectral sensitivity, Q.C.E. (quantum capture efficiency) values are theoretical estimates derived as described in the text, and normalized to 1.0 for Rl-6. *indicates $\text{APS}_{50}$'s and Q.C.E.'s corrected for daylight (see text). Both $\text{APS}_{50}$ and Q.C.E. values are with respect to a point source, as explained in the text, in relative terms, values for an extended source are simply obtained by multiplying the value for Rl-6 by 1.64.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta\rho$ (frontal)</th>
<th>$S(\lambda)_{\text{nm}}$</th>
<th>PS</th>
<th>$\text{APS}_{50}$ (quanta$^{-1}$s.cm$^{-2}$)</th>
<th>$\text{Q.C.E}.$</th>
<th>$\text{Q.C.E.}^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calliphora:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1-6</td>
<td>1.6° (1.3°)</td>
<td>360, 490 (450)</td>
<td>2.0 (1.9)</td>
<td>2.8 x 10$^{-11}$</td>
<td>2.8 x 10$^{-11}$</td>
<td>1.0</td>
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<tr>
<td>R7</td>
<td>1.26°</td>
<td>340-360</td>
<td>2.2</td>
<td>8.6 x 10$^{-11}$</td>
<td>2.3 x 10$^{-11}$</td>
<td>0.37</td>
</tr>
<tr>
<td>R8</td>
<td>1.2°</td>
<td>540</td>
<td>1-3</td>
<td>5.8 x 10$^{-11}$</td>
<td>2.8 x 10$^{-11}$</td>
<td>(0.37)</td>
</tr>
<tr>
<td><strong>Musca:</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>R1-6</td>
<td>2.3°</td>
<td>360, 490</td>
<td>1.9</td>
<td>NOT MEASURED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td>1.6°</td>
<td>340-360</td>
<td>5</td>
<td>NOT MEASURED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td>NOT MEASURED</td>
<td>540</td>
<td></td>
<td>NOT MEASURED</td>
<td></td>
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</tr>
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</table>
spatial resolution in the different receptor classes (Kirschfeld and Franceschini, 1968). However, an unsuspected factor (namely the voltage gain per quantum) means that in voltage terms R7 and R8 are as sensitive as individual R1-6 cells. Further, light-adaptation narrows the angular sensitivity of R1-6 so that $\Delta \rho$ values are approximately the same as those found in R7 and R8 when dark-adapted. If the pupil is indeed responsible for the smaller acceptance angles, then it is to be expected that over a certain intensity range, R1-6 angular sensitivity will be more or less the same as that in R7/8, since the pupil mechanism in the central retinula cells is only activated at intensities ca. 2 log units higher than those which already saturate the pupil mechanism in R1-6 cells (Franceschini and Kirschfeld, 1976).

The results call for a reappraisal of the function of the two receptor systems in behaviour. Thus, the evidence for the hypothesis that either R1-6 or R7/8 can drive the optomotor pathway under appropriate conditions (Eckert, 1971; McCann and Arnett, 1972) is no longer tenable — a conclusion reached independently by Heisenberg and Buchner (1977) from their behavioural results in receptor deficient Drosophila mutants. However, the interaction of R7 and R8 in the pathway is by no means excluded. It has also been possible to resolve the paradox concerning polarisation sensitivity in R7/8 (Kirschfeld and Lutz, 1974; Järvilehto and Moring, 1974; Smola and Meffert, 1975).
Finally, a role for R7 and R8 in colour vision must be considered as a strong possibility. The spectral sensitivities presented here form a basis for this suggestion. In addition it has recently been demonstrated that flies (*Drosophila*) can be conditioned to discriminate colour in a phototactic choice situation (Menne and Spatz, 1977) and there is good evidence (again in *Drosophila*) demonstrating a strong influence of R7/R8 in phototactic behaviour; (Heisenberg and Buchner, 1977; Hu and Stark, 1977). If R1-6 cells are involved as well, the major peaks of spectral sensitivity in the different cell classes (R7, 340-360 nm; R1-6, 490 nm, or 450 nm when light-adapted; and R8, 540 nm) are well suited for a role in colour vision.
CHAPTER III

A NEW FUNCTIONAL CLASS OF PHOTORECEPTOR
SUMMARY

1. An anomalous class of photoreceptors, believed to represent receptors Rl-6, have been analysed by intracellular recordings in Calliphora stygia.

2. The cells (referred to as BR cells) were only recorded from frontal medial eye regions of flies caught in the wild, where they were the commonest class of photoreceptor encountered.

3. Classification as a BR cell was based upon the spectral response curve which has a single broad peak when determined with high intensity test flashes, but two peaks (at ca. 360 nm and 490 nm) with low intensity test flashes (Fig. 3.1). BR cells, classified on this criterion, also differed from normal Rl-6 cells in the following respects. a) The slopes of the \( V/\log I \) curves in BR cells vary consistently with wavelength, whereas the \( V/\log I \) curves of normal Rl-6 cells are parallel at all wavelengths (Fig. 3.3). b) Polarisation sensitivity (PS) in BR cells is strongly dependent upon both the intensity, and the wavelength with which it is measured. Thus, as the test intensity is increased, PS rises from ca. 1.0 to values as high as 10 (Fig. 3.6); PS is also highest when measured at ca. 490 nm (Fig. 3.7). In normal Rl-6 cells, PS is independent of intensity (Fig. 3.6) and the wavelength dependence of PS is quite different (Fig. 3.7).

4. Since the spectral properties of BR cells determined using an axial point source were similar to those determined when only a single facet was illuminated (Fig. 3.8), and the angular response curves in BR cells were independent of
wavelength (Fig. 3.9), it is concluded that the anomalous spectral properties of BR cells probably reflect the events taking place in single cells rather than the interaction between cells.
INTRODUCTION

Since the first intracellular recordings from fly photoreceptors (Kuwabara and Naka, 1959; Burkhardt and Autrum, 1960) the majority of electrophysiological and microspectrophotometrical evidence has indicated that the large peripheral receptors, Rl-6 form a functionally, as well as anatomically, homogeneous population. Numerous authors have reported that these receptors have two peaks in their spectral sensitivity function, at ca. 360 nm and 490 nm in Calliphora (Burkhardt, 1962; McCann and Arnett, 1972; Horridge and Mimura, 1975). Spectrophotometry of the retina (Hamdorf et al., 1963), and of the deep-pseudopupil in living, intact flies (Stavenga, 1976) has indicated that the basis for such spectral sensitivities is a rhodopsin absorbing maximally at ca. 490 nm, and a photostable, ultraviolet (uv) absorbing sensitising pigment (Kirschfeld et al., 1977).

A few reports, however, indicate that the situation may not be quite so simple. Thus both Autrum and v.Zwehl (1962) and Moring and Järvilehto (1977) have reported that a significant proportion of Rl-6 cells fail to show any differential sensitivity to the plane of polarised light, whilst others do. Further, Järvilehto and Moring (1976) have reported that not all Rl-6 cells have the same spectral sensitivity, however the limited number of cells and data points (only 5 wavelengths were used) precludes any meaningful conclusions from their results. Unusual spectral and polarisation sensitivities have also been reported from an
unidentified cell type in the fly *Eristalis* (Tsukahara and Horridge, 1977b), and interpreted as the result of intercellular interactions, on the basis of the angular dependence of the spectral properties. In the present study, a new functional class of cells was discovered in *Calliphora*, and a detailed description of their properties forms the basis of this Chapter. Whilst, in some respects these resemble the properties of the cells described by Tsukahara and Horridge (1977b), they differ in the crucial respects that led, in *Eristalis*, to the proposal of cellular interactions.
METHODS

General details of the preparation, recording and optical stimulation are described in Appendix A. Female specimens of Calliphora stygia only were used. Specific points relevant to the present study are described below.

Recording conditions were normal, except that for some experiments, the indifferent electrode was placed subcorneally at the lateral margin of the same eye into which the recording electrode was lowered. This electrode configuration results in a negligible electroretinogram (e.r.g.) response to even the brightest test flashes, and was employed to ensure that the unusual properties to be described were derived purely from intracellular potentials. As described in Appendix A, this method was later deemed to be unnecessary, as a control experiment indicated that there was no detectable difference between peak responses, recorded in this manner, and when the indifferent electrode was placed in the contralateral eye.

Most experiments were performed using a small axial source (1.8° in visual angle, unless otherwise stated), however, in some cases only single facets were illuminated (following Shaw, 1969). For this purpose, a miniature light guide was formed from a length of quartz rod, pulled in a high temperature (1700°C) glass-blower's flame to a tip diameter of ca. 20 µm. Subsequently the tapering light-guide was coated with aluminium in a vacuum depositor, and then sprayed with black paint. The latter was to minimize stray light, whilst the aluminium deposit helped to maintain internal reflection. This light guide was coupled to the quartz optics, fibre light guide (see Appendix A) and mounted
on a micromanipulator that allowed the tip of the miniature light-guide to be placed on any desired facet. The correct facet could be found quite easily as this was the only facet through which illumination evoked a response, except when using very high intensities.

The majority of the spectral data is presented as spectral response curves (Figs. 3.1, 3.5 and 3.8) since spectral sensitivity could not be calculated by the normal method (see Results, Section 2). In this case the responses were not corrected for the relative transmission of the interference filters which were only approximately isoquantal. This results in a slight distortion of the spectral response curves. However, this is similar for all the curves, and the major purpose of all the curves shown is the comparison of spectral response curves determined in different situations. In general, filters were isoquantal to within ± 15%, except for the 358 nm filter, which was ca. 50% brighter than the average.
RESULTS

1. Discovery, identification and occurrence

The anomalous class of cells, whose properties form the basis of this Chapter were discovered whilst recording from photoreceptors in the frontal eye regions of female Calliphora stygia. The first characteristic feature noticed was the shape of the spectral response curve. This was determined by measuring the peak responses to a series of approximately isoquantal flashes of light, over the wavelength range 320 nm-600 nm. When the flashes were of an intensity sufficient to generate responses greater than ca. 40% $V_{\text{max}}$, the spectral response curve was rather broad and could peak anywhere between 410 nm and 530 nm, quite unlike the twin-peaked spectral response curve typical of the majority of Rl-6 cells (Fig. 3.1). However, when the intensity was only sufficient to elicit responses of ca. 10-20% $V_{\text{max}}$, then the typical twin-peaked function was observed (Fig. 3.1). To avoid confusion, cells showing such spectral properties are referred to as BR cells (indicating their broad spectral response curve) whilst normal Rl-6 cells are referred to as UG cells (indicating the ultraviolet and green peaks of their spectral response curve). Subsequent experiments described in the following sections, showed that BR cells also differed consistently from UG cells in a number of other respects.

On no occasion was an attempt made to identify a BR cell by intracellular dye injection, and subsequent histological recovery. However, BR cells may be confidently identified with the anatomical class Rl-6 on the following grounds.
Figure 3.1 Spectral response curves in a typical BR cell determined from the peak responses to a series of isoquantal (±15%) flashes at different wavelengths. Three such curves were determined with increasing intensities. The numbers by each curve indicate the relative intensity of the test flashes in log units. The maximum response ($V_{\text{max}}$) in this cell was 63 mV. For comparison the spectral response curve of a normal R1-6 cell (UG) is shown.
a) In eye regions where they occur, BR cells are virtually the only functional class of receptor encountered and on several occasions at least 5 BR cells were penetrated in succession.

b) The other two anatomical photoreceptor classes (R7 and R8) have been recorded from and stained during the course of this work (see Appendix B). In response to low light levels R7 and R8 both showed higher levels of noise than UG cells (which have been identified as R1-6 cells - Appendix B) whereas all BR cells showed noise levels similar to those seen in UG cells.

c) In addition, all cells identified as R7 or R8 were characterised by the instability of the recordings (average penetration lasted ca. 10 minutes - up to a maximum of 1 hour) whereas BR cells showed comparable stability to UG cells, and good penetrations could be maintained for several hours.

BR cells were only penetrated in flies that had been caught in the wild. Even specimens from first generation cultures appeared to have no such cells. In addition, all recordings of BR cells were made in the frontal medial region of the eye (cells having visual axes, both vertically and horizontally, within ± 25° of the fly's longitudinal axis). Typically, the electrode track would start in a more dorsal eye region; then the first cells encountered were normal (UG) cells, but as the electrode was lowered vertically into more medial regions, the cells encountered began to show properties characteristic of the BR class. In fact, there appeared to be a continuum between the properties of BR cells and UG
cells, the characteristics of the BR class becoming more pronounced as the electrode moved from dorsal (frontal) to medial (frontal) eye regions. Other eye regions were not systematically explored for BR cells.

The experiments described below were performed upon BR cells from the more extreme end of the continuum, and these are contrasted with parallel (control) experiments performed upon typical (UG) cells (from dorsal eye regions in the same animals, or from cultured flies).

2. Spectral properties

a) $V/\log I$ curves and spectral sensitivity

The standard method of calculating spectral sensitivities used throughout this work, and by many other authors (e.g. Burkhardt, 1962; Autrum and V.Zwehl, 1964) converts responses to sensitivities by referring them to a single $V/\log I$ curve (Appendix A). This method assumes that $V/\log I$ curves measured at different wavelengths are parallel, and if this is so, spectral sensitivity is independent of response criterion. The fact that the spectral response curves in BR cells change their form with the test intensity (Fig. 3.1) indicates that this assumption is false. For these cells therefore, the standard method should be abandoned and spectral sensitivity must be defined with respect to a fixed response criterion, and calculated from the intensity required to generate that fixed response at each wavelength.
To accurately describe the spectral properties of BR cells, \( V/\log I \) curves were determined at a variety of wavelengths, and analysed with respect to slope and position on the \( \log I \) axis. Such data describe the systematic deviation of the curves from the parallel situation, and spectral sensitivity can be defined with respect to any response criterion desired. This analysis requires particularly stable recording conditions, and only recordings in which the resting potential, \( V_{max} \), and response to a standard test flash (a monitor of absolute sensitivity) varied by less than 2 mV, were accepted. To avoid human error, or bias, the \( V/\log I \) curves were fitted to a third order polynomial expansion, by a least squares fit (Laughlin, 1974a) and the slopes computed as the tangent at the 50% \( V_{max} \) level.

The \( V/\log I \) slopes in BR cells showed a consistent dependence upon wavelength that may be seen by eye in Figure 3.2, and is plotted quantitatively in Figure 3.3. The most pronounced feature is the low slope value at 358 nm, whilst steepest slopes are found at ca.400 nm and 570 nm. Interestingly, the shape of the \( V/\log I \) slope vs wavelength curve is inversely correlated with the shape of the normal Rl-6 cell's spectral sensitivity. As a control similar experiments were performed in normal (UG) cells. In contrast, the \( V/\log I \) curves were parallel at all wavelengths tested (essentially as found by Streck, 1972). On average the slopes in BR cells were less steep than in normal (UG) cells, except at the wavelengths where BR cell slopes reach their maximum values (Fig. 3.3). This suggests that the
Figure 3.2 A series of V/log I curves determined in a single BR cell at four different wavelengths. The curves are all normalised to the same $V_{\text{max}}$ (65 mV) as this was independent of wavelength. Each curve is labelled with the wavelength (in nm) and it can be seen by eye that those in the ultraviolet (333 and 358 nm) are less steep than those measured in the green/yellow (541 and 572 nm). Intensity scale is relative only, and the curves at 541 and 572 nm have been shifted (to the left) by 0.5 log units to prevent overlap.
mechanism(s) responsible for the peculiar spectral properties of BR cells act by reducing the effectiveness of quanta of certain wavelengths at higher intensities.

Spectral sensitivity was measured in one BR cell at a variety of response criteria from a family of V/log I curves. The result essentially confirms the observation from the spectral response curves, namely, that at low response criteria a twin-peaked function, similar to that seen in normal R1-6 cells (UG), is observed, and that this is converted to a single-peaked function as the criterion is raised (Fig.3.4).

b) Chromatic adaptation

Some preliminary observations were made upon the effects of intense chromatic adaptation in BR cells, in an attempt to gain evidence regarding photo-pigment identity and function. Illumination by intense blue light (451 nm) was found to induce a prolonged depolarising afterpotential (PDA) similar to that seen in normal (UG) cells following such illumination (Muijser et al., 1975). Again as in normal cells, this PDA could subsequently be knocked down by illumination with intense red (600 nm) light.

Following illumination with intense blue light, the spectral response curve of BR cells showed the twin-peaked appearance characteristic of UG cells, however, following intense illumination by red light, the broad spectral response curve typical of BR cells was observed (Fig. 3.5). These observations suggest that the manifestation of the BR cell's anomalous spectral properties is dependent upon the rhodopsin/metarhodopsin equilibrium.
Figure 3.4 Spectral sensitivity of a single BR cell as a function of response criterion. The curves were derived from a series of V/log I curves at ten different wavelengths. At the lowest response criterion used, the characteristic twin peaked function of normal Rl-6 cells (UG cells) is obtained. However, as the response criterion is raised, there is a gradual suppression of the uv peak, and a shift of the visible peak towards shorter wavelengths.

o...o 10% $V_{\max}$ response criterion

•---• 20% $V_{\max}$ response criterion

Δ---Δ 40% $V_{\max}$ response criterion

□ — □ 60% $V_{\max}$ response criterion
Figure 3.5  Spectral response curves in a BR cell determined following adaptation with monochromatic light of 451 nm and 600 nm. The adapting intensity of approximately $10^{13}$ quanta. cm$^{-2}$.s$^{-1}$ was applied for several minutes. The test intensity was the same for each determination. The overall lower values following adaptation at 451 nm may be explained by the effect of bleaching and metarhodopsin screening. (Rosner, 1975).
3. Polarisation sensitivity

a) Polarisation sensitivity as a function of intensity

As was the case with spectral sensitivity, it was discovered that polarisation sensitivity (PS) in BR cells was dependent upon the intensity at which it was measured. If the responses to flashes of polarised light (E-vector direction being rotated 10° between successive flashes) were converted to sensitivities through a V/log I curve, the PS varied from insignificant levels when using low intensity test flashes, up to values as high as 12, when using intensities sufficient to generate responses of ca. 75% \( V_{\text{max}} \). Once again, therefore, it is, strictly speaking, incorrect to use the above method for calculating sensitivity. Consequently, having first determined the orientation of the preferred and orthogonal E-vector directions (directions generating maximum and minimum responses respectively), V/log I curves were determined, first with the preferred E-vector direction, and then with the orthogonal E-vector direction. The experiment was then repeated to ensure that any effect was reproducible. As shown in Figure 3.6 such curves are not parallel, the curve for the orthogonal direction being considerably less steep than that for the preferred direction. A PS value for any chosen response level may be determined from the separation of the two curves at the desired response criterion\(^1\). This, more correct method validates the

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\(^1\) This method assumes that the preferred E-vector direction is independent of intensity, and this was confirmed by complete polarisation 'runs' at different intensities.
previous observation, showing that PS rises from ca. 1.0 to values as high as 10 with higher response criteria (Fig. 3.6).

Similar experiments were also performed in normal (UG) cells as a control. In this case the V/log I curves at different E-vector directions appear parallel, and PS remained constant, at ca. 2.0, for all response criteria (Fig. 3.6).

b) Polarisation sensitivity as a function of wavelength

PS in BR cells was also found to depend quite critically upon the wavelength with which it was measured. The intensity dependence of PS just described was investigated using light at 499 nm, which was found to generate the largest polarisation sensitivities. To determine PS at each wavelength from V/log I curves (as described above) is a prohibitively long experiment, and therefore a simpler method was devised. For this purpose, isoquantal spectral runs were performed using polarised light first with the preferred E-vector direction, and then the orthogonal E-vector direction. At each wavelength, the difference in responses between runs was converted to polarisation sensitivity through a V/log I curve. Reproducibility was ensured by repeating the experiment at least once in each cell. The use of this method for calculating sensitivities when V/log I curves are not parallel may be justified as follows. During an isoquantal spectral run with polarised light, response amplitudes typically vary by less than 5 mV in the range 350-550 nm. From curves, such as those shown in Figure 3.6, it can
be estimated that the variation of PS due to different response levels, would amount to less than ± 0.15. However, PS varies by at least ± 0.5 between 350-550 nm (Fig. 3.7), and one can thus be confident that this variation is largely due to the influence of wavelength, rather than the varying response heights. This method for determining the wavelength dependence of PS also assumes that the preferred E-vector direction is the same at all wavelengths. In each cell this was checked at a few selected wavelengths, and generally speaking found to be true, except that in two cells it was found that the preferred E-vector direction shifted by 40-80° at wavelengths longer than 570 nm. In other BR cells there was little or no PS detectable at such wavelengths.

The wavelength dependence of PS was determined in 9 BR cells. The results were consistent between cells, all showed maximum PS in the green at ca. 490 nm, and PS values fell quite sharply at both longer and shorter wavelengths (Fig. 3.7).

Similar experiments were also performed in normal (UG) cells. The results were also consistent between cells and are clearly different from those of BR cells. Over most of the range, PS appeared relatively independent of wavelength, however, values fell to ca. 1.0 in the uv, and increased slightly at wavelengths longer than 500 nm (Fig. 3.7). Again the preferred E-vector direction was the same at all

1 Even assuming the most severe PS/intensity dependence observed in any BR cell, at any wavelength.
Figure 3.7  Polarisation sensitivity (PS) as a function of wavelength in UG cells and BR cells. The method used for the determination is described in the text. Each point represents the averaged PS from 9 BR cells (●-●-●) or 8 UG cells (○-○-○). Error bars indicate ± 1.0 S.D. Although these appear rather large, this is mainly due to the variation in absolute values, rather than relative trends. In fact every BR cell investigated showed maximum PS at 484 or 499 nm. For BR cells, the PS/wavelength functions were determined at a response level of ca.50-60% \( V_{\text{max}} \).
wavelengths, except that in two cells, the direction was again found to be shifted at wavelengths longer than 570 nm. The phase-shifted PS in the uv, described in the same species by Horridge and Mimura (1975) was not observed however, and in general PS could not be detected in the uv, in either UG or BR cells.

4. Spatial properties

Receptor cells with properties, similar in some respects to those of BR cells, have been reported previously in the fly *Eristalis* (Tsukahara and Horridge, 1977b). Their conclusion was that the properties could be explained by the interaction between cells with different spectral and polarisation sensitivities. To test whether such a mechanism could account for the present results, the spatial dependence of spectral properties in BR cells was examined in some detail.

Presumably, at least two sorts of cell-cell interactions could occur: a) between cells in the same ommatidium, b) between cells in different ommatidia whose rhabdomeres have the same field of view and whose axons converge upon a single lamina cartridge (Braitenberg, 1967; Kirschfeld, 1967) where interactions could take place. The latter group are referred to as members of the same neuroommatidium. The normal method of stimulation (by a small axial source) stimulates both these groups of cells, since the visual fields of neighbouring cells
in the same ommatidium overlap considerably. To test whether one or other of these possible interactions is responsible for the anomalous response properties, however, it is desirable to restrict stimulation to only one of these groups. To stimulate only the members of one anatomical ommatidium, therefore, light was delivered through a miniature quartz light guide (tip diameter 20 µm) onto the facet in front of the recorded cell. By testing the response to stimulation of neighbouring facets, it could be demonstrated that the effective light entering a neighbouring ommatidium was less than 1% of that in the ommatidium directly illuminated.

Having first characterised a BR cell with an axial point source, its spectral properties were measured again, but now using the technique of single facet illumination. Spectral response curves determined by either method were very similar (Fig. 3.8). In addition, the characteristic wavelength dependence of the V/log I slopes (Fig. 3.3) was also demonstrated using the single facet technique.

Because so little light enters neighbouring facets with this technique, these results can only be explained on a cell-cell interaction hypothesis if the interacting cells are in the same ommatidium. Since these cells all have divergent visual axes (except for R7 and R8 which share a common, tiered rhabdom) wavelength specific interactions should be

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1 This can be estimated from the ratio between the acceptance angle, Δ and the interommatidial angle, Δ. When \( \frac{\Delta}{\Delta} = 0.9 \) as estimated for *Musca* by Snyder (In press) neighbouring visual fields overlap at the 40% sensitivity level (approx.).
Figure 3.8 The spectral response curve of a BR cell determined, first with an axial point source (o-o-o), and then using a miniature quartz light guide placed on a single facet (●-●-●). Each point represents the peak response to a 100 ms flash of light. Flashes at each wavelength were isoquantal to within ±15%.
reflected in wavelength dependent angular response functions. To search for indications of such interactions, angular response functions were determined at different wavelengths. For this experiment, pairs of wavelengths were presented sequentially (separated by 200 ms) through the same point source (0.1°), making use of the mixed beam method of stimulation (Appendix A). Angular runs were then made simultaneously at each wavelength by moving the point source in 1/2° or 1/4° steps through the cell's visual axis in horizontal and vertical directions. Figure 3.9 shows the results of a number of such runs made with a variety of wavelength pairs. In no case was there a significant difference between responses at different wavelengths, and in particular the position of the highest response was the same at each wavelength (cf. Tsukahara and Horridge, 1977b). Furthermore, at all wavelengths tested the receptive fields were narrow and symmetrical (\( \Delta \rho \approx 1.5^\circ \)), consistent with the responses being derived from only one cell.

All measurements thus far described, have been made from the heights of the peak responses. The paired flash presentation just described was also used to investigate other aspects of the waveform. Again two different wavelengths were chosen, and the intensities adjusted so that the peak responses to each were identical. Plateau height was then investigated with a variety of wavelength pairs and in different angular positions. In no case could a combination be found that resulted in different plateau heights when the peak responses were the same (cf. Tsukahara and Horridge, 1977b).
From such observations it is concluded that measurements of the peak height alone are sufficient description of the cell's voltage responses.
Figure 3.9  Angular response curves from a single BR cell. The curves were determined simultaneously at two wavelengths, as described in the text. The 'point' source used subtended 0.1° at the eye, and two runs were made for each wavelength pair. The wavelengths used (in nm) are indicated on each plot. The runs shown were all made in the horizontal direction, but similar results were also obtained in the vertical direction.
DISCUSSION

1. The possibility of interaction between cells

The anomalous class of BR cells was originally defined on the basis of their intensity (or response level) dependent spectral response curve, which shows a single broad peaked function when using a high test intensity. Subsequent experiments showed that BR cells, thus defined, also differed from typical (UG) cells in the following respects. 1. The \( V/\log I \) slope varies consistently as a function of wavelength (Fig. 3.3); 2. The \( V/\log I \) slope also varies consistently with E-vector direction (Fig. 3.6); 3. Polarisation sensitivity shows a characteristic dependence upon the wavelength with which it is measured (Fig. 3.7).

The first question asked is whether these properties reflect the events taking place in one cell, or whether cell-cell interactions are responsible for this anomalous behaviour.

The arguments presented in the last section (Results, Section 4) make it very unlikely that intercellular interactions are responsible for the anomalous properties of BR cells. However, they cannot rigorously exclude, for example, a diffuse interacting 'network' of cells. The critical experiment requires the determination of spectral properties in BR cells when only a single rhabdomere is illuminated. However, the optical techniques required for such stimulation (Kirschfeld, 1972) were not available in the present study.
Because the conclusion that intercellular interactions are not responsible for the properties of BR cells, is exactly the opposite to that reached by Tsukahara and Horridge (1977b), it is worthwhile to compare the two sets of data in some detail. The following points of similarities exist between BR cells and the anomalous cell type reported from *Eristalis*.

1. Both show a broad spectral response curve with no clearly defined peak

2. Both have $V/\log I$ curves whose slopes vary with wavelengths

3. Both were recorded in frontal medial regions of the eye

4. Both can show a phase-shifted PS at long wavelengths.

However, the two cell types differ in the crucial respects that allow the conclusion of interaction. Thus, in *Eristalis* it was demonstrated that the angular position of a point source generating maximum response was wavelength dependent, whereas, in the present study the opposite was observed (Fig. 3.9). In addition, in *Eristalis*, the wavelength and angular position of the point source were found to influence the response waveform (with respect to relative peak and plateau heights), whereas no such effect could be observed in the BR cells in *Calliphora*. Finally, the phase-shifted PS at longer wavelengths, was not found to be a unique property of BR cells in the present study.
Despite some striking similarities, therefore, the initial conclusion that the properties of BR cells reflect the events taking place in single cells, is adhered to.

2. Interactions within a single cell

The finding, that the spectral sensitivity of photoreceptors is a function of response criterion, is not unique. For example, Adolph (1968) described a reduction in short wavelength sensitivity at higher response criteria in Limulus lateral eye, and Chappell and DeVoe (1975) described non-parallel V/log I curves in the green and uv in dragonfly ocellar photoreceptors. The latter authors concluded that such phenomena can only result from the interaction of two photopigment systems, whether they be in the same or different cells.

As argued in the last section, the hypothesis that cell-cell interactions can account for the anomalous spectral properties of BR cells is more or less excluded. Therefore, following the logic of Chappell and DeVoe (1975), one might consider the possibility that two or more photopigments exist in the same rhabdomere, and that these have different absorption spectra, dipole alignments and intensity/response characteristics.

There are two lines of evidence suggesting that BR cells contain (at least) a similar photopigment system to that found in normal (UG) cells. 1) Under certain conditions BR spectral response curves resemble those of normal (UG) cells (namely at low intensities, Fig. 3.1, and following
blue adaptation, Fig. 3.5). 2) PDA's and knock-down effects are elicited by similar wavelengths in both BR and normal cells. The normal photopigment system in R1-6 cells is already known to consist of two components, a 490 nm rhodopsin and a uv-sensitising pigment (Kirschfeld et al., 1977). If the energy transfer from the uv sensitising pigment to the rhodopsin became less efficient at higher intensities in BR cells, then the loss of the uv sensitivity peak at higher response criteria (Figs. 3.1, 3.4) might be explained. However, this comparatively simple notion cannot account for the remaining spectral properties, or the dependence of PS upon intensity and wavelength (Figs. 3.6, 3.7). Whilst further photopigments might be hypothesised to account for these properties, it is felt, that in contrast to the view of Chappell and DeVoe (1975), it is not in fact necessary to invoke extra photopigments to explain such phenomena. Instead it can be proposed that there may be interactions between similar photopigment molecules that are dependent upon the local density of quantal absorption. For optical reasons (e.g. pigment absorbance spectra, exponential absorption and rhabdomeres twisting) the density of quantal absorption within a rhabdomere is expected to depend upon both wavelength and E-vector as well as intensity. To produce a satisfactory model at this stage, however, would require too many ad hoc assumptions regarding mechanisms of transduction and the arrangement of photopigment in rhabdomeres. Consequently, with the available data, and our current knowledge of transduction, it is not considered profitable to speculate further. Instead, it is noted that a successful theory must
explain how the effectiveness of incident quanta is reduced at higher intensities, in a manner that is strongly dependent upon the E-vector direction in particular, and also the stimulus wavelength. That a satisfactory theory cannot be formulated with our current knowledge of transduction, implies that new concepts may have to be developed to explain these and similar phenomena.

4. Comparison with other data

The present data have already been compared with those of Tsukahara and Horridge (1977b), however, there are two other sets of data that warrant discussion in light of the present results.

Firstly, Horridge and Mimura (1975) described a significant polarisation sensitivity in the uv, that was phase-shifted from that seen in the green. In view of the demonstration that a sensitising pigment is largely responsible for uv sensitivity in R1-6 cells (Kirschfeld et al., 1977), this result may find an explanation in the relative dipole alignments of the sensitising pigment and the rhodopsin. In the present study, although the same species was used, PS in the uv was never detected in either BR or UG cells. The reason for this discrepancy is not obvious, however, it should be mentioned that the diet of the cultures used in the earlier work included less liver (rich source of vitamin A) than the diet of the cultures used in the present study (Horridge, personal communication). Furthermore, the spectral sensitivities of the cell's in Horridge and Mimura's study were different from those seen
here (in UG cells). In particular the uv peak was higher, and the green peak, more sharp. Therefore, it might be expected that the rhodopsin concentrations, and the ratio between rhodopsin and the uv-sensitising pigment, were different in the two studies. An explanation for the discrepancy may thus be sought in terms of the mechanism of energy transfer between the uv-sensitising pigment and the rhodopsin, which, if a resonance transfer (Förster, 1965) is assumed, may depend upon both the molecular spacing, and the dipole alignments of the molecules involved. Until more is known about the details of the mechanism of energy transfer in this system, a complete explanation may not be given, however, any hypothesis that accounts for the details of energy transfer, should also account for these findings.

Secondly, it has been reported that ca. 50% of Rl-6 cells in Calliphora were insensitive to the plane of polarised light (Moring and Järvillehto, 1977). In the present study, however, Rl-6 cells always showed a significant PS, except that some BR cells showed very little, or no PS when measured with low intensities, and this possibly is what Moring and Järvillehto (1977) observed. The possibility of an artifact being responsible for their results must also be considered however. This is suggested by an observation made during the present study, namely that some Rl-6 cells fail to show any PS when first penetrated, but when tested, perhaps half an hour later, a normal PS (ca. 2.0) is observed. An interpretation for this (and also possibly for Moring and Järvillehto's results) is that initially the electrode
penetrates two cells simultaneously, and since PS in adjacent cells is ca. 60° out of phase, the resultant may be expected to be approximately flat. Later, the hole may seal around the electrode, thus isolating a single cell and restoring PS in the responses. Such artificial coupling might also account for the different temporal properties of polarisation sensitive and insensitive cells reported by Moring and Järvilehto (1977). Alternative explanations, such as degree of rhabdomere twist (Smola, 1977), physiological coupling or some molecular difference in membrane architecture may also be considered, and without further information the significance of Moring and Järvilehto's results is not apparent.

5. Implications and conclusions

The description of a new functional class of photoreceptors is important because it can aid the interpretation of data from other levels in the visual system. It is even possible that some existing behavioural data may find an explanation in the properties of BR cells. Thus both Eckert (1971) and McCann and Arnett (1972) reported that the spectral sensitivity of the optomotor response in Musca changes from a twin-peaked (360 nm and 490 nm) function at low intensities to a single-peaked blue function at higher intensities. However, as discussed at length in Chapter II, alternative explanations for this behaviour also exist.

Notwithstanding, the major significance of these results may eventually prove to be with respect to concepts of
transduction in photoreceptors since current knowledge seems insufficient to explain the unusual spectral and polarisation properties. Moreover, it seems unwise to dismiss these results as a peculiar feature of an insignificant class of cells. Thus the apparent continuum between BR cells and normal (UG) cells suggests that there is only a quantitative difference between the two sorts of cells. Possibly this might be as simple as the rhodopsin concentration, since following bleaching with light at 451 nm the spectral response curve in BR cells appears similar to that in normal (UG) cells (Fig. 3.5). Further as already mentioned, these sorts of results are not unique, and also might be expected to be overlooked, bearing in mind the prolonged stable recording required for their analysis. It is thus possible that properties, such as those described here, may be an indication of some widespread feature(s) of transduction yet to be discovered.
SUMMARY

1. Peripheral effects of light-adaptation are studied from intracellular recordings of the photoreceptors, R1-6, and the large monopolar cells (LMC's) in the fly, Calliphora stygia (wild type).

2. The use of identical stimulus regimes for each cell type allowed the isolation and identification of consecutive stages of the light-adaptation process.

3. The receptors show two components of adaptation, a fast phase occurring within 100 ms, and a slow phase taking several seconds to complete (Fig. 4.1). This adaptation translates the receptor V/log I curves along the log intensity axis without affecting their shape or slope (Fig. 4.3). Adaptation is negligible at low intensities, but with stronger adapting lights, changes in range sensitivity (Table A.1, Appendix A) become equal to background increments (Fig. 4.7).

4. The responses of LMC's reveal strong adaptation mechanisms acting in the lamina at intensities which induce negligible adaptation in the receptors. These mechanisms generate hyperpolarising 'on' transients and depolarising 'off' transients, and annihilate the response to maintained backgrounds (Fig. 4.2).

5. At all background intensities investigated the slope of the LMC V/log I curve remains ca. 8x that of the receptors (Fig. 4.5), suggesting that the first synapse operates with a constant gain, independent of background intensity. This
results in the steep slopes and narrow dynamic ranges (± 0.5 log unit about the mean) of the light-adapted LMC curves (Fig. 4.4).

6. During dark-adaptation, fast lamina mechanisms can be seen superimposed upon slower receptor processes (Fig. 4.9).

7. A comparison of these results with studies of behaviour and of higher order visual interneurons, suggests that the peripheral processes of light-adaptation play an important limiting role in the performance of the visual system as a whole.

8. There are probably several mechanisms of adaptation in the receptors, including membrane/ionic effects, photopigment equilibria, and the longitudinal pupil mechanism (Kirschfeld and Franceschini, 1969). In LMC's, it is suggested that a dominant effect is one of subtraction of the voltage signal at the receptor terminals, possibly by a depolarisation of the extracellular space.

9. A comparison of the results with data from other animals, including the lower vertebrates show that a "log transform-subtraction-amplification" strategy of light-adaptation is widely used, and the advantages of this in preserving maximal detail of contrast excursions expected in the real world are discussed.
INTRODUCTION

Light-adaptation must be considered a fundamental function of any sophisticated visual system. Environmental light intensities range over ten orders of magnitude, whilst the intensity range over which peripheral neurons and photoreceptors operate is limited to perhaps 4 or 5 log units or less.

The ways in which visual systems cope with this problem have been studied most in vertebrates, where a variety of mechanisms have been identified that help to extend this range. These include photomechanical attenuation of light flux (e.g. pupil mechanism), intensity dependent spatial summation, and ionic and neural processes acting upon the receptors and neurons of the retina. (Dowling, 1967; Barlow, 1972; Werblin, 1973).

There are comparatively fewer studies of light-adaptation amongst the arthropods. The majority have concentrated at the most peripheral level, describing photomechanical responses (incl. pigment migration, Walcott, 1975; Kirschfeld and Franceschini, 1969), photochemical effects (Hamdorf et al., 1973; Rosner, 1975) and receptor membrane and ionic processes (Bader et al., 1976; Lisman and Brown, 1975). There are also several accounts of light-adaptation at a neural or behavioural level (Glantz, 1971; 1972; Rowell and O'Shea, 1976; Reichardt, 1969; Eckert, 1973; Wolf, 1933; Labhart, 1974), but apart from a preliminary report (Laughlin, 1975), no one has followed the vertebrate lead, and isolated consecutive stages of the light-adaptation process by starting
at the receptors and working inwards. (Normann and Werblin, 1974; Werblin, 1974; Werblin and Copenhagen, 1974).

In the retina and lamina of the fly there are two classes of cells which are well suited for this approach. Firstly, the receptors R1-6, which have short axons terminating in the lamina; and secondly the large monopolar cells (LMC's), L1 and L2 which receive a direct input from 6 R1-6 cells with the same field of view. With the reservations mentioned in Chapter III, both these classes of cells form effectively homogeneous populations.

A comparison of light-adaptation processes in these two classes of cells has been undertaken with a view to identifying the sites and mechanisms of adaptation, and to see which of the phenomena, observed at this peripheral level, play a limiting role in the visual system as a whole. Finally, the results are compared with data from other insects and also the lower vertebrates. The analogies drawn suggest that a common strategy is employed by a variety of animals, and the functional significance of this is discussed.
METHODS

General details of the preparation, recording, and optical stimulation are described in Appendix A. Criteria for identifying cells are explained in Appendix B.

Rather specialised stimulus paradigms were employed for the present study, and these are described as follows:

Optical stimulation

The majority of experiments were performed using broad field illumination, with spatially coincident test and adapting beams (mixed beam method, Appendix A). The broad stimulus (17° in visual angle) was achieved by placing a diffusing screen made from lens tissue (spectrally flat in the range 350 nm-650 nm) in front of the light guide. The screen could be readily swung out of the light beam, so as to allow the whole assembly to be accurately centred on the cell's visual axis, by using the light guide as a point source.

All parameters (intensity, duration, timing, spectral content) could be accurately and independently controlled for each beam. In most experiments, incremental test flashes were superimposed upon the adapting light, but for some experiments, an alternating shutter arrangement was employed (Appendix A), whereby the test beam is instantaneously substituted for the adapting beam, so that responses to intensity decrements, as well as intensity increments, could be investigated.
Experimental protocol

Most experiments involved the determination of light and dark-adapted intensity/response functions, the steady membrane potential set up by backgrounds and the changes in response waveform with adaptation. Unless otherwise specified, all experiments were performed with white light. The experimental protocols were as follows.

\textit{i) Twin beam experiments.} The end of the light guide was centred upon the axis of the unit and a dark-adapted intensity/response function was determined with a point source at peak wavelength by delivering a series of flashes with intensities incremented in approximately 0.25 log unit steps. The diffuser was placed in position and using white light, a further dark-adapted intensity/response function determined with a test beam. The first adapting background was applied with the adapting beam\textsuperscript{1}, and following the equilibration of membrane potential and sensitivity (usually 2 mins.) a series of superimposed test flashes was delivered to determine the light-adapted intensity/response function at that background. This process was repeated for several backgrounds, each one brighter than the one before. Following the brightest background, the unit was dark-adapted for as long as recording conditions permitted, or until fully dark-adapted. During this dark-adaptation the unit's sensitivity was monitored with low intensity test flashes. Full dark-

\textsuperscript{1} The intensity of the adapting beam was adjusted to equal (in effective intensity) a value set by the test beam, using the alternating shutter arrangement described below.
adaptation was usually possible and a final intensity/response function, for test and adapting lights showed that sensitivity was restored.

**ii) Alternating shutter experiments.** The intensity/response function for a monochromatic point source was determined (as above) with the test beam and repeated for the broad field stimulus with white light. The adapting beam was then adjusted in intensity so as to equal (in effective intensity) a value set by the test beam. This was achieved by alternating the shutters and adjusting the adapting intensity (via a neutral density wedge) until a null response was observed. This method means that only one of the beams needs to be calibrated. Once the adapting intensity is set, and 2 minutes allowed for steady state to be reached, the intensity/response function is determined by substitution of the test beam for 100 ms intervals, starting with intensity decrements and increasing in 0.25 log unit steps, through the null position to the new saturating value. As before, the process is repeated for successively brighter backgrounds and the experiment concluded by following the course of subsequent dark-adaptation.

**Normalisation of stimulus intensities and cell responses**

**Intensities:** Both photoreceptors and LMC's show a scatter in absolute sensitivities, although this is greatest for LMC's (Laughlin, 1973). To enable results from different cells to be best compared, all intensities are expressed as log units above the intensity required to give a 50% $V_{\text{max}}$
'on' response in the dark-adapted LMC. For LMC's this value (the LMC DAI_{50}) is simply determined from the dark-adapted intensity/response function routinely determined in every unit studied, and intensities for that unit are then expressed relative to this value. For retinula cells however, a conversion must be made if the intensity values are to be compared with LMC's. The receptor DAI_{50} is determined from the intensity/response function, and then intensities are expressed as log units above this value plus a factor derived from the average difference (in log units) between receptor and LMC DAI_{50}'s (2.5 log units). In absolute terms, the LMC DAI_{50} is equivalent to $1.3 \times 10^8$ quanta.cm$^{-2}$.s$^{-1}$, when referring to axial quanta of peak wavelength (484 nm) from a point source. Values for fly photoreceptors are found in Table 2.2 (Chapter II).

Responses: All voltage response amplitudes were measured relative to the dark-adapted resting potential because there was little drift during recording. Response amplitudes were normalized for both retinula cells and LMC's to % maximum 'on' response from the dark-adapted unit (% $V_{\text{max}}$).
RESULTS

Retinula cells, R1-6, and the large monopolar cells (LMC's), L1 and L2 were subjected to identical regimes of light- and dark-adaptation, and their performance investigated over a 5 log unit range of intensities. The ways in which the peripheral visual system light-adapts is described and those sensitivity changes occurring in the receptors are distinguished from those generated at the level of the second order cells. The results summarise the responses of receptors and LMC's to sustained backgrounds and superimposed test flashes, both in terms of response waveforms, and intensity/response functions. The rapid time course of light-adaptation is described, and some observations on dark-adaptation presented. Finally, an account of some spectral aspects of light-adaptation is given.

The broad background (17° in visual angle) should illuminate uniformly at least 40 ommatidia, so that the stimulus approaches that pertaining to natural conditions. The test stimulus was spatially coincident with, and had the same angular subtense as, the adapting beam. Preliminary experiments established that there were no major differences between the light-adapted LMC responses to smaller, axial, test spots (1.8°) and the larger fields used here.

1. Response waveforms

a) Photoreceptors.

As previously described by several authors (e.g. Burkhardt, 1962; McCann and Arnett, 1972) dark-adapted fly retinula cells show responses typical of arthropod
photoreceptors. At low intensities (up to 2.0 log units above the LMC DAI$_{50}$) there is a maintained depolarisation with little or no adaptation in response height during continuous light. At higher intensities two phases of adaptation (expressed in response level) may be detected. The first (fast phase) occurs within 100 ms, and consists of a rapid attenuation of the peak transient to an initial plateau level. Following this there is a slow phase, lasting up to 60 seconds, during which the plateau level slowly declines to a steady state level (Fig. 4.1). During maintained light, the receptor potential is never observed to return to the resting potential, and thus the receptors always monitor the presence of a continuous light with a maintained depolarisation.

If incremental test flashes are superimposed upon a constant background, incremental responses are seen to ride on top of the plateau response (see Chapter II, Fig. 2.2). These differ from the dark-adapted waveforms in that the transient-plateau (fast phase) is quicker, and at the termination of the incremental flash, there is a transient after-hyperpolarisation, the magnitude of which is dependent on both the intensity and the duration of the incremental test flash (Fig. 4.1). It seems probable that this after-hyperpolarisation represents a transient suppression of sensitivity to the background, induced by the additional adaptation generated during the test flash.

b) LMC's

The responses of dark-adapted LMC's in the fly have also been described previously (Järvinehto and Zettler, 1971).
Figure 4.1 Dark and light-adapted response waveforms in a photoreceptor (R1-6). For the dark-adapted responses (DA), the stimulus intensities were: 1.2, 2.6 and 4.5 log units (all intensities expressed as log units above LMC DAI_{50}). The cell was then weakly light-adapted (I_a = 2.3 log units) and incremental responses of similar sizes were elicited (LA). The lowest trace shows the response of the same cell (initially dark-adapted) to a prolonged saturating stimulus, and illustrates the fast (F) and slow (S) phases of light-adaptation.
Similar responses have been observed in LMC's of dragonfly (Laughlin, 1973), bee (Menzel, 1974) and locust (Shaw, 1968).

At intensities where receptors show little or no adaptation in response levels, the LMC's show a highly phasic hyperpolarising response, which anatomical (Strausfeld, 1976b) and electrophysiological (Järvilehto and Zettler, 1971) evidence suggests is generated by synaptic input from the photoreceptors R1-6.

At least three phases of the waveform may be distinguished: a) a rapid hyperpolarising 'on' transient, which is rapidly attenuated to, b) a plateau phase which, itself usually returns to the resting level within 1/2 second; c) at the termination of the flash there is a depolarising 'off' transient which returns to the resting potential within 100-200 ms. (Fig. 4.2). The exact shape of the waveform depends upon the area of stimulation. Compared to the response generated by an axial point source, a broad field stimulus (17°) generates a response which is characterised by a more severe and rapid attenuation of both 'on' and 'off' transients (Fig. 4.2). Thus lateral, or intercartridge inhibition, previously inferred from measurements of LMC receptive fields (Zettler and Järvilehto, 1972; Laughlin, 1974b), is partially responsible for the annihilation of the response to backgrounds.

An additional feature, not always observed, is the presence of a spike-like response on the leading edge of the depolarising 'off' transient. By analogy with a similar response observed in second order cells of the locust
Figure 4.2: The response waveforms of dark and light-adapted LMC's and the effect of stimulus area on adaptation. The responses of dark-adapted units to an axial point source (DA 0.4°) and to a wide field stimulus (DA 17°), together with the light adapted responses to wide field of illumination (LA) are recorded from a single cell. The waveforms of the dark-adapted responses depend upon stimulus area. The adapting intensity (2.3 log units) was the same as that in Fig. 4.1, demonstrating that the LMC waveform undergoes significant changes at adapting intensities that hardly affect the receptor waveform.
ocellus (Wilson, 1978b), it is suggested that this spike is an action potential generated some distance from the recording site, possibly on an area of excitable membrane in the medulla.

During a constant adapting background, the LMC registers no maintained response, aside from a significant noise level whose peak-peak amplitude is related to the intensity of the background. Like shot noise in the receptors (Dodge, Knight and Toyoda, 1968) this initially increases with intensity, and then declines to levels lower than the membrane noise in the dark. There is evidence to suggest that similar noise in locust ocellar neurons represents responses to single quanta of light, transferred across the first synapse from 'quantum bumps' in the receptors (Wilson, 1978a).

Incremental test flashes, presented during such a maintained background, again generate triphasic waveforms in the light-adapted LMC's. They differ from the dark-adapted responses in having more rapid transients and no clear plateau phase. In addition the relative amplitudes of the 'on' and 'off' transients change, the 'off' transient becoming larger, whilst the 'on' transient reduces in size (Fig. 4.2).

2. Intensity/response functions

a) Photoreceptors.

Intensity/response functions were determined by measuring the responses to 100 ms test flashes given in the dark, or superimposed upon a variety of constant adapting backgrounds. For light-adapted determinations, at least 2 minutes was
allowed after application of the background to ensure that sensitivity had reached equilibrium. Intensity/response functions were plotted in terms of total voltage (measured from the dark resting potential, and normalised to $V_{\text{max}}$) against the log total intensity (adapting intensity plus test intensity) = log ($I_a + I_t$) (Table A.1, Appendix A).

This convention was used for the following reasons. a) The absolute voltage scale expresses responses in the context of the fixed voltage operating range determined by the membrane and ionic environment of the cell. b) There is no reason to suppose that the transduction mechanism can distinguish between test and adapting quanta when applied together and therefore the total intensity is the relevant parameter. c) A log (total intensity) axis was used, since then equal log increments represent equal contrast modulations. Contrast is an important object parameter, which, in general, is independent of the mean illumination level, thus preserving contrast constancy aids in the functional interpretation of the intensity/response curves.

The dark- and light-adapted intensity/response functions determined in the present study are similar to those previously reported in fly photoreceptors by Dörrscheidt-Käfer (1972), however it was necessary to repeat these measurements accurately for a comparison with the LMC intensity/response functions determined in the same population of flies with identical stimuli. The curves are also similar to those reported from comparable studies of other retinula cells (Naka and Kishida, 1966; Glantz, 1968; Bader et al., 1976; Laughlin, 1975).
The dark-adapted V/log I curve measured from the peak response has a dynamic range of ca. 4 log units. The curves measured with respect to the initial, or steady state plateau also saturate at similar intensities (Fig. 4.3). Light-adapted curves (measured from the peak response) retain a similar slope and shape, and are merely translated along the log I axis as a function of the adapting intensity. A few determinations made using the alternating shutter arrangement described in the Methods, reveals that this is true for both increments and decrements of intensity.

The sensitivity of a light-adapted unit can be defined by the range sensitivity - a measure which indicates the position of the V/log I curve on the log I axis. The change in range sensitivity, as the V/log I curve shifts along the log I axis with increasing background intensities is defined as the range shift (Table A.1, Appendix A). In Figure 4.7 the range sensitivity is plotted as a function of the adapting intensity. At low intensities, the range shift is very small, but accelerates as the adapting intensity is increased, so that, under the brightest adapting regimes employed, the range shift is equal to the adapting increment.

As also demonstrated in bee photoreceptors (Bader et al., 1976), range shift is seen to be closely correlated with the attenuation of receptor potential during light-adaptation. Thus if the decrement in response from peak to the steady state plateau is converted to sensitivity terms through the dark-adapted (peak) V/log I curve, this sensitivity decrement as a function of adapting intensity falls on the same curve.
Figure 4.3  The dark and light-adapted intensity/response functions of a typical photoreceptor (R1-6) presented as V/log I curves. The total amplitudes of the peak voltage responses, measured from the dark-adapted resting potential, are plotted against the log of total intensity during the test flash, log \( I = \log (I_a + I_t) \). The normalisations of voltage and intensity are described in the METHODS. \( \circ-\circ \) = peak responses from dark-adapted cell; \( \bullet-\bullet \) = peak responses from cells light-adapted at intensities indicated by arrows; \( \ldots \ldots \ldots \) = amplitudes of steady state responses to continuous background illumination. \( \ldots \ldots \) = dark-adapted intensity/response function determined from the initial plateau response.
as the range sensitivity (Fig. 4.7). This suggests that the mechanisms which affect range sensitivity and response attenuation during light-adaptation are one and the same.

b) LMC's

The dark-adapted LMC V/log I function has been adequately described previously (Järvilehto and Zettler, 1971; Laughlin, 1973). The basic features are, the inversion of signal polarity, amplification resulting in a smaller dynamic range (ca. 2 log units), and a curve lying at considerably lower intensities on the log I axis than the receptor's.

Light-adapted V/log I curves in the LMC's were determined from the peaks of both the 'on' and 'off' transients (Fig. 4.4). These differed from those of the receptors in 5 major respects.

1. There is no background signal during maintained light, and the 'off' transient must code for decrements of sensitivity. V/log I curves determined using the alternating shutter arrangement reveal that the 'on' and 'off' transients form a continuous curve passing through the intensity axis at the background intensity value (Fig. 4.4b).

2. The slope of the V/log I curve (=contrast efficiency—Table A.1, Appendix A) is always steeper in the LMC's. In the dark, it is approximately 50-60%/log unit and increases rapidly to values at high as 300%/log unit in light-adapted states, for both 'on' and 'off' transients (Fig. 4.5). The increase in contrast efficiency occurs over the same intensity range as the accelerating phase of the receptor V/log I function, and within the limits of the
Figure 4.4  The dark and light-adapted intensity/response functions of a typical LMC, plotted as $V/\log I$ curves and derived in two different experimental situations. (a) incremental test flashes are superimposed upon a background and the responses at flash onset and offset are plotted against the total stimulus intensity during the test flash. (b) brighter or dimmer test stimuli are instantaneously substituted for the background using the alternating shutter method described in the METHODS. Again the hyperpolarising or depolarising response is plotted against intensity during the test period. In all cases there is no sustained response to background and background intensity is indicated by the intersect of the $V/\log I$ curve with the axis indicating zero voltage response. $\bullet-\bullet$ = dark-adapted responses; $\blacktriangle-\blacktriangle$ = light-adapted responses. The normalisation of response amplitude and intensity is described in the METHODS.
considerable scatter the LMC contrast efficiency appears to maintain a constant ratio with the contrast efficiency in the receptors (approximately 8x at all intensities) (Fig. 4.5). This correlation implies that the voltage gain at the first synapse is constant, and independent of the adapting intensity.

As this point is of great importance to both functional and mechanistic interpretations of light-adaptation, two independent methods were used to measure contrast efficiency in LMC's: a) graphical measurement of the slopes of the light-adapted V/log I curves as they pass through the log I axis, b) measurement of voltage responses to modulations of the intensity of the adapting beam generated by means of a 0.1 log unit neutral density filter (gelatin) mounted on the vane of a high speed shutter placed in the light path of the adapting beam. Averaged voltage responses to ca. 20 repeated modulations were then converted to contrast efficiencies for an independent estimate, thus confirming the dramatic increase of this parameter in LMC's. Receptor contrast efficiencies (Fig. 4.5) were determined by the second method only.

3. Since the voltage bandwidth of the LMC response remains approximately constant with light-adaptation, the increase in contrast efficiency necessarily results in a significant contraction of the dynamic range (Fig. 4.4). From 2 log units in the dark, the dynamic range, under moderate adapting regimes, is limited to ca. 0.5 log units either side of the background.
Figure 4.5  The dependence of LMC contrast efficiency upon photoreceptor contrast efficiency and hence background intensity. Contrast efficiency, is defined as the slope of the V/log I curve measured as \(\%V_{\text{max}}/\text{log unit}\). The contrast efficiencies determined from LMC 'on' and 'off' transients are approximately equal. ○ = values obtained from V/log I curves; ▲ = values obtained using the neutral density shutter method described in the text. ⋆⋆⋆⋆ = retinula cell contrast efficiency; -■- = contrast efficiency expected in LMC's if the first synapse operates with a constant voltage gain of x8. Data taken from 7 LMC's.
4. Although the absolute voltage bandwidth is little affected by light-adaptation it is increasingly biased towards more positive values (Figs. 4.2, 4.4, 4.6). Thus the saturated amplitude of the 'on' transient is progressively reduced to less than half its dark-adapted value, whilst the saturated amplitude of the 'off' transient may be doubled. This positive bias correlates quite closely with the level of plateau depolarisation in the receptors (Fig. 4.6). This observation suggests that the recorded LMC potentials are the sum of the true LMC transmembrane potentials and the extracellular lamina field potentials (Shaw, 1978). Light induced depolarisations of the extracellular space in the lamina (lamina depolarisations - Laughlin, 1974a) are generated by an inflow of receptor current through the retinula cell axon terminals, and approach the size of the receptor potential with the large field stimuli used in these experiments (Shaw, 1978). However, the positive bias of the LMC's responses is still observed when recording in the chiasm\(^1\) where extracellular field potentials are minimal. If the interpretation of the bias is correct, therefore, it appears to be a functionally significant observation rather than a misleading artifact.

\(^1^\) An electrode location can be readily identified as being in the chiasm between the lamina and medulla, by the sequential recording of units with visual axes first anteriorly directed, and then posteriorly directed.
Figure 4.6 The effect of light-adaptation upon the voltage bandwidth of LMC's at any one adaptation state, as defined by the saturated amplitudes of transient 'on' and 'off' responses. • = dark adapted saturated response amplitude; △ = light-adapted saturated response amplitude. •••• sustained background signal in receptors normalised to the same voltage scale. Log I = log (I_a + I_t) is the total intensity that just causes saturation at any one adaptation level. Data from 8 LMC's
5. The light-adapted range sensitivity in LMC's is more severely attenuated than in the receptors. Thus the range shift is proportional to background increments at all adapting intensities. Such proportionality is expected when the steady state background signal is annihilated and the V/log I curves at different backgrounds remain parallel. Although the latter proviso does not strictly hold (Figs. 4.4, 4.5) the curves are so steep that the deviation from direct proportionality is very small (Fig. 4.7). This means that adaptation in LMC's is much more severe than in the receptors and the sensitivity in light-adapted LMC's is reduced by ca. 2 log units, compared to the attenuation seen in the photoreceptors (Fig. 4.7).

In summary, a comparison of the waveforms and intensity/response functions of LMC's and receptors reveals that the LMC's act as temporal high pass filters of the receptor signal, and that there must be additional adaptation mechanisms acting at the level of the first synapse. Further, since the LMC's retain the high amplification of the receptor signal at all adapting intensities investigated, it follows that their 'view' of the receptor signal must be restricted to a small 'window' of approximately 5-10 mV under all adapting conditions.

3. Time course of the lamina adaptation process

The relatively rapid decay of the LMC response to sustained stimuli (Fig. 4.2) suggests that the lamina adaptation mechanisms act quickly. This was confirmed by delivering saturating backgrounds to LMC's and testing their
Figure 4.7 The shift of receptor and LMC range sensitivity (log R.S.) plotted as a function of adapting intensity. • = LMC 'on' transient; ▲ = LMC 'off' transient; ○ = photoreceptor; ▣ = photoreceptor range shift predicted from decay of receptor potential during light adaptation (see text). The points for LMC range shift have been fitted with a linear regression line of slope -0.94. The curve for photoreceptor range sensitivity has been fitted by eye. Data are derived from 5 LMC's and 6 photoreceptors R1-6.
responsiveness with flashes delivered at a number of intervals following background onset. A background intensity of 1-2 log units above the saturating intensity for the dark-adapted LMC was chosen because such intensities induce significant adaptation in LMC's but not in the photoreceptors (Fig. 4.7). Consequently all time dependant processes observed in this situation originate from the lamina mechanisms. The test flashes were carefully selected to produce large but definitely unsaturated responses when the LMC was fully light-adapted to the chosen background. For control experiments performed upon receptors, the same background intensities were used but brighter test flashes were selected so as to produce well defined responses capable of accurately monitoring changes in receptor sensitivity. As judged from the absence of any decrease in response to the background following the test flash (i.e. after-hyperpolarisation), these brighter test flashes did not themselves produce significant adaptation.

Several rapid time course experiments were performed upon 4 LMC's. All gave consistent results, of which those presented in Figure 4.8 are typical. The LMC response to the background is a decaying hyperpolarisation upon which the transient 'on' and 'off' responses to the test flashes are superimposed. Any totally unresponsive period produced by the saturating background could not be resolved. Clear 'on' and 'off' responses to test flashes are found within 50 ms of background onset and these rapidly increase in amplitude so that after 200 ms, responses of constant incremental amplitude are riding upon the decaying background response. Clearly the lamina
Figure 4.8 Rapid changes in LMC and receptor responsiveness after turning on (LA) or turning off (DA) a moderate background of $I_a = 2.0$ log units. This background initially saturates the dark-adapted LMC but causes little receptor adaptation (upper graphs). Responsiveness is monitored by applying brief non-saturating test flashes at various intervals following the change in background. To obtain high temporal resolution the experiments are repeated many times on the same unit using different delays between adapting and test stimuli. -A- = 'on' response; -o- = 'off' response; -•- = 'background' membrane potential level between test flashes.
mechanisms adjust the LMC range sensitivity extremely rapidly during light-adaptation.

4. Time course of dark-adaptation

Dark-adaptation is the reversal of the effects of light-adaptation. This preliminary investigation has been undertaken to separate the roles of retina and lamina processes and to help identify those rate limiting steps which may be of behavioural significance.

At low intensities the rapid lamina mechanisms of LMC's dominate dark-adaptation, as illustrated in Figure 4.8 where delayed test flashes are used to explore the rapid time course of LMC dark-adaptation. The experiments are complementary to the light-adaptation experiments described above but in this case the test stimuli follow the switching off of a sustained adapting light. The test intensity was carefully determined before light-adaptation to produce a large but nonsaturating (about 75% \( V_{\text{max}} \)) response in the dark-adapted cell. The results show that a LMC range shift of over one log unit is completed within 500 ms and this is accompanied by a very small change in receptor response.

Following adaptation at higher intensities the rapid lamina mechanisms of adaptation are superimposed upon slower receptor processes. Thus, as shown in Figure 4.9, LMC range sensitivity increases extremely rapidly during the first seconds of dark-adaptation. After this the slower receptor processes limit LMC sensitivity.
Figure 4.9  The long term time course of dark adaptation in a typical receptor (---) and LMC (---), following complete adaptation to a bright background ($I_a = 5.0$ log units). Log sensitivity is plotted against the time following the onset of darkness.
5. Spectral components of light-adaptation

Out of the photoreceptors, only R1-6 are known to form direct synapses on LMC's in the lamina, however, it is possible that the other receptors, R7 and R8, could affect the LMC response via another path. Evidence for such a possibility was sought by investigating the spectral properties of LMC's under various conditions, since it is known that the spectral sensitivities of the different receptors are markedly different (see Chapters II and V).

With respect to excitation, the spectral sensitivity of the LMC peak 'on' response was determined in both the dark-, and light-adapted states, in case there is any reorganisation of synaptic inputs as a function of light-adaptation. The result (Fig. 4.10) is very similar to the typical spectral sensitivity observed in the majority of receptors R1-6, with two peaks at ca, 360 nm and 490 nm. No significant effect of light-adaptation could be detected. The adapting intensity used in the light-adapted determinations was 3.0 log units, which is sufficient to saturate the LMC, but not sufficient to cause the shift in spectral sensitivity observed in R1-6 cells during brighter adaptation (Chapter II). Different classes of LMC spectral sensitivity, as reported by Moring (1978) were not observed in this study.

The possibility also exists, that the lamina adaptation mechanisms might be mediated by cells R7 and/or R8. In particular it has been suggested (Strausfeld and Campos-Ortega, 1977) that adaptation in the lamina may be mediated by a uv sensitive interneuron described by Mimura (1976), which
Figure 4.10 The spectral sensitivity of dark-adapted (□) and light-adapted (●) LMC's. Each point is the average light or dark-adapted sensitivity derived from 12 cells. The adapting light ($I_a = 2.3$ log units) caused no significant change in spectral sensitivity, and a single curve is drawn through both sets of points.
presumably has a major input from R7. However, two lines of evidence indicate that the lamina adaptation mechanisms have the same spectral sensitivity as the excitatory ones. Firstly, responses generated with different wavelengths (389 nm, and 513 nm were used) of equivalent excitatory intensity, both show similar transients, and the cut back of the 'on' transient to the plateau is accomplished to the same degree and at a similar rate with either wavelength.

Secondly, light-adapted V/log I functions showed the same shift in range sensitivity when the backgrounds used were of different wavelengths (389 and 513 nm) adjusted in intensity to the same equivalent excitatory effectiveness.
DISCUSSION

The results have demonstrated a variety of adaptation mechanisms acting in both the retina and lamina. The significance of these results will be discussed in three respects. 1) The relative roles of receptor and LMC's in determining visual performance as a whole; 2) possible mechanisms of light-adaptation; and 3) by comparing the present results with studies in other animals, it is hoped to highlight the functional significance of the strategies employed for light-adaptation.

1. Peripheral adaptation and central function

When studies of visual interneurons, or visually mediated behaviour reveal properties similar to those seen in the peripheral elements driving them, then one can infer that the peripheral elements are limiting the performance. With such correlations one can separate peripheral from central function, and thus gain a better understanding of both.

a) Photoreceptors

The results obviously indicate the existence of powerful adaptation mechanisms operating at the level of the first synapse, however, the comparison of photoreceptors and LMC's revealed at least two properties of the photoreceptors that are transferred across the first synapse, and thus might be expected to be seen at higher levels. These receptor characteristics were, the slow recovery of sensitivity during dark-adaptation (Fig. 4.9), and the increase of contrast efficiency (slope of the V/log I curve) with intensity (Fig. 4.5).
The slow recovery of sensitivity following light-adaptation is a common feature of retinula cells (e.g. Baumann, 1968; Glantz, 1968; Autrum and Kolb, 1972). Correlates of the slow phase of receptor dark-adaptation have been seen in the sensitivity increase during the slow phase of the crab optomotor memory response (Erber and Sandeman, 1976).

The accelerating slope of the receptor V/log I curve means that at higher background intensities a given contrast produces larger receptor signals. At higher levels in the visual system correlates of this may be looked for in terms of increment thresholds. Indeed, behavioural studies of bees and flies have shown that the Weber fraction (increment threshold/background intensity) falls as the background rises (Wolf, 1933; Reichardt, 1969; Labhart, 1974). Such a decrease in the Weber fraction can only be partly attributed to statistical fluctuations in quantum catch (e.g. Reichardt, 1969) and it is proposed that much of the remaining decrease is explained by the rise in receptor contrast efficiency with intensity.

b) LMC's

Apart from the properties just mentioned, the LMC's have been observed to undergo additional strong adaptation, and thus in general, one cannot expect to see the receptor sensitivity expressed in visual behaviour when this is mediated by the receptor-LMC pathway. Close correlates of LMC performance, however, may be found at higher levels, suggesting that the process of light-adaptation is largely
completed at this early level. In the crayfish optic tract, for example, the "sustaining" fibre response rises more rapidly to a peak, and saturates at lower intensities than the receptor potential (Glantz, 1971). This is a finding that is readily explained if the crayfish LMC's are functionally, as well as anatomically (Hafner, 1973) similar to those of the fly.

One of the most complete studies of light-adaptation at a higher level in the insect visual system, is that of the locust descending contralateral motion detector, or DCMD (Rowell and O'Shea, 1976). The DCMD is a command interneuron running from the optic lobes to the thoracic ganglia. Intensity/response functions of this unit in dark- and light-adapted states were investigated by counting the number of spikes generated in response to either brightening (ON) or dimming (OFF) of small test areas. These correlate rather well with those of the fly LMC's in the following respects.

1. There is no sustained response to backgrounds
2. The intensity/response functions show shifts in range sensitivity proportional to background increments.
3. The dark-adapted dynamic range of the ON response is ca. 1.5-2.0 log units
4. The saturated ON response decreases, whilst the saturated OFF response increases, as a function of adapting intensity.
5. Light-adaptation increases the contrast efficiencies and contracts the dynamic ranges of both ON and OFF responses.
Locusts also have hyperpolarising units in the lamina, whose responses resemble those of fly LMC's (Shaw, 1968). It is thus suggested that these are responsible for much of the light-adaptation observed at the level of the DCMD, which represents an output of the optic lobe. The 'on' and 'off' transients of the LMC response have opposite polarities and if these feed into different pathways in the medulla, there is no a priori reason to suggest that the long visual fibres act as an ON pathway to the medulla (cf. Rowell and O'Shea, 1976).

Although these results suggest that the majority of light-adaptation is completed at the level of the LMC, there is at least one aspect that must have its origins elsewhere, namely spatial summation, or neural pooling. A recent study of the activity of motion detecting units in the lobula plate of the fly *Lucilia* indicates that neural pooling is required to explain the decrease in acuity observed at low light luminances (Dvorak and Snyder, 1978). Since the dark-adapted LMC's have visual fields of the same order of size as the receptors (Zettler and Järvilehto, 1972; Laughlin 1974b), they cannot be the units responsible for this spatial summation.

2. Adaptation mechanisms
a) Photoreceptors.

During light-adaptation the sensitivity of the photoreceptors decreases as a function of the background intensity, as depicted in Figures 4.3 and 4.7. The correlation of sensitivity decrements, measured as range shifts, with
equivalent sensitivity decrements calculated from the response attenuation (peak-steady state plateau), suggests that this adaptation in sensitivity can be completely described in terms of the voltage response attenuation seen during a constant light (Fig. 4.7). This attenuation consists of a fast and a slow phase (Fig. 4.1). Two general classes of mechanism can be suggested that might reduce sensitivity: a) those reducing the number of quanta effectively absorbed by the visual pigment; b) those reducing the contribution of each absorbed quantum to the response. Both classes would be predicted to shift the V/log I curve parallel to the log I axis (Laughlin, 1975), and the available evidence suggests that both contribute to the reduction of sensitivity in photoreceptors seen during light-adaptation.

Much evidence has accumulated in other arthropod species to suggest that the fast phase of adaptation is associated with an increase in intracellular calcium concentration (possibly stimulated by the influx of sodium ions during the receptor potential). Thus in bee retinula cells, intracellular injection of calcium mimics the sensitivity decrease seen during light-adaptation, whilst injection of EGTA (which binds with calcium) abolishes the major effects of light-adaptation (Bader et al., 1976). In Stimulus ventral eye direct measurements of intracellular calcium concentrations, using aequorin, reveal a transient increase on exposure to light (Brown and Blinks, 1975). The presumed effect of the calcium is to reduce the current produced by each absorbed quantum.
The mechanisms of slow phase adaptation almost certainly include several components.

a) Photochemical: light of appropriate chromatic content has been shown to alter the rhodopsin/metarhodopsin ratio in fly and many other invertebrate species (Hamdorf et al., 1973). If the rhodopsin concentration is reduced, then sensitivity is expected to decrease, not only because there is less rhodopsin, but also because the metarhodopsin produced acts as an inert self-screening agent with respect to excitation (Hamdorf and Rosner, 1973; Tsukahara and Horridge, 1977a). The component due to this mechanism will depend upon both the colour of the adapting light, and the cell's previous history of illumination. Indeed, under certain circumstances the effect can be an enhancement of sensitivity - e.g. when red illumination follows a period of blue pre-adaptation, a transient increase of the plateau potential can be observed before other adapting mechanisms pull the response down.

b) Another candidate mechanism for slow phase adaptation is the longitudinal pupil mechanism (Kirschfeld and Franceschini, 1969). This consists of the light-induced migration of pigment granules in the retinula cells towards the rhabdomere boundary where they have been estimated to attenuate the light flux in the rhabdomere by ca. 0.7 log units (Srinivasan and Bernard, In preparation).

c) The two foregoing mechanisms, can attenuate sensitivity, by reducing the number of quanta effectively absorbed by at most, 1.5 log units. The sensitivity decrease observed in the decline of voltage during the slow phase may be as
much as 1.5-2 log units (Fig. 4.3). It thus seems that there must be a further mechanism(s) to completely account for the slow phase. This conclusion is supported by the observation that a significant slow phase component of adaptation may be observed in chalky mutants, which lack the pupil pigment, when exposed to adapting regimes that are not expected to greatly decrease the rhodopsin/metarhodopsin ratio (Dörrscheidt-Käfer, 1972). Such mechanisms, it is suggested, are probably at the membrane level.

b) LMC's

During light-adaptation, sensitivity in the LMC's is seen to be reduced in direct proportion to the background intensity at all intensities (Fig. 4.7). In voltage terms, the adaptation mechanisms in the lamina produce large 'on' and 'off' transients, and annihilate the response to sustained backgrounds.

Two general classes of mechanisms can be suggested that result in range sensitivity decreasing in proportion with the background intensity, namely multiplicative and subtractive. In a multiplicative model, the voltage gain at the first synapse is inversely proportional to the background intensity, and responses to both the background and the increment test flash should be reduced.

In the subtractive model, the background signal is effectively backed off, leaving the LMC free to use all its voltage bandwidth, at the same high gain, for any intensity modulation within its dynamic range.
Two facts, namely that the background signal is annihilated at all background intensities, and the ratio of the contrast efficiencies of LMC's and photoreceptors (a measure of voltage gain) is more or less independent of the adapting intensity, suggest that the subtractive mechanisms dominate adaptation in the lamina.

A candidate for this mechanism is the extracellular field potential (lamina depolarisation) that may be recorded from an extracellular electrode location in the lamina cartridge layer (Laughlin, 1974a). Recent studies in locust (Shaw, 1975; 1978) have shown that the extracellular space in the lamina is surrounded by a high resistance electrical barrier, so that the receptor current which flows into the lamina through the receptor terminals depolarises the extracellular space.

These slow depolarisations could provide a delayed signal that would effectively oppose the depolarisation in the receptor terminals, thus backing off a portion of the background signal at the first synapse (Laughlin, 1974a; Shaw, 1978). This proposed mechanism would generate the transients observed in the LMC's, due to the phase lag between the intracellular potential of the receptors and the extracellular potential. Note that this mechanism is one of presynaptic inhibition.

The lamina depolarisation is particularly suited for such a role in adaptation since: a) it is a centred weighted mean response, averaged over several ommatidia; b) the lamina depolarisation is far less noisy than the receptor potential due to averaging and temporal smoothing. In particular, there is no intrinsic noise added, as would be
the case with a synaptic mechanism. It is important in a lateral inhibitory system that the noise be minimal, since uncorrelated noise in the surround will add to the noise level of the centre signal, and thus degrade it.

It is by no means excluded however, that synaptic interactions can also play a role in adjusting LMC sensitivity, and indeed the complex neural circuitry of the lamina contains several pathways that might mediate such interactions (Strausfeld and Campos-Ortega, 1977). In particular, there is a strong possibility that the depolarising 'off' transient may be synaptically produced, because in locust ocellar neurons, whose responses closely resemble those of fly LMC's, the depolarising 'off' transient is generated by an increase in conductance, which is most probably synaptically mediated (Wilson, 1978b).

One particular suggestion, that a broad field uv sensitive lamina unit described by Mimura (1976) plays a dominant role in adaptation mechanisms (Strausfeld and Campos-Ortega, 1977), can be more or less excluded, however. If this were the case, it would be predicted that uv illumination would be far more effective than green light in shifting the range sensitivity, however, in this study it was found that the effectiveness of uv and green light in light-adaptation is that expected if the predominant influence is that of cells with the same spectral sensitivity as R1-6.
3. **Functional similarities with other visual systems**

a) Other insects

Experiments effectively identical to those reported here, have been performed on receptor and LMC's in the dragonfly, *Hemicordulia tau* (Laughlin and Hardie, In press).

The comparison of light-adaptation in the peripheral visual system of fly and dragonfly reveal striking similarities and the results presented here essentially hold for the dragonfly as well. The only significant difference appeared to be that in the dragonfly, the dark-adapted 'on' transient is consistently larger ($V_{max} \approx 50 \text{ mV}$), whilst the 'off' transient is not usually seen in dark-adapted units. Even this difference, however, might be attributable to a difference in preparative techniques. Thus, because in the dragonfly, glass micro-electrodes cannot penetrate the basement membrane without breaking, a small sector was cut from the eye to expose both retina and lamina, thus possibly causing a significant short-circuiting of the retina and lamina resistance barriers (Laughlin and Hardie, In press).

Fly and dragonfly are distantly related insect species occupying rather similar visual environments (thus both are diurnal, highly manoeuvrable flying insects). Since in other insects lamina architecture exhibits considerable adaptive radiation, and indeed fly and dragonfly retinæ and laminaæ are based upon different anatomical patterns (Kirschfeld, 1967; Braitenberg, 1967; Strausfeld, 1976b; Armett-Kibel et al., 1977). The functional similarities imply convergent evolution of optimal mechanisms. Similar studies have not been made.
in the second order cell of other insects, however, studies of light-adapted intensity/response functions in photoreceptors have been made in bees (Naka and Kishida, 1966; Bader et al., 1976) and found to be essentially similar to those reported here.

To further identify the important features of the proposed optimal mechanism, a functional comparison is made with analogous studies in vertebrate retina and outer plexiform layer.

b) Vertebrates

Studies of the performance of first and second order units in the outer plexiform layer have been made in several lower vertebrates including skate (Green et al., 1975), the mudpuppy, Necturus (Normann and Werblin, 1974; Werblin, 1974) and the tiger salamander (Werblin, 1978).

The intensity/response curves of the receptors are very similar to those in the fly (though inverted in polarity). Thus they have a dynamic range of ca. 4-5 log units, and during light-adaptation are translated along the log I axis, whilst retaining their slope and alignment (Normann and Werblin, 1974). Since Weber's law (ΔI/I = constant) is maintained over a large range, it is clear that they effectively perform a log transform.

The range fractionation between receptors (rods and cones in vertebrates with duplex retinas) however, has not been observed in insects (see Chapter II for a comparison of different receptors in the fly).
The bipolar cells also show striking similarities to their functional analogues, the LMC's (Laughlin, 1976b). Thus both amplify the receptor signal, resulting in a contracted dynamic range lying at lower values on the log I axis. A comparison of the V/log I slopes of receptors and bipolars during dark- and light-adaptation in the tiger salamander, also shows that the voltage gain is relatively unaffected by light-adaptation, and again a subtractive mechanism acting at the level of the receptor terminals is proposed as the dominating mechanism of light-adaptation (although synaptically mediated) (Werblin, 1978).

The division into receptoral and 'network' (Green et al., 1975) mechanisms of light-adaptation is also well established, with significant adaptation occurring at the first synapse at intensities where the receptors show little or no adaptation. In the skate, this extra adaptation at the first synapse represents an extra sensitivity decrement of ca. 2.0 log units (Green et al., 1975) which is very similar to that seen in fly LMC's (Fig. 4.7).

In summary the common strategy of light-adaptation found in the visual systems of animals with widely differing evolutionary backgrounds is one of: log transform - subtraction - and amplification. The majority of light-adaptation appears to have been achieved at the level of the first synapse.

Presumably these analogies are of functional significance, and this possibility is explored further by looking at the functional implications of the shapes and dynamic ranges of the measured intensity/response functions under environmental conditions.
4. Functional implications of light-adaptation

The results have shown that fly receptors and LMC's remain sensitively responsive to light over at least a 5 log unit range of intensities, without saturating. The way in which they match their response range to the intensity range at any one mean luminance is illustrated, graphically in Fig. 4.11.

Since the majority of contrasts in the real world are generated by differences in object reflectances, the range of object intensities should be proportional to the mean level of illumination. Thus as the mean level rises, a proportionally expanding envelope of expected object intensities is generated. In Fig. 4.11 this is illustrated for a contrast modulation of 0.5. The lower curves are generated by transferring this through the experimentally derived receptor and LMC intensity/response functions.

Receptors respond to such a contrast modulation with a voltage modulation that initially rises as adaptation moves the receptor into the log-linear region of the V/log I curve, and then levels off, contrast constancy being maintained by a log transform at all except the lowest intensities. Notice that the stronger adaptation observed at higher intensities keeps the receptor in the log-linear region at the brightest adapting intensities investigated. This voltage modulation is superimposed upon a maintained depolarisation that increases as a function of intensity. At the first synapse this background signal is removed in the LMC, but the modulation is amplified to fill most of the voltage bandwidth.
SIGNAL

log

average
subtract
multiply

RECEPTOR

signal strength

response (mV)

mean intensity

mean intensity

LMC
Again contrast constancy is maintained at all but the lowest intensities.

At first sight it seems that the dynamic range of the LMC's is so small that they would be saturated very easily, and thus lose much information about contrast values. However, a consideration of expected environmental intensities shows that this is probably not the case.

Thus, 1) At any mean illuminance, object reflectances generate the majority of contrasts to be seen in the real world. Object reflectances vary at most by 1.2 log units (black soil to fresh snow, Wyszecki and Stiles, 1967, p.108). Thus at any one state of adaptation the LMC dynamic range (± 0.5 log units about the mean) in fact neatly encompasses most of the range of natural reflectances (which on average must be lower than the figure just presented). 2) Since the LMC's have small receptive fields, they are unlikely to simultaneously monitor object space with more than one mean illuminance (e.g. shadows and highlights) and 3) thanks to the extremely rapid mechanisms of light-adaptation in the lamina (Fig. 4.8), as the LMC encounters a new mean level of illuminance, its range will be quickly adjusted to the new conditions. 4) Finally, the foregoing 'justification' of the LMC's small dynamic range has not taken into account the demodulation of high temporal and spatial frequencies that occurs at the receptor level, thus further reducing the probability of large contrast changes.

In summary, the small dynamic range of the light-adapted LMC in fact can be considered well tailored to the expected
range of environmental intensities. Indeed the LMC would appear maladapted if it had a larger range, as then it would not be using its voltage bandwidth (and hence, signal to noise capabilities) to the full, except for the rare occasions when an LMC's visual field crosses visual boundaries such as shadows and horizons.

The question rather becomes, why do the receptors have such a large dynamic range? The answer for this probably lies in the fact that the receptors must contain all the information available to the visual system. Whereas the LMC's are units specialised, probably, for contrast detection, and thus can afford to filter out certain information (such as rare, large, intensity changes, and low temporal frequencies), the receptors cannot afford this luxury without compromising other functions of the visual system. One such function has indeed already been suggested, and this is the role of the lamina depolarisations (directly derived from the receptor potentials) which may well be the essential component of light-adaptation at the level of the first synapse.
CHAPTER V

SPECTRAL MECHANISMS IN R7 AND R8
SUMMARY

1. Intracellular recordings have been made from over 100 central retinula cells (R7 and R8) in the flies *Calliphora stygia* (wild type), *Calliphora erythrocephala* (chalky) and *Musca domestica* (white), with the aim of analysing pigment identity and function in the different rhabdomeres.

2. All retinula cells R7 investigated had a major peak of spectral sensitivity in the ultraviolet (Figs. 5.1, 5.3, & 5.7). One class with a peak at 341 nm and less than 10% sensitivity remaining at wavelengths longer than 400 nm, are termed UV cells. Another class with a peak at 359 nm, and a long tail of sensitivity (> 10%) extending to 500 nm, are termed UT cells.

3. The ratio of UT to UV cells was approximately 7:3, this is similar to the ratio between the two classes of central rhabdomeres, seven yellow (7y) and seven pale (7p) (after Kirschfeld and Franceschini, 1977). This correlation (Table 5.1) and the results from studies of spectral sensitivities (Fig. 5.7), afterpotentials (Fig. 5.2), and the dependance of polarisation sensitivity upon wavelength (Table 5.2 and Fig. 5.5) are consistent with the following interpretation: UV cells have 7p rhabdomeres which contain an ultraviolet-absorbing rhodopsin that can be reversibly photoisomerised to a blue-absorbing metarhodopsin. UT cells have 7y rhabdomeres which contain a photostable pigment (Kirschfeld and Franceschini, 1977); this filters the light reaching the active photopigments. The active photopigment system in UT (= 7y) cells is different to that found in UV (= 7p) cells and possibly consists of a
blue-absorbing rhodopsin and a UV sensitising pigment. Fig. 5.7).

4. The R8 cells encountered probably all had rhabdomeres lying beneath 7y rhabdomeres; their spectral sensitivity could be accurately modelled by the screening action of the pigments present in 7y rhabdomeres upon a visual pigment with a peak absorbance at ca. 520 nm (Fig. 5.8).
INTRODUCTION

Characterisation of the properties of the central retinula cells R7 and R8 of the dipteran retina has been a major goal of many researchers interested in the analysis of visual function in the fly. Because of the technical difficulties associated with the small size of these cells, indirect methods have often been used in attempts to understand their function. These include electroretinograms (e.r.g.'s) from flies in which the peripheral receptors (R1-6) have been inactivated by selective adaptation (Minke et al., 1975), e.r.g.'s from mutants deficient in certain receptor types (Harris et al., 1976), inferences made from behavioural studies (Eckert, 1971), and theoretical modelling (Snyder and Pask, 1973). Recently, the direct method of microspectrophotometry (MSP) has revealed that the rhabdomeres of the central retinula cells occur in two classes, recognisable by their appearance in transmitted light: seven yellow (7y) which contain a highly absorbing photostable pigment in addition to photopigment, and seven pale (7p) which lack this pigment (Kirschfeld and Franceschini, 1977; Kirschfeld et al., 1978). Since the indirect methods mentioned above have failed to reveal the physiological correlates of this subdivision, we argue that the direct approach of single cell analysis is necessary for a more complete appreciation of R7 and R8's properties. Previously, intracellular recordings from cells identified as R7 and R8 have been rare, and the spectral data obtained in this way (Smola and Meffert, 1975; Eckert et al., 1976; Järvilehto and Moring, 1976) suffer from the small number of cells impaled. As described in
Chapter II, however, it has now proved possible to record with some reliability from these cells.

In Chapter II, the intracellular recordings from R7 and R8 were used to make a functional comparison of their properties with those of R1-6. In the present study, we again make use of these intracellular recordings, but now address ourselves to questions concerning the origin of R7 and R8's properties, particularly in relation to the pigments in their rhabdomeres. These questions include: what are the characteristics of the photopigments in the different rhabdomeres, what is the effect of the photostable pigment upon spectral and polarisation sensitivities, and how are the properties of R8 affected by the absorption of light in the overlying rhabdomere of R7? Although results from intracellular recordings are insufficient to settle such questions by themselves, in combination with MSP, they can be a powerful tool. The basic results are therefore compared with the existing data from MSP, and a theoretical analysis performed in an attempt to decide amongst conflicting hypotheses.

The situation regarding pigments and function is shown to be more complex than previously supposed, and thus emphasises the value of the direct approaches of intracellular recording and MSP when analysing receptor function.
The preparation, recording and optical stimulation have been described in Appendix A. The present study draws upon data from *Calliphora stygia* (wild type) and white-eyed mutants of *Musa domestica* (white) and *Calliphora erythrocephala* (chalky). The great majority of experiments were performed on females. Unless otherwise stated, all stimulation was by a small source (1.8° in visual angle) centred on the cell's visual axis.

The 'in vivo recovery' technique for identifying stained cells (see Appendix B) was employed in the present study for the following experiments: a) identification of R7; b) correlation of microvillar direction with E-vector direction generating maximum response in R7 (microvillar direction in R7 is parallel to the line of rhabdomeres R1-3 — Trujillo-Cenoz and Melamed, 1966; Boschek, 1971); c) identification of 7y and 7p rhabdomeres, which may be recognised by this technique because 7y rhabdomeres fluoresce green whilst 7p rhabdomeres show no fluorescence (Franceschini et al., 1978). An attempt was made to identify the rhabdomere type of individual R7 and R8 cells by staining an R1-6 cell in the same ommatidium as that in which an R7 or R8 had been recorded from, and then identifying the central rhabdomere type by its autofluorescence (or lack of it).

The advancement of the electrode was monitored by a micrometer gauge with resolution of 2 µm, mounted on the micromanipulator.
RESULTS

The findings for R7 and R8 are presented separately.

The criteria for identification are given in Appendix B, but are recapitulated here. R7 cells are identified by a spectral sensitivity with a single peak in the ultraviolet (uv), and R8 by a major peak of sensitivity at ca. 540 nm, with a much smaller secondary peak (ca. 30%) at ca. 360 nm. In both R7 and R8 responses are characterised by higher noise levels than seen in R1-6 cells. The validity of these criteria in determining cell type was confirmed by dye injection and histological recovery (Appendix B), 3 times for R8 and twice for R7 (Hardie, 1977b). Using the 'in vivo recovery' technique (Appendix B), R7 was identified on a further 9 occasions. Both spectral classes of R7 (Chapter II, Fig. 2.3) were identified in this manner. From these positive identifications, it was felt unnecessary to stain every cell recorded from to be confident of anatomical identity.

An additional feature of R7 and R8 is the instability of the recordings (presumably related to the small size of the cells), and only the finest electrodes produced favourable results. Although over 100 units were penetrated at least long enough for a spectral sensitivity determination, less than half of these could be held stably sufficiently long for more detailed analyses to be performed.

To sample rapidly the large number of cells required before encountering an R7 or R8, a cell's spectral class was identified from its responses to two successive flashes (uv and green). R1-6 cells, which give equal responses to the
two flashes, were rejected and further penetrations made until eventually finding an R7 or R8.

1. R7 Spectral sensitivity

Intracellular recordings were made from 91 cells identified as R7 on the above criteria. Spectral sensitivity was measured routinely by measuring the responses to axial, isoquantal (±15%) flashes of monochromatic light at a variety of wavelengths, and then referring the responses to the V/log I curve determined in the same cell. The cells fell into two major spectral classes (Chapter II, and Fig. 5.7) For convenience, one class, with a major peak in the ultraviolet (uv) and a long tail of sensitivity extending into the green are referred to as UT cells (for uv plus tail), whilst the other class, which has less than 10% sensitivity remaining at wavelengths longer than 400 nm, are referred to as UV cells. These two classes also differed with respect to the shape and position of the uv peak (Fig. 5.7). Since our data points were limited by the interference filters used (322 nm, 333 nm, 358 nm and 381 nm in the uv), the position of the peak was estimated by fitting a parabola to the three highest points. Following this procedure, the peak of sensitivity in UV cells is estimated to occur at 341 nm ± 5 (S.D.), and the peak in the UT cells, at 359 nm ± 4 (S.D.) (n = 13 cells for each sample); the difference is clearly significant (p << .001, on the Student's t test). Table 5.1 shows the frequency of occurrence of the two classes in the different species studied.
**TABLE 5.1**

Frequency of occurrences of spectral classes and rhabdomere types of R7 cells in the different species studied. uv/bl refers to cells showing two peaks of spectral sensitivity (at 360 nm and 440 nm), but as explained in the text this may be an artifactual result and the cells could represent UT cells, hence the indicated uncertainty in the results. NM = not measured.

<table>
<thead>
<tr>
<th></th>
<th>UT</th>
<th>UV</th>
<th>(uv/bl)</th>
<th>7y:7p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calliphora stygia</strong></td>
<td>39-44</td>
<td>12</td>
<td>0-5</td>
<td>NM</td>
</tr>
<tr>
<td>(wild type)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Musca domestica</strong></td>
<td>12-16</td>
<td>8</td>
<td>0-4</td>
<td>260:102 (11 flies)</td>
</tr>
<tr>
<td>(white)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Calliphora erythrocephala</strong></td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>NM</td>
</tr>
<tr>
<td>(chalky)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>56-65</td>
<td>25</td>
<td>0-9</td>
<td>260:102</td>
</tr>
<tr>
<td><strong>PERCENT</strong></td>
<td>62-72</td>
<td>28</td>
<td>0-10</td>
<td>72:28</td>
</tr>
</tbody>
</table>
For comparison, we also estimated the frequency of occurrence of the two rhabdomere types (7y and 7p) in *Musca* (white) in the same region in which the recordings of R7 were made. Use was made of the *in vivo* method of examining rhabdomere autofluorescence (see Appendix B; Franceschini, 1977), and we determined the 7y/7p ratio in the same flies in which we had recorded and marked R7 cells (or a nearby R1-6 cell). The counts were then made (in 11 flies) from ca. 30 ommatidia surrounding the marked site. The results (ca. 70% 7y : 30% 7p) are similar to those measured independently in wild and cultured strains of *Musca*, *Calliphora* and *Drosophila* (Kirschfeld et al., 1978) and agree rather well with the UT/UV cell ratio (Table 5.1).

A few cells (9) presumed to represent R7 in *Calliphora stygia* and *Musca* showed a subsidiary peak of sensitivity at ca. 440 nm in addition to a major peak at 360 nm, but only in one case was it possible to repeatedly determine the spectral sensitivity with the point source demonstrably aligned with the cell's visual axis. As explained below, we are not confident that these few results genuinely indicate the existence of a third spectral class.

In several cells, spectral sensitivity was also determined with the point source aligned approximately 20° away from the cell's visual axis (= off-axis). There were two reasons for this experiment: firstly to see if the twin-peaked spectral sensitivity curves just mentioned might be explained by the use of off-axis stimulation; and secondly for comparison with results from other workers who have used non-axial illumination for determining spectral sensitivity (e.g. Burkhardt, 1962). Apart
from the obvious overall decrease in absolute sensitivity, UV cells show no significant change in spectral sensitivity. However, in UT cells there is a consistent relative suppression of uv sensitivity that results in either a double-peaked (uv + blue) spectral sensitivity, or even a single-peaked blue function (Fig. 5.1). On these grounds we regard with caution spectral sensitivities from cells poorly penetrated, where time did not allow both accurate axial alignment of the point source and repeated determinations of spectral sensitivity. These include the majority of the cells with uv + blue peaks, and also the two single-peaked blue cells previously reported by Hardie (1977b).

On several occasions we attempted to stain an R1-6 in the same ommatidium in which an R7 had been physiologically characterised, so as to correlate spectral class with rhabdomere type directly. Unfortunately in every case this technique failed, as the R7 cell was also stained, thus obscuring its own autofluorescence. It is not clear whether this was due to leakage of the dye during the recording from R7 or whether the damage caused to the R7 cell's membrane by withdrawing the electrode and advancing it again to find an R1-6 cell allowed dye to be taken up by the R7 during the injection of the R1-6 cell. In any case this result served as a valuable control demonstrating that one can reliably tell when one has recorded from two cells in the same ommatidium, purely by reference to the depths of the penetration (monitored by a micrometer gauge on the micro-manipulator).
Figure 5.1 Relative spectral sensitivity of a typical UT cell measured using a small source (1.8° in visual angle), first centred on the cell's visual axis (ON-AXIS), and then aligned 20° away from the cell's visual axis (OFF-AXIS). Both curves are normalised to 100%. Data from an R7 cell recorded in Calliphora erythrocephala (chalky).
2. Chromatic adaptation in R7

The effects of intense chromatic adaptation can provide information about the nature of photopigments. In cells R1-6 for example, it has been established that illumination with metarhodopsin(M)-generating wavelengths induces a prolonged depolarising afterpotential (PDA), whilst illumination with rhodopsin(R)-generating wavelengths produces a knockdown effect (KDE), returning the PDA to the dark resting potential (Muijser et al., 1975; Tsukahara et al., 1977). Most invertebrate photopigment systems studied have the interconvertible R/M system (e.g. Hamdorf et al., 1973), and it is likely that PDA's and KDE's are widespread phenomena. In addition the presence of a high proportion of M (induced by adaptation with M generating wavelengths) affects the spectral sensitivity of the cell by acting as a screening agent. Although the limited recording time did not allow us to perform a complete analysis of pigment spectra making use of these phenomena, as has been achieved in the elegant study of Tsukahara and Horridge (1977a) in dronefly R1-6 cells, it did prove possible to gain some clues about pigment absorption.

In UV cells, intense illumination with light at 358 nm results in a PDA which is characterised by large amounts of noise as it slowly returns to the baseline. If the cell is illuminated with intense blue light (442 nm) during this PDA, a KDE is observed, whereby the potential returns to the baseline and the noise is abolished (Fig. 5.2). Further, following 358 nm adaptation, spectral sensitivity shows a slight relative decrease in the violet (Fig. 5.3). These
Figure 5.2. Original recording from a UV cell in Calliphora stygia (wild type) showing repeatable prolonged depolarising afterpotentials (PDA) and knock-down effect (KDE). Following illumination at 358 nm, a small PDA is observed characterised by high levels of noise. Illumination at 442 nm produces a KDE. Figures denote the quantal flux (quanta/cm²·s⁻¹) employed from the axial source (2.5° in visual angle). The dotted line indicates the dark-adapted resting potential. At the point indicated by the arrow there is a 3 minute break in recording.
Figure 5.3  Spectral sensitivity measured in a UV cell after adaptation to either 358 nm (●●●) or 442 nm (○○○). Each point is the average of two determinations. Both curves are normalised to 100% so as to reveal the relative suppression of sensitivity in the violet (at 381 nm) following adaptation at 358 nm. Absolute sensitivity at peak wavelength was reduced by 0.25 log units following 358 nm adaptation (relative to that following adaptation at 442 nm). Data from Calliphora stygia (wild type).
results are all consistent with there being a uv-absorbing rhodopsin that can be interconverted with a blue-absorbing metarhodopsin. Similar experiments were performed in UT cells, but although a small, short lived depolarising after-potential could be induced by several wavelengths, no wavelength was found that could produce a KDE. There was also no consistent effect on absolute or spectral sensitivity following a variety of chromatic adaptation regimes. These negative results are not, however, considered definitive, as often the penetration deteriorated markedly following a series of intense adapting lights.

3. Polarisation sensitivity in R7

When recording time and stability allowed, polarisation sensitivity (PS) was also investigated. Contrary to previous reports (Smola and Meffert, 1975; Järvilehto and Moring, 1974), significant PS was detected in almost every case, and when it was not, it was possible that it was masked by variability in the responses or recording noise. PS was determined routinely in the uv (358 nm) and blue (442 nm) by recording the responses to 100 ms flashes as a uv polariser was rotated in $10^\circ$ steps through $180^\circ$, and then back again to check that maxima and minima were clearly repeatable (Fig. 5.4). The results, summarised in Table 5.2, reveal a clear correlation of polarisation properties with spectral class. Thus, UV cells always showed a rather low PS in both the blue and the uv, whereas UT cells were never seen to have an obvious PS in the uv, but in 15 out of 17 cases they showed a significant PS
Figure 5.4  Original recordings (retouched for clarity) of polarisation sensitivity (PS) in UT cells of a) *Musca* (white) and b) *Calliphora stygia* (wild type). Each response is to a 100 ms flash of linearly polarised monochromatic light of wavelength 358 nm or 442 nm; the E-vector direction is rotated 10° between flashes. In *Musca* the PS runs are followed by a V/log I determination, with test flashes incremented in 0.25 log unit steps. In *Calliphora* repeated PS runs are made in reverse directions to demonstrate that the maxima and minima are repeatable. Notice the suggestion of a phase-shifted PS at 358 nm in *Calliphora.*
when measured in the blue (Table 5.2). In a few UT cells, there was a suggestion of a low PS in the uv, ca. 90° out of phase with that measured in the blue (e.g. Fig. 5.4b). However, the response modulation was so small, that it is not possible to be confident on this point.

In two UT cells a more detailed analysis of PS as function of wavelength was performed by determining the spectral sensitivity with the polariser first in the preferred direction and then in the orthogonal direction. PS at each wavelength was then simply determined by comparing the maximum (preferred) and minimum (orthogonal) responses at each wavelength and referring them to the V/log I curve to convert the difference in responses to polarisation sensitivity (cf. Chapter III). In one of these cell, the PS/wavelength function was also determined by performing complete PS runs at 10 different wavelengths. The resulting plots of PS against wavelength show a conspicuous peak in the blue at ca. 450 nm (Fig. 5.5).

The photostable pigment in 7y rhabdomeres absorbs polarised light maximally when the E-vector direction is perpendicular to the microvillar direction (Kirschfeld et al., 1978), whereas known rhodopsins absorb maximally when the E-vector direction is parallel to the microvilli (review: Waterman, 1975). In considering theories of pigment function in R7, therefore, it was felt important to determine at which E-vector direction UV and UT cells showed maximum sensitivity to polarised light.
TABLE 5.2

Polarisation sensitivities in R7 cells: a) Calliphora stygia; b) Musca. ND = none detected (<1.2); NM = not measured. Each value is the average of two repeated determinations with the polariser rotated in opposite directions, and measured with light at either 358 nm or 442 nm. An additional two UT cells in Calliphora failed to show any PS at either wavelength.

* Cells in which a low, phase-shifted PS was suspected in the uv (358 nm) (e.g. Fig. 5.4b).

a) Calliphora stygia (wild type)

<table>
<thead>
<tr>
<th>UV</th>
<th>358 nm</th>
<th>442 nm</th>
<th>UT</th>
<th>358 nm</th>
<th>442 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
<td>1.8</td>
<td></td>
<td>ND</td>
<td>2.2</td>
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<td>1.6</td>
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<td>3.0</td>
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<td>ND</td>
<td>3.0</td>
</tr>
<tr>
<td>Mean ± S.D. (n = 11 cells)</td>
<td>ND</td>
<td>2.3±0.3</td>
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</table>

b) Musca (white)

<table>
<thead>
<tr>
<th>UV</th>
<th>358 nm</th>
<th>442 nm</th>
<th>UT</th>
<th>358 nm</th>
<th>442 nm</th>
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</tr>
<tr>
<td></td>
<td>ND</td>
<td></td>
<td></td>
<td>ND</td>
<td>5.8</td>
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<td>Mean ± S.D. (n = 4 cells)</td>
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</tbody>
</table>
Figure 5.5  Polarisation sensitivity (PS) as a function of wavelength determined in two UT cells from Calliphora stygia (wild type). •-•-•, results from one cell determined from the responses to spectral runs with polarised light (method described in text). o-o-o, results from a second UT cell determined by the same method, and ■■■■ results from a series of polarisation runs at different wavelengths, also in the second cell. All the curves show a similar shape with maximum sensitivity being generated at ca. 450 nm.
In 2 UT cells and one UV cell in *Musca* it was possible to correlate the E-vector direction generating maximum sensitivity with the microvillar direction, making use of the 'in vivo recovery' of stained cells (see Methods and Appendix B). Since the electrode is lowered into the eye vertically, the orientation of the microvilli in the stained R7 with respect to the vertical is given by the angle between the electrode track (the line between the hole in the cornea and the stained cell) and the line of rhabdomeres R1-3 (which is parallel to the microvillar direction in R7). The preferred E-vector direction (with respect to the vertical) is taken from the position of the calibrated polariser. In each of the three cells maximum sensitivity (measured at 442 nm) was found when the E-vector direction was parallel (± 10°) to the microvilli in R7.

With respect to all other properties measured or observed, no other significant differences were observed between the two major spectral classes of R7. These properties included angular sensitivity, absolute sensitivity (with respect to monochromatic light of peak wavelength), intensity/response functions, maximum response levels, and stability of recordings.

4. R8 Spectral sensitivity

Cells identified as R8 on the criteria mentioned earlier were encountered less frequently in the retina than R7, presumably because they only occur in the proximal half of the retina. However, the recordings of R8 in the retina of *Calliphora stygia* (wild type) and the white-eyed mutants of
Musca domestica (white) and Calliphora erythrocephala (chalky) (a total of 11 cells) confirm the spectral sensitivity reported previously from recordings in the chiasm of wild type Calliphora stygia and Lucilia sericata (Hardie, 1977b). In the present study this was the only spectral class of R8 encountered, but as argued in the Discussion we strongly suspect the existence of an additional spectral class of R8.

A striking feature of R8's spectral sensitivity is the exceptionally low sensitivity in the blue. In this study it was discovered that this feature is dependent upon precise axial alignment of the stimulus within the visual field. When stimulated with a point source ca. 20° off the cell's visual axis, sensitivity in the 400-500 nm range is now increased to around 50% (Fig. 5.6). A confirmatory observation is that the angular sensitivity function determined in the blue is broader than in the green (Fig. 5.6).

On one occasion, in Musca (white), it was possible to inject an Rl-6 cell with Procion red, in the same ommatidium as that in which an R8 cell had been recorded from. In this case the electrode passed into the Rl-6 cell with less than 5 µm advancement of the manipulator, and thus we could be confident that the ommatidium was indeed the same. In vivo inspection of the eye (see Methods and Appendix B) subsequently revealed that the central rhabdomere of this ommatidium fluoresced green, indicating rhabdomere type 7y (whilst one of the peripheral rhabdomeres - R3 - fluoresced orange from the injected dye). Thus it was possible in this one instance to correlate the unusual spectral sensitivity of R8 with
Figure 5.6 Above: spectral sensitivity in an R8 cell in *Calliphora stygia* (wild type) determined, as in Figure 5.1, with a small source (1.8°) centred on the cell's visual axis (ON-AXIS) and also when aligned 20° away from the cell's axis (OFF-AXIS). Below: angular sensitivity functions in the same cell, determined with monochromatic light at wavelengths of 541 nm and 442 nm, and using a point source (0.4°).
the presence of photostable pigment in the overlying rhabdomere of R7.

5. Adaptation in R8

Two R8 cells were adapted with intense monochromatic lights. All wavelengths tried (442, 484, and 600 nm) produced a short lived depolarising afterpotential (less than 90 seconds), but no wavelength was found that could produce a knock-down effect. Following adaptation (ca. 1 minute) at either 442 nm or 600 nm, no significant difference in spectral sensitivity could be detected. As for R7 (UT) cells, however, these negative results are not considered definitive.

6. Polarisation sensitivity in R8

Polarisation sensitivity (PS) was only tested in 7 R8 cells, and of these, only 3 showed a significant PS. The cells were tested with monochromatic light of wavelengths 442 nm and 541/572 nm. From this small sample of cells, PS at 442 averaged 1.6 ± 0.7 (S.D.) (5 determinations on the 3 cells) and PS at 541 nm or 572 nm averaged 1.7 ± 0.6 (6 determinations on the 3 cells). The orientation of the E-vector direction generating maximum sensitivity, in any one cell, was the same at all wavelengths tested. On no occasion did we correlate the preferred E-vector direction with microvillar direction, however Järvi-lehto and Moring (1976) report that they are parallel.
DISCUSSION

1. Properties of R7

Microspectrophotometry (MSP) has shown the presence of two classes of central rhabdomeres: seven yellow (7y) which appear yellow in transmitted white light, due to the presence of a photostable pigment absorbing highly in the blue, and seven pale (7p), which lack this pigment and look pale and brighter in transmitted light (Kirschfeld and Franceschini, 1977). The absorbance spectrum of the photostable pigment resembles that of β-carotene with a major peak at 460 nm and subsidiary peaks or shoulders at 430 nm and 485 nm (Kirschfeld et al., 1978). The absolute absorbance of this photostable pigment is so high (McIntyre and Kirschfeld, In preparation) that it should have some effect upon the spectral properties of R7 when present. The electrophysiological results show two classes of spectral sensitivity, and in the absence of a direct identification of spectral class with rhabdomere type, we must consider three hypotheses.

I. UV cells correspond to 7y rhabdomeres, and UT cells to 7p.

II. UT cells correspond to 7y rhabdomeres and UV cells to 7p.

III. There is no strict correlation of spectral class with rhabdomere type.

In addition we must consider at least two possibilities for the function of the photostable pigment: a) it acts as
an inert screen or filter; 

b) it has a sensitising function for either rhodopsin or metarhodopsin, a function demonstrated with respect to rhodopsin, for a uv-absorbing photostable pigment in receptors Rl-6 (Kirschfeld et al., 1977).

If there is a definite correlation between spectral class and rhabdomere type, then the frequency of penetrations in the two spectral classes argues strongly for the correspondence of UT cells with 7y rhabdomeres (Hypothesis II). Both occur with a frequency of ca. 70% (Table 5.1), and there is no reason to suppose there is a sampling bias as 7y and 7p rhabdomeres appear to be randomly distributed in the retina (Kirschfeld et al., 1978).

As to the function of the photostable pigment, we notice that if it acted as a sensitising pigment for the rhodopsin, then energy transfer would occur between a blue-absorbing donor (the photostable pigment) and uv-absorbing acceptor (a uv rhodopsin). Such an uphill energy transfer is rare (Knox, 1975) and thus, initially, we consider the screening function as more probable (see below and Appendix I).

These considerations lead us to take the following as a working hypothesis (Hypothesis IIa): UT cells may be identified with 7y rhabdomeres, and UV cells with 7p rhabdomeres. The photostable pigment has a screening function (at least with respect to the excitation of rhodopsin) in the manner of a lateral filter (Snyder et al., 1973). This hypothesis has the disadvantage of complexity because it inevitably predicts that UT (= 7y) cells should have a different active photopigment system from UV (= 7p) cells, since the broader UT spectral
sensitivity obviously cannot be derived from a UV-type spectral curve screened by the photostable pigment. However, as will be shown, the available data are consistent with this hypothesis, whilst each of the alternative hypotheses considered has at least one major shortcoming.

The following additional lines of evidence are also consistent with our working hypothesis.

1) UV and UT cells have different polarisation sensitivities (PS). UV cells have a moderate PS in both the blue and the UV. This is predicted from the simple case of one rhodopsin, the slightly higher blue PS being explained by less self-screening (see Appendix III). UT cells never show an obvious PS in the UV, which already suggests that UV sensitivity in these cells is generated by a different pigment to that in the UV cells. However UT cells show a high PS in the blue; this is predicted from the orthogonal absorption of the photostable pigment acting as a screen, which should enhance the PS without changing the preferred E-vector direction (Appendix III).

2) The position of the uv peak in the two classes is consistently, and significantly, different (UV cells - 341 nm; UT cells - 359 nm), strongly suggesting the involvement of different photopigments. Waveguide effects cannot account for this difference (see Appendix I).

3) The spectral sensitivity of UV cells can be accurately modelled by a single rhodopsin with peak absorbance at 344 nm (Fig. 5.7a). In contrast, the spectral sensitivity of UT cells cannot be modelled by a single rhodopsin.
4) In response to intense uv light, UV cells show a PDA that may be knocked down by blue light (consistent with a uv rhodopsin/blue metarhodopsin system) (Fig. 5.2), whereas no wavelength could be found that would give a KDE in UT cells, again consistent with our prediction that the two cell classes have different photopigments.

5) The prediction that 7γ and 7p rhabdomeres have different photopigments, has recently been confirmed directly from difference spectra measured by MSP (Kirschfeld, In preparation).

We now consider the alternative hypotheses.

**Hypothesis Ia.**

*UV cells represent 7γ and UT, 7p rhabdomeres.* This hypothesis is initially attractive because it might be expected that the UV spectral sensitivity could be generated by screening of a UT-type spectral sensitivity by the photostable pigment. However, it is in fact impossible to generate the sharp spectral sensitivity curve of the UV cells in this way by the lateral filter model (Fig. 5.7a and Appendix I). The frequency of penetrations also argues against this hypothesis since there would have to be a large sampling bias in favour of UT cells (Table 5.1). Finally the orthogonal absorption of the photostable pigment predicts that PS in the blue would be ca. twice that in the uv (Appendix III). This is not observed in UV cells but is in UT cells.

**Hypothesis IIIa.**

*No strict correlation between spectral class and rhabdomere type.* In other words, UT cells (and also UV cells) may have
either 7y or 7p rhabdomeres. Although we consider this hypothesis unlikely because of the extra complexity of function it implies, it is difficult to rule out completely. The screening effect of the photostable pigment would be unlikely to show up in the spectral sensitivity of UV cells, as the extinction spectrum of the photostable pigment in 7y rhabdomeres (Kirschfeld et al., 1978) barely overlaps the UV spectral sensitivity. In UT cells the scatter in sensitivity (between cells) is a result of the orthogonal absorption by the photostable pigment and spectral sensitivity (between cells) in the range where the photostable pigment absorbs strongly (400-500 nm) might conceal both cells with 7y rhabdomeres and those with 7p rhabdomeres, although there is no indication of a bimodal distribution of measurements at each wavelength as would be expected. 

The argument we have against this hypothesis comes from a comparison of polarisation sensitivity (measured in the blue) and spectral sensitivity in the blue. Since we suppose that the high PS in the blue in UT cells is a result of the orthogonal absorption by the photostable pigment, then this hypothesis (H) predicts that there should be two classes of UT cells: those with high blue PS and low blue spectral sensitivity (=7y), and those with low blue PS and high blue spectral sensitivity (=7p). However, a scatter diagram of PS vs blue spectral sensitivity shows no indication of this predicted bimodality. Since the sample was small however (13 cells), it could be argued that it was insufficient to reveal the two subclasses.
Hypothesis IIb.

UT cells represent \( ?y \), and UV cells, \( ?p \) rhabdomeres, and the photostable pigment has a sensitising function (with respect to a rhodopsin). This hypothesis has the advantage of simplicity, in that it suggests that the UT spectral sensitivity can be generated from the same photopigment as in UV cells, extended into the blue by absorption and energy transfer by the photostable pigment. It is also hard to rigorously exclude, but if we consider the specific hypothesis that the photostable pigment acts as a sensitising pigment to a uv rhodopsin, then the following points lead us to feel that it is a most unlikely solution (see Appendices I, III for details).

1. Energetic considerations make it very unlikely that significant energy transfer could occur between a blue-absorbing photostable pigment (donor) and a uv rhodopsin (acceptor), especially if a resonance transfer is assumed (Förster, 1965) (Appendix I).

2. Even if there were significant energy transfer, the shape of the UT spectral sensitivity curve (Fig. 5.7) is not closely predicted by this model (Appendix I).

3. If we consider theoretically the expected polarisation sensitivity from such a system, it is not possible, with reasonable assumptions, to generate the high values of PS in the blue, with maximum excitation parallel to the microvilli, as actually observed in UT cells (Appendix III).
4. In addition, as it stands, the hypothesis does not account for the difference in PS between spectral classes observed in the uv. However, it is conceivable that some other factor could account for this (e.g. the degree of rhabdomere twisting, which, it has been claimed, differs in different R7's: Smola, 1977).

5. This hypothesis also does not, by itself, account for the difference in the positions of the uv peak of sensitivity in the two spectral classes of R7.

In conclusion we are reasonably confident that most, if not all, UT cells have 7y rhabdomeres, whilst UV cells have 7p rhabdomeres, and that the major effect of the photostable pigment is one of screening. On this assumption, we now consider the nature of the photopigments in each cell class.

As already argued, it seems that the properties of the UV (= 7p) cells can be readily explained by a single uv-absorbing rhodopsin that can be photoisomerised to a blue-absorbing metarhodopsin (a pigment system demonstrated in R7 cells in Drosophila: Harris et al., 1976). The dichroic ratio of the rhodopsin should be about 1.8 (Appendix III).

In UT cells, uv sensitivity is most probably conferred by a different pigment, as the sensitivity peak occurs at a different wavelength and little or no PS can be detected in the uv. A candidate is a uv sensitising pigment, possibly the same as that demonstrated in receptors R1-6 (Kirschfeld et al., 1977). In favour of this suggestion we notice that:

a) the peak of sensitivity in UT cells (359 nm) is virtually
identical with that in Rl-6 cells; b) PS in the uv is similar
in Rl-6 cells - i.e. as in UT cells, either one detects no
PS in the uv (See Chapter III), or else a low PS, considerably
phase-shifted from the PS observed in the blue/green (Horridge
and Mimura, 1975).

Certainly the strange shape of the UT spectral sensitivity
argues for complex spectral mechanisms. Since we propose that
the photostable pigment of 7y rhabdomeres acts as a screen in
UT cells, we can gain a better picture of the photopigments
in UT cells by theoretically removing the effect of the
photostable pigment and self-screening (Fig. 5.7b) and
Appendix I). The results indicates that if, as suggested, uv
sensitivity is conferred by a uv sensitising pigment similar
to that found in Rl-6, then the remaining sensitivity might
be generated by a rhodopsin absorbing maximally in the range
430-450 nm. Note that according to this view, the blue-
absorbing photostable pigment in 7y (UT) rhabdomeres would
reduce direct excitation of the blue-absorbing rhodopsin,
without affecting the indirect excitation by energy transfer
from the uv sensitising pigment. The novel results is a
spectral sensitivity that shows no indication of the rhodopsin's
absorption peak. At present we have no data that gives any
indication of the absorption of the presumptive metarhodopsin
in UT cells. Finally, none of our evidence excludes the
possibility that the photostable pigment has a sensitising
function with respect to a metarhodopsin.
2. Properties of R8

a) Spectral sensitivity

The only cells identified as R8 had a distinctive spectral sensitivity of which the major features are a narrow peak at ca. 540 nm, very low sensitivity in the range 400 nm-500 nm, and a second small peak at ca. 360 nm (Fig. 5.6 and 5.8). In one case a cell with this spectral sensitivity was proven to lie in an ommatidium containing a 7y rhabdomere. When using axial illumination, all light absorbed by photopigments in R8 must first pass through the rhabdomere of R7, and thus the absorption in R7 should profoundly affect the spectral sensitivity of R8. Making use of the measured absorption of 7y rhabdomeres (Kirschfeld et al., 1978; McIntyre and Kirschfeld, in preparation), we found that the spectral sensitivity of R8 could be accurately modelled in the range 400 nm-600 nm by assuming R8 contains a rhodopsin absorbing maximally at ca. 520 nm. The high absorption by the photostable pigment in 7y rhabdomeres then generates both the sharp peak at ca. 540 nm and also the exceptionally low sensitivity in the 400 nm-500 nm region (Fig. 5.8 and Appendix II). In this we disagree slightly with the conclusion of Kirschfeld et al. (1978) who claim that R8's spectral sensitivity may be modelled by screening of a 490 nm visual pigment by the photostable pigment. In fact, on this assumption the long wavelength flank of R8's spectral sensitivity deviates considerably from the predicted curve.

Because we have no accurate data on uv extinction in 7y rhabdomeres, we did not attempt to model R8 spectral sensitivity
Figure 5.8 The measured spectral sensitivity in R8 cells (●-●-●) compared with the theoretical prediction, which assumes a 520 nm rhodopsin is screened by the photostable pigment in 7γ rhabdomeres (see APPENDIX II). The 7γ extinction curve is taken from the data of Kirschfeld et al. (1978). The unscreened R8 curve represents the absorption of the 520 nm nomogram adjusted for self-screening, and the predicted curve represents this function after taking into account the screening action of the 7γ rhabdomere. Spectral sensitivity data are averaged from 5 cells in Calliphora stygia (wild type), chosen for the quality of the recording. The error bars represent ± 1.0 S.D.
below 400 nm. At present therefore, we cannot say whether the secondary peak at 360 nm in R8 is likely to derive from the β-peak of the rhodopsin, or, for example, a uv sensitising pigment (see Appendix II).

The conclusion, that R8's spectral properties are largely influenced by absorption in R7, gains further support from the observations that the relative spectral sensitivity in the range 400-500 nm is considerably enhanced when using off-axis stimulation, and that the angular sensitivity function determined with light of a wavelength near the peak of the photostable pigments's absorption spectrum is rather broad (Fig. 5.6). Both these effects are predicted when the stimulating light no longer passes predominantly through the rhabdomere of R7 before reaching that of R8.

This interpretation of R8's spectral properties leads us to postulate the existence of a second class of R8 that went undetected in the present study. These would represent cells whose rhabdomeres lie beneath 7p rhabdomeres. If we assume they contain the same photopigment as other R8 cells, then their spectral characteristics can be predicted from a knowledge of the absorption of 7p rhabdomeres. It is to be expected that the spectral sensitivity will depend upon the state of the visual pigment in R7 (7p): thus when it is in the rhodopsin state (e.g. following blue adaptation) the result will be close to the 520 nm nomogram adjusted for self-screening and waveguide effects - Fig. 5.8). However, when the R7 photopigment is in the metarhodopsin state (following uv adaptation) we
predict an attenuation of sensitivity in the blue, though not as extreme as in R8's lying beneath 7y rhabdomeres.

The failure to identify the second spectral class of R8 in the present study is puzzling but might be explained by the search regime used; thus if the uv peak is sufficiently high in this class, the paired flash presentation might not have generated responses significantly different from those seen in R1-6 cells. In addition, the sample of R8 cells was small (compared to R7 penetrations) and on statistical grounds we could only have expected to penetrate 4 or 5 of this second class.

b) Polarisation sensitivity

The results from polarisation sensitivity determinations in R8, although from a limited sample (3 cells), indicate no wavelength dependence of this parameter. This is puzzling as the obvious interpretation of the spectral results just discussed indicates that light absorbed by R8 must first have passed through the 7y rhabdomere. Since this has a significant dichroism, with maximum extinction parallel to the microvilli in R8, and in a limited spectral range only (i.e. ca. 400 nm-500 nm) we predict a wavelength dependent PS. However, in view of our limited results we feel that this apparent paradox is best left an open question (see Appendix III).

3. Comparison with existing data

Since the present results differ in some respects from those previously published for the spectral properties of the central retinula cells, we shall attempt, in this section, to
resolve the various discrepancies.

a) Intracellular recordings. Burkhardt (1962), in his pioneering work on fly photoreceptors, reported three classes of spectral sensitivity from cells in the retina of Calliphora when using broad field, non-axial, stimulation. Apart from a class with two peaks (at 360 nm and 490 nm), presumably representing R1-6 cells, he described one class with a major peak in the uv and a subsidiary peak in the blue, and a yellow/green class with peaks at 360 nm and 520 nm. In the present study it was possible to obtain similar spectral profiles using off-axis stimulation in UT cells and R8 cells respectively (Figs. 5.1 and 5.6), and it is therefore possible that these early recordings in fact represented the three anatomical receptor classes, although the cells were not identified histologically.

Meffert and Smola (1976) reported different classes of central retinula cells identified by injection of Procion yellow. One spectral class resembled the present UV class of R7. Another class had two peaks, at 360 nm and 440 nm (uv + blue), again similar to our UT class when stimulated off-axis. Meffert and Smola (1976) do not indicate if their stimulus was centred on the cell's visual axis, however, and therefore it is not possible to say whether their uv + blue cells could represent our UT cells or not.

Two other groups have identified R7 and R8 cells histologically after studying their spectral properties (Järvišento and Moring, 1976; Eckert et al., 1976), but as sensitivity was only measured at 5 wavelengths and 2 wavelengths
respectively, and also in only one or two cells, their data are insufficient for comparison.

b) Electretinogram (e.r.g.) data. The spectral sensitivities of R7 and R8 in Drosophila have been inferred from the e.r.g.'s of flies that have had the R1-6 system inactivated by intense blue adaptation (Minke et al., 1975) or by genetic elimination (Harris et al., 1976). Although these techniques do not resolve different classes of R7 or R8, the R7 data are in general agreement with our results, showing a major peak of sensitivity in the ultraviolet. With respect to R8, the techniques used obviously fail to identify the effects of absorption by R7, and, taking this into account, the results may be compared quite favourably. Thus the peak of absorption measured in Drosophila R8 is at ca. 490 nm which is approximately 20-30 nm longer than the sensitivity peak in Drosophila R1-6, and, as just argued, we predict that the actual photopigment in R8 of Calliphora and Musca has a peak absorbance at 520 nm which is also 20-30 nm longer than peak absorbance of R1-6 in these species. The exact nature of this photopigment is still open to question however, as neither the Drosophila studies nor the present work managed to identify any adaptational phenomena that demonstrate the presence of a metarhodopsin.

c) Behavioural data. As discussed in Chapter II, earlier work inferring spectral properties of R7 and R8 from optomotor behaviour (Eckert, 1971) or units of the optic lobe presumed to be involved in this behaviour (McCann and Arnett, 1972) must be discounted. At present the only clear behavioural
data indicating spectral properties of R7 is that of high uv sensitive phototaxis (Hu and Stark, 1977). To our knowledge, no clear indication of R8's spectral properties in behavioural responses have been demonstrated.

d) *uv + blue cells.* Although we are confident that the majority of R7 cells have the UT-type spectral sensitivity and that double-peaked uv + blue functions may often be artifactual, we do not discount the possibility of a genuine, but rare, class of cells with a double-peaked uv + blue spectral sensitivity. Such cells may represent cells with the UT photopigment system but lacking the blue-absorbing photostable pigment. Interestingly, it is occasionally possible in *Musca* to identify a rare (less than 10%) third class of central rhabdomeres from their red autofluorescence (Franceschini, 1977). On statistical grounds it is possible that such cells might represent a rare uv + blue spectral class.

4. *Spectral mechanisms in dipterans.*

The recent results of microspectrophotometry (Kirschfeld *et al.*, 1977; 1978) and the more recent of the electrophysiological data (Meffert and Smola, 1976; and the present study) indicate that the situation with regards to spectral mechanisms in dipteran photoreceptors is considerably more complex than previously supposed (e.g. Snyder and Pask, 1973). Thus there are probably at least 2 spectral classes of both R7 and R8, and considering all the photoreceptors, the available evidence suggests that at least 6 separate pigments may be involved in the 8 rhabdomeres (not including the metarhodopsins):
Rl-6 cells: 490 nm rhodopsin (in *Musca* and *Calliphora*) plus a uv sensitising pigment (Kirschfeld *et al.*, 1977).

R7: UV cells: uv rhodopsin (Harris *et al.*, 1976; present study)

UT cells: blue rhodopsin and uv sensitising pigment (tentative conclusion of the present study) plus photostable pigment - probably β-carotene (Kirschfeld *et al.*, 1978)

R8: rhodopsin absorbing maximally at wavelengths 20-30 nm longer than that in Rl-6 (Harris *et al.*, 1976; present study), and possibly, a uv sensitising pigment also.

An obvious explanation of this complex situation is not readily forthcoming. The major function of the photostable pigment in 7y rhabdomeres may well be with respect to R8, where there is a severe effect upon the spectral sensitivity resulting in a more sharply 'tuned' curve (Fig. 5.8). In contrast, the effects in R7 cells are probably relatively minor, as the lateral filter action that occurs when two or more pigments share the same waveguide (Snyder *et al.*, 1973) is less drastic than the direct filter action appropriate for the effect of 7y absorption upon R8 (Appendices I and II).
The apparently random distribution of R7 rhabdomere type over the eye as a whole, and the apparent existence of two distinct spectral mechanisms in R7 cells, both generating a spectral sensitivity with a single peak in the ultraviolet (Fig. 5.7), at present confound our attempts at a rational explanation in terms of function.
The central rhabdomere R7 of fly acts as a light guide propagating only the fundamental mode (Kirschfeld and Snyder, 1975). Assuming that the sensitivity of a retinula cell is proportional to the number of photons absorbed by the visual pigment and that only one absorbing pigment is present in the rhabdomere we can therefore write the sensitivity as a function of wavelength as (Snyder and Pask, 1973):

\[ S(\lambda) = 1 - e^{-\gamma \eta \ell} \]  \hspace{1cm} (5.1)

where \( \gamma(\lambda) = \alpha(\lambda)c \), \( \alpha(\lambda) \) is the normalised dimensionless absorption coefficient of the pigment, \( c \) is its concentration per unit length, \( \ell \) is the length of the rhabdomere and \( \eta(\lambda) \) is the fraction of modal light within the rhabdomere.

When there is a photostable pigment present, Eq. (5.1) must be modified to include lateral screening (Snyder et al., 1973). If we assume that the photostable pigment acts only as a screening pigment (cf. sensitising pigment), then

\[ S(\lambda) = \frac{\gamma_{ap}}{\gamma_{ap} + \gamma_{pp}} \left[ 1 - e^{-(\gamma_{ap} + \gamma_{pp})\eta \ell} \right] \] \hspace{1cm} (5.2)

where the subscripts ap, pp stand for active visual pigment and photostable (screening) pigment respectively.

The parameter \( \eta \) is a function of the waveguide parameter

\[ \nu = \frac{\pi d}{\lambda} (n_1^2 - n_2^2)^{1/2} \]

where \( d \) is the diameter of the rhabdomere, \( \lambda \) the vacuum wavelength of light, and \( n_1, n_2 \) are the refractive indexes of the rhabdomere and the surrounding medium respectively. \( \eta \) for R1-6 and R7/8 has been measured directly.
by Kirschfeld and Snyder (1975). In calculating $\eta(\lambda)$ here we use a value of $V = 1.6$ at $\lambda = 500$ nm. This lies near the upper limit of the values of Kirschfeld and Snyder, but this higher value is indicated by later measurements on R7 using a different technique (McIntyre and Kirschfeld, In preparation). Our results and conclusions do not depend sensitively on the $V$ value chosen.

1. We first consider hypothesis I, that UV cells have 7$\gamma$ rhabdomeres and that $S_{UV}(\lambda)$ is a result of screening by the photostable pigment, of the pigment in the UT rhabdomeres, i.e. $S_{UT}(\lambda)$ is given by Eq. (5.1) and $S_{UV}(\lambda)$ by Eq. (5.2), where $\alpha_{ap}(\lambda)$ is the same in both cases. $\alpha_{ap}(\lambda)$ is calculated from Eq. (5.1) by assuming a value for the maximum extinction $\gamma_{ap}^{max}$ in UT cells. This $\alpha_{ap}(\lambda)$, together with $\alpha_{pp}(\lambda)$ from the results of Kirschfeld et al. (1978), are then substituted into Eq. (5.2) to give $S_{UV}(\lambda)$. The results are shown in Fig. 5.7a. We have deliberately used a high value for the maximum extinction of the photostable pigment ($\gamma_{pp}^{max} \lambda = 3.9$, giving 98% absorption), in order to generate the maximum possible screening effect. The result shown is insensitive to the value of the maximum extinction $\gamma_{ap}^{max}$ of the active pigment; thus values of 1.0 and 3.0 give an almost identical result.

We conclude from this analysis that it is not possible to generate $S_{UV}(\lambda)$ by screening the UT pigment with the measured photostable pigment.
2. It has been suggested (Smola, 1977) that two classes of R7's exist with different diameters, although no values were given. Snyder and Pask (1973) show that a decrease in rhabdomere diameter can have a profound effect on the spectral sensitivity because of waveguide effects. In particular, the uv sensitivity is increased with respect to the visible, and the peak of sensitivity is shifted towards the uv. To exclude the possibility that the difference between UV and UT sensitivities in the uv is due to different rhabdomere diameters, we calculated the effect on the UT sensitivity of decreasing the diameter, using Eq. (5.1). We find that, even with extreme changes in diameter, it is not possible to generate the UV sensitivity from UT, nor to shift the UT peak more than about 5 nm, by waveguide effects.

3. The measured $S_{uv}(\lambda)$ corresponds quite well to a single rhodopsin nomogram (Dartnall, 1972) with maximum absorption at 344 nm, i.e. Eq. (5.1). The agreement is best when the maximum extinction is not too large (Fig. 5.7a).

4. We next consider the pigments in UT cells, assuming that UT cells correspond to 7y rhabdomeres. To calculate the absorption spectrum of the active pigment(s), we use Eq. (5.2) to remove the effects of selfscreening and screening by the photostable pigment, assuming various values for $\gamma_{\text{ap}}^{\text{max}}$ and $\gamma_{\text{pp}}^{\text{max}}$. Curves of $\alpha_{\text{ap}}(\lambda)$, calculated in this manner, are shown in Fig. 5.7b. It is clear that these curves cannot be generated by a single nomogram. However, the shoulder at 430-450 nm, and the fact that a nomogram with a maximum in this range fits the right-hand portion of these curves
(λ>λ_{max} \text{ of the nomogram}), suggest that two pigments, one a rhodopsin given by the nomogram, the other a uv-absorbing pigment, might be present. A uv sensitising pigment with a maximum at about the same wavelength has already been found in R1-6 of fly (Kirschfeld \textit{et al.}, 1977).

5. Finally we consider the hypothesis that the measured UT sensitivity is generated by the same uv rhodopsin as in UV cells, extended into the blue by the 460 nm photostable pigment acting as an antenna pigment (Hypothesis IIb). Antenna pigment molecules (the donors) absorb light and are thought to pass the energy on to the visually active rhodopsin molecules (the acceptors) by means of a resonance transfer (Kirschfeld and Franceschini, 1977; Förster, 1965). This transfer depends upon, among other things, (a) there being sufficient energy available from the donors to excite the acceptor rhodopsin molecules, (b) the amount of overlap of the fluorescence spectrum of the donor with the absorption spectrum of the acceptor and (c) the relative orientation of the donor and acceptor molecules (Förster, 1965). We argue below that, because of these factors, the hypothesis of this section can be precluded.

(a) In the present situation, the excitation of the uv rhodopsin (λ_{max} \approx 360 \text{ nm}) requires more energy than is required for the excitation of the photostable pigment (λ_{max} \approx 460 \text{ nm}), so that an "uphill" transfer must take place. The energy deficit must be supplied by the heat bath of the donor (Knox, 1975). For the two pigments here we find a deficit of about 30 kT, where T is room temperature (20°C). Uphill transfers
have been observed (Knox, 1975), but the energy deficit in
the case reported is only about 3.6 kT, and the transfer rate
"uphill" only about half of that in the opposite direction

The energy difference between the uv rhodopsin acceptor
and the photostable pigment donor is therefore a powerful
argument against significant nett energy transfer taking
place in the "uphill" direction.

(b) Again because of the separation of the absorption
peaks, the amount of overlap between the donor fluorescence
spectrum (λ_max > 460 nm, and probably λ_max ≈ 520-540 nm -
N. Franceschini, unpublished) and the acceptor absorption
spectrum (λ_max ≈ 360 nm) is likely to be small, resulting
in a small energy transfer, even if the energy deficit is
overcome.

(c) The relative orientation of the donor and acceptor
molecules, based on present knowledge of the microvillar
membrane structure, also further reduces the total energy
transfer. Microspectrophotometric measurements indicate that
the photostable pigment molecules are probably predominantly
aligned perpendicular to the membrane surface, i.e. radially
with respect to the microvillar axis (Kirschfeld et al., 1978;
McIntyre and Kirschfeld, In preparation), whereas the
rhodopsin chromophores probably lie at a small angle to the
membrane surface (Snyder and Laughlin, 1975; Schlecht and
Täuber, 1975; Goldsmith and Wehner, 1977). A calculation
incorporating the angular dependence of resonance transfer
( Förster, 1965), together with the possible alignments of the molecules, shows that this relative orientation reduces energy transfer by 75%-90% compared with that at optimal (predominantly parallel) alignment. Relative orientation also has a significant effect on the dichroic absorption of the microvilli. This is discussed in detail in Appendix III, in which a further argument against the present hypothesis is put.

We do not preclude here the possibility that the photostable pigment acts as an antenna for a rhodopsin with \( \lambda_{\text{max}} \approx 430 \) nm, the existence of which is suggested above. This arrangement would tend to increase the sensitivity at these wavelengths, but as the nett transfer of energy is still likely to be small, the predominant role of the photostable pigment would still be one of screening and our overall conclusions would remain unaffected.

(d) Finally we examine the expected shape of the spectral sensitivity curve of a 7y cell, assuming a uv rhodopsin (given by a Dartnall nomogram) and the blue-absorbing photostable pigment (extinction spectrum from Kirschfeld et al., 1978) acting as a sensitising pigment. Then

\[
S(\lambda) = \frac{\gamma_R + \delta \gamma_P}{\gamma_R + \gamma_P} \left[ 1 - e^{-(\gamma_R + \gamma_P) \eta L} \right],
\]

where \( \delta \) is the efficiency of power transfer from the sensitising pigment to the rhodopsin and \( \gamma_R, \gamma_P \) are the absorption coefficients of the rhodopsin and photostable pigments respectively. Various values for \( \gamma_{R_{\text{max}}}, \gamma_{P_{\text{max}}} \) were used and \( \delta \) chosen to give the best fit to the measured UT spectral sensitivity curve. A nomogram with maximum at 360 nm was
taken as corresponding most closely to the UT sensitivity in the uv. We find that the spectral sensitivity curve given by Eq. (5.3) falls off more rapidly than the measured UT sensitivity in the region 360 nm-400 nm, and from then on is practically flat, in contrast to the slow fall off of the measured curve. We conclude that, even if there were significant energy transfer, the shape of the UT spectral sensitivity curve is not that predicted by the above model.
APPENDIX II  $S(\lambda)$ OF R8

On-axis light reaching R8 must first pass through R7, so that the absorption properties of R7 will have a significant effect on the sensitivity of R8. The spectral sensitivity of R8, with the screening of R7 included, is given by (Snyder, 1973):

$$S_8(\lambda) = e^{-\gamma_7 \eta_7 \lambda_7} S_8'(\lambda)$$

(5.4)

where $S_8'(\lambda)$ is the sensitivity of R8 alone, given by Eq.(5.1) (or Eq. 5.2 if a screening pigment is present). $\gamma_7(\lambda)$ and $\lambda_7$ are the absorption coefficient and length of R7, respectively.

Strictly speaking, we should calculate $S_8(\lambda)$ for unpolarized (UP) light (as was used in the measurements) by taking the average of the sensitivities when light is polarised parallel and perpendicular to the microvilli of R7, i.e.

$$S_8^{UP}(\lambda) = 1/2[S_8^P(\lambda) + S_8^P(\lambda)].$$

However, the error introduced by ignoring dichroism in Eq. (5.4) is small for reasonable values of the dichroic ratios, and this has the advantage of two less parameters to consider. If dichroism is included, it must be remembered that the microvilli of R8 are perpendicular to those of R7.

Figure 5.8 shows the predicted $S_8(\lambda)$ from Eq. 5.4). The values for $\gamma_7 \eta_7 \lambda_7$ were taken from the measurements of R7y by Kirschfeld et al. (1978). Nomograms with different $\lambda_{max}$ were tried, but $\lambda_{max} \approx 520$ nm was found to provide the only reasonable fit to the longer-wavelength part of $S_8(\lambda)$, where screening by R7y is negligible and where agreement between
the nomogram and the measured values is therefore to be expected. The goodness of fit, and certainly the qualitative agreement, are relatively insensitive to the other parameters. As well as the measured and predicted $S_8(\lambda)$, Fig. 5.8 shows the normalised extinction of R7y used in the calculation and the predicted sensitivity, $S'_8(\lambda)$, of an R8 unscreened by R7y, but with the same amount of self-screening as used for the predicted $S_8(\lambda)$. The peak of the measured $S_8(\lambda)$ was estimated by fitting a parabola to the highest three measured points.

The measured $S_8(\lambda)$ has a second maximum of about 30% in the uv. While it is possible to obtain a reasonable agreement between the measured and predicted curves in the uv by using a nomogram with a $\beta$ peak, we have not included this in Fig. 8, as there is a large uncertainty in several of the parameters in the uv, in particular in the extinction of R7y (Kirschfeld, personal communication). More data are needed before $S_8(\lambda)$ in the uv can be predicted with certainty.

We have not included the effect of screening of R8 by the active visual pigment(s) in R7y. No significant difference in the measured $S_8(\lambda)$ was in fact found after adaptation with uv, blue or green light. Whereas absorption by the active pigment as measured in UT cells would have little effect on $S_8(\lambda)$ (except perhaps on the uv peak), a metarhodopsin with maximum absorption in the range 500-600 nm could theoretically be of significance. This point remains to be considered once the pigments in R7y have been identified.
APPENDIX III  POLARISATION SENSITIVITY (PS)

(a)  \textit{R7}

1. In 7p rhabdomeres, which lack the photostable pigment, maximum absorption of light occurs when light is linearly polarised parallel to the microvilli (Kirschfeld and Franceschini, 1977). Assuming only a single rhodopsin is present, the PS is given by (Snyder, 1973):

\[
PS(\lambda) = S\#(\lambda)/S\perp(\lambda) \quad S(\lambda)+\text{Eq. (5.1)} \quad (5.5)
\]

The subscripts \# , \perp indicate that the absorption coefficients \( \alpha\# , \alpha\perp \) for light polarised parallel, perpendicular to the microvilli are to be used. Note that because the rhabdomeres are birefringent, \( \eta\# \neq \eta\perp \) (Kirschfeld and Snyder, 1975), but this effect is small and we ignore it here.

The behaviour of Eq. (5.5) is discussed in detail by Snyder (1973). In general the higher the absorption (the larger \( \gamma\# \)), the lower the PS, owing to self-screening. At 442 nm, the absorption by UV cells is very small (see Fig.5.3); the predicted PS in this case (assuming 7p = UV) is equal to the dichroic ratio \( \Delta \) of the microvillar medium containing the rhodopsin. Taking \( \Delta = 1.8 \) (PS measured at 442 nm for Calliphora UV cells, Table 5.2), and assuming an absorption of approximately 1% per micron in R7p, the predicted PS at 358 nm (near the maximum of absorption) is 1.5, in agreement with the measured value. The overall trend in the measured Musca (white) UV PS values is the same, although the agreement with predicted values is not as good. The measured values for...
Musca (white) indicate a higher dichroic ratio and possibly a lower absolute absorption, but the evidence is not yet conclusive.

2. In 7y rhabdomeres it was found that maximum extinction as measured by microspectrophotometry (MSP), occurs when the light is polarised perpendicular to the microvilli and that the photostable pigment in R7y is at least partly responsible for this observation (Kirschfeld and Franceschini, 1977; Kirschfeld et al., 1978; McIntyre and Kirschfeld, In preparation). As we show in this paper, maximum sensitivity with respect to direction of polarisation occurs when light is polarised parallel to the microvilli. This argues strongly for a screening role for the photostable pigment (see 3 below). The PS of R7y (and, by our hypothesis, UT cells), assuming a screening role for the photostable pigment, is given by

$$PS(\lambda) = S_{\parallel}(\lambda)/S_{\perp}(\lambda) ; \quad S(\lambda) \text{+ Eq. (5.2)} \quad (5.5)$$

From a knowledge of lateral polarisation filters (Snyder et al., 1973), we predict, that because the maximum absorption of the photostable pigment occurs for light polarised perpendicular to the microvilli, the PS, will be enhanced at those wavelengths at which the photostable pigment absorbs strongly. Figure 5.5 shows that the PS of UT cells does in fact peak at around 450 nm, in confirmation of this prediction. A dichroic ratio of 1.6-1.8 for the active pigment absorbing at these wavelengths is sufficient to generate the maximum PS measured in Calliphora.

It is difficult to explain the measured $PS(\lambda)$ results -
low PS in the uv and $PS = 2.3$ (Calliphora) in the blue - on the basis of one active pigment and the photostable pigment. A dichroic ratio in the range 1.6-1.8 is necessary to generate the maximum measured PS, yet this value leads to a PS in the uv of about 1.5-1.6, well above that found. This suggests the presence of a third pigment, absorbing in the uv - either an active (sensitising?) pigment or another screening pigment with maximum absorption parallel to the microvilli.

3. We now examine the PS under the hypothesis that UT cell sensitivity results from a uv rhodopsin as in UV cells, plus a photostable pigment ($\lambda_{\text{max}} = 460$ nm) acting as a sensitising pigment (see Appendix I, # 5). The dependence of the amount of energy transferred to the rhodopsin on the direction of polarisation of the incident light can be calculated using the angular factor from the Förster resonance transfer theory (Förster, 1965), and by making certain assumptions about the distribution of the rhodopsin (acceptor) and photostable pigment (donor) molecules. For the latter we use a Boltzmann distribution with axis in the radial direction with respect to the microvillar axis and parameter chosen to give the observed dichroic ratio ($\simeq 2$) due to the photostable pigment (Kirschfeld et al., 1978; McIntyre and Kirschfeld, In preparation). For the rhodopsin, both fixed positions and a random distribution within the membrane surface were considered, but with various fixed tilt angles with respect to the membrane surface. Partially ordered distributions, such as a Boltzmann distribution, lie between these two extremes.
From these calculations we obtain the effective dichroic ratio $\Delta_T$ for sensitivity, i.e. the ratio of energy transferred to the rhodopsin when the E-vector direction is parallel to the microvilli to that when it is perpendicular. The PS is given by:

$$PS = \frac{\Delta_p \Delta_T}{1 - e^{-\gamma_\perp \lambda / \Delta_p}} \cdot \frac{1 - e^{-\gamma_\parallel \lambda}}{1 - e^{-\gamma_\perp \lambda}}$$

(5.7)

where $\Delta_p (> 1)$ is the actual dichroic ratio of the microvilli ($\gamma\perp / \gamma\parallel$), resulting from the dichroic absorption of the photostable pigment, and $\gamma\parallel$ is the extinction of the photostable pigment for light polarised perpendicular to the microvilli. Using the values $\gamma_\parallel\lambda = 3$, $\Delta_p = 2.1$ (McIntyre and Kirschfeld, in preparation) we obtain

$$PS = 1.7 \Delta_T$$

(5.8)

A PS $> 1$ ($< 1$) indicates maximum sensitivity to light polarised parallel (perpendicular) to the microvilli. Therefore provided that $\Delta_T > 0.6$, there is greater sensitivity to light polarised parallel than perpendicular to the microvilli, despite the fact that only 76% of incident light polarised parallel is absorbed compared with 95% of light polarised perpendicular. The relative orientation of the two types of molecules involved accounts for the different between the direction of maximum sensitivity and maximum absorption.

However, the restrictions on the rhodopsin molecules' orientation must be quite severe in order to achieve a high enough $\Delta_T$. In particular, to achieve a value $PS = 2.3$, as measured in Calliphora at 442 nm, the rhodopsin molecules
must be tilted out of the plane of the membrane by at most about 7° and must deviate by not more than about 10°, on average, from the microvillar axis. This compares with values of 20° tilt and ± 50° axial deviation deduced by Goldsmith and Wehner (1977) in crayfish. A tilt angle of 20° in our analysis gives a PS of about 1.1 when the rhodopsin molecules are perfectly aligned along the microvillar axis and a value of 0.8 when the angle with the axis is random.

We conclude, on the basis of this model, that it is unlikely that the measured PS in Calliphora could be generated by the photostable pigment acting as an antenna pigment for a uv rhodopsin, and that it is impossible to explain the PS measured in Musca. The same conclusions apply incidentally if the rhodopsin has its maximum around 430 nm, as proposed above, again arguing for a predominantly screening role for the photostable pigment.

(b) R8

The expression for the PS of R8 is more involved, because the dichroic absorption properties of R7 as well as R8 must be taken into account. The PS is given by:

$$\text{PS}(\lambda) = S \parallel(\lambda)/S \perp(\lambda) \text{, \quad } S(\lambda) + \text{Eq. (5.4)} \text{ (5.9)}$$

The directions $\parallel, \perp$ are with respect to the microvilli direction of R8. As the microvilli of R8 are aligned perpendicular to those of R7, the photostable pigment absorbs maximally parallel to the microvilli of R8; it is therefore possible that at some wavelengths $\text{PS}_8(\lambda) < 1$, i.e. greater absorption perpendicular to the microvilli of R8.
In view of the limited PS data of R8 and the large number of variables, no comparison of theory and experiment is given here. However we note the following points:

1. The PS of an R8 lying beneath a R7y rhabdomere should be wavelength dependent in the region of the photo-stable pigment absorption in R7y.

2. The good fit of the 520 nomogram plus screening to $S_g(\lambda)$ suggests that the active pigment in R8 is a normal rhodopsin, and as such would be expected to result in higher absorption parallel to the microvilli than perpendicular, as is the case with all known invertebrate rhodopsins (reviews: Goldsmith, 1975; Waterman, 1975). It would seem then in this case that the combination of R8/R7y is not designed for high PS in R8, at least in the range 400 nm-520 nm. The microvilli in R8 and the direction of maximum absorption of the photo-stable pigment in R7y should be perpendicular to maximize PS in R8 (Snyder, 1973; Menzel and Snyder, 1974).

3. R8's lying beneath R7p rhabdomeres should have their PS enhanced by the filtering effect of the R7p, but as the dichroism of R7p (UV cells) appears to be small and their absorption in the range 400-600 nm low, the enhancement will not be large.

We have ignored the possible effects of twisting of the rhabdomeres R7 and R8 in the above analysis. If twisting is present, as suggested by Smola (1977), higher dichroic ratios will be necessary to explain the observed PS. For example, we require a dichroic ratio for rhodopsin of about 1.8 to
explain the measured PS of UV cells in Calliphora. If these were twisted a total of 50°, the dichroic ratio would have to be 2, at 120° 3, and at 200° of twist 5. We cannot discount at this stage the possibility that the difference between the PS of UV and UT cells is due to UV rhabdomeres twisting and UT not, although there is good quantitative agreement between measured and predicted results for Calliphora without such twisting. The difference in PS between Calliphora and Musca may also arise from twisting—Smola's results are for Calliphora. The resolution of these questions awaits further experimental data.

For a more detailed discussion on twisting of rhabdomeres, including its effects in fly, see McIntyre and Snyder (1978).
GENERAL APPENDICES
APPENDIX A

GENERAL METHODS

Specific experimental details relevant to particular studies are discussed in the Methods sections of each Chapter, but since many features - such as the animals, recording conditions, optical stimulation, measuring procedures and calibrations were essentially the same throughout the work, it is appropriate to describe them under a separate heading to avoid unnecessary repetition.

1. Animals

All experiments described were performed upon dipterans. Details of the species used, and their culture conditions are as follows.

a) Calliphora stygia (wild type)

This species was used for the majority of the experiments as, of the flies tried, it was found to be the easiest species to prepare and record from. Calliphora stygia is the commonest species of blowfly occurring in the wild around Canberra, and all specimens used were caught locally, or taken from cultures initiated from wild specimens. Cultures were periodically renewed, so that no specimen used had been bred in captivity for more than four or five generations. In general, there were no significant qualitative or quantitative differences in the results obtained from wild or cultured flies, except that the anomalous class of 'BR' cells, described in Chapter III, were all recorded from wild flies.
For the cultures, flies were induced to lay eggs on rotting liver in a darkened container. The maggots were fed on liver, and after pupation, the pupae were kept in moist vermiculite for approximately 10 days until they hatched. Adults were fed on sugar, water, and fresh liver, and kept in cages under a 16 hr light: 8 hr dark cycle, in a glasshouse whose temperature was maintained between $15^\circ C$ and $30^\circ C$, with humidity kept at 50%. Specimens for experiments were taken from between 4 and 20 days post-emergence.

b) *Lucilia sericata* (wild type)

This species was also cultured from wild specimens caught locally. It was used for very few experiments - in particular to demonstrate that the spectral classes of central retinula cells R7 and R8 (most thoroughly investigated in *Calliphora stygia*) also occurred in another dipteran genus (see Chapter V). Culture conditions were similar to those used for *Calliphora stygia*.

c) *Musca domestica* (white-eyed mutant)

This species was used in a variety of experiments discussed in Chapters I, II and V. The cultures originated from pupae kindly supplied by Dr. N. Franceschini (Tübingen). Maggots were fed on an artificial medium consisting of bran, milk powder, baker's yeast and water. The adults were fed on liver, sugar and water. The culture environment was the same as that described for *Calliphora stygia*. 
d) *Calliphora erythrocephala* (chalky mutant)

This species was only used for a few experiments described in Chapter V. The pupae were also supplied by Dr. N. Franceschini (Tübingen), and the culture conditions were the same as those described for *Calliphora stygia*.

Both these mutants, *Musca* (white) and *Calliphora* (chalky), lack all accessory pigments in the retina, including the pupil granules.

2. Preparation of animals.

In all experiments the surgery on the animal was minimal. Unanaesthetised flies were mounted intact on a perspex platform attached to a ball and socket stand fixed to a small magnet. First the wings were pressed into some soft periphery wax moulded onto the platform, and then the fly further immobilised in a low melting point wax (resin and bee's wax). The major thoracic spiracles were left unobstructed, and the abdomen was free to move, so as to encourage normal respiration. The head and thorax, however, were completely immobilised.

The indifferent electrode (a fine platinum wire) was inserted just subcorneally, at the ventral margin of the eye. Initially the eye used was the same as the one from which recordings were made, as this minimises the contribution of the e.r.g. to the responses. However, at a later stage, a control experiment, in which two indifferent electrodes were inserted (one in each eye) revealed that the effect of the e.r.g. on the peak responses of the intracellularly recorded
receptor potentials was minimal, since intensity/response functions, determined with either configuration were virtually identical. Since inserting the indifferent electrode in the contralateral eye minimises the damage done to the eye recorded from (a particularly important consideration when investigating angular properties), this configuration was subsequently used, except when investigating plateau response levels, which were shown to be affected by e.g. contamination.

So far the stages mentioned were performed in normal light, from then on the fly was only exposed to dim red light.

Next, the fly was mounted, via the magnet, on the stage of a Zeiss grease-plate micromanipulator, and a small hole cut in the cornea using a chip of razor blade. With care this hole may be made only ca. 5 facets in diameter, and often smaller. On occasion, and particularly after making angular measurements, the optics of the eye were checked after the experiment by observing the deep pseudopupil pattern of the eye in orthodromic, reflected light (Franceschini and Kirschfeld, 1971b). The characteristic pattern was then usually found to be unaffected except in the immediate vicinity of the hole.

Immediately after the hole was cut, it was covered with a high viscosity silicone vacuum grease, to prevent dessication, and also to allow repeated electrode tracks to be made through the same hole.

Animals thus prepared, typically continued to show healthy intracellular responses for 10-24 hours.
3. Electrodes

Electrodes were pulled from fibre-filled capillary glass (Borosilicate, Karl Hilgenberg) using a vertical two stage pipette puller (David Kopf 700C). For the best recordings - particularly from the small central retinula cells - it was necessary to be rather critical about selecting only the electrodes with the finest tips, which were always examined under a high power objective before filling.

Electrodes were simply filled by injection of 3M potassium acetate through a 30 gauge hypodermic needle. Electrode resistances were typically 70-200 megohms when measured in tissue.

4. Recording conditions

Electrodes were always lowered vertically into the eye so that it was necessary to orient the fly to choose the direction of the electrode track. For retinula cells, this was usually chosen to be parallel to the front surface of the eye, so that the electrode had a good chance of penetrating numerous cells in the retina layer itself. When making recordings in the lamina or chiasm, the fly was tilted back so that the electrode penetrated the basement membrane, thus entering the lamina compartment, and later, the chiasm.

Responses were amplified through a Grass P16 preamplifier that had some additional circuits added to the input stage. These included: a bridge circuit coupled to a pulse generator (for measuring electrode resistances, and detecting conductance changes in cell membranes); a voltage back-off unit to
neutralise tip potentials; a calibration pulse unit; and finally a relay break-away unit, that simultaneously disconnected the electrode from the amplifier and allowed current to be passed directly across the preparation to ground (for purposes such as dye injection or clearing clogged electrode tips). Current passed through the electrode could be monitored by taking the indifferent electrode to ground through the 1 megohm input impedance of the Tektronix CRO (5103N). Responses displayed on the CRO were simultaneously recorded on a Devices chart recorder from which the majority of the data was measured. The preparation was shielded from electrical interference and stray light by a Faraday cage covered in thick black curtain material.

5. Optical simulation

Light was delivered by a high pressure, 900W Xenon arc lamp through one, or both, of two beams equipped with quartz condensers (Fig. A.1). Each beam was first focussed through a small (5 mm) aperture in a heavy duty transistor cooling vane. This cut out much of the infra-red heat from the arc electrodes. The infra-red in the light was further reduced by a heat filter, or cold mirror. The shutters (thin vanes of blackened steel mounted on high speed rotary solenoids) were situated just beyond the apertures. The beams were then collimated through quartz lenses, and passed through sets of interchangeable quartz neutral density filters (Schott or Balzer) and narrow band interference filters (Schott DIL) before being focussed onto one end of a quartz fibre optics light guide (Schott). The other end of this served as the
Figure A.1  The set-up used for optical stimulation in all experiments. Light from a 900 Watt Xenon arc lamp (Xe) may be delivered via one or both of two beams (Test and Ad). The adapting beam (Ad) may be used in one of two modes. (1) The collimated beam is focussed through its own light-guide (LG). (2) A front surface mirror (M) deflects the collimated beam onto a half-silvered quartz mirror (HM) in the collimated path of the test beam. Thereafter, the adapting beam is 'mixed' with the test beam and delivered through a common light guide. The beams are thus spatially coincident. Details of filters etc., in both beams are described in the text. CM, cold mirror; A, aperture (5 mm); S, shutter; ND, neutral density filters (quartz); IF, interference filters mounted on wheels; W, neutral density wedge (quartz). The other ends of both light-guides (LG) are mounted on a 'Cardan' arm.
stimulus and was mounted on a Cardan arm perimeter device, whose vertical and horizontal axes of rotation passed through the eye of the animal. The effective stimulus size could be adjusted between $0.1^\circ$ and $25^\circ$ (in visual angle) in a variety of ways: i.e. by changing the distance between the light guide tip and the eye, by placing small apertures in front of the light guide or by placing a diffusing screen in front of the light guide. When investigating polarisation sensitivity an ultraviolet polarizer (HNP'B - Polaroid Corp.) was mounted in front of the light guide on a device that allowed the polariser to be accurately rotated in $10^\circ$ steps.

The details of the filters in the two beams were as follows:

**Test Beam (Main test beam)**

*ND filters* (in terms of $\log_{10}$ units attenuation): 3.0, 2.0, 1.0, 0.75, 0.5, 0.25 - thus allowing the intensity to be varied through 7.5 log units in 0.25 log unit steps.

*Interference filters:* at the following wavelengths: 322, 333, 358, 381, 405, 421, 434, 442, 464, 484, 499, 523, 541, 572, 600, 621 (nm). Bandwidth (at 50% transmission) 5 nm. With the exception of the 322, and 333 and 358 nm filters, transmission of the filters was adjusted (by means of different thicknesses of clear plastic film) so as to give isoquantal delivery ($\pm 15\%$). The 358 nm filter transmitted ca.50% more quanta than the average, the 322 nm and 333 nm filters ca.3x less.
Ad. Beam (Secondary test beam, or adapting beam).

**ND filters:** $2 \times 2.0$ log unit filters, plus a quartz neutral density wedge, covering 2 log units of intensity. Intensity of this beam could thus be adjusted continuously over 6 log units.

**Interference filters:** at the following wavelengths: 389, 412, 451, 513, 535, 561, 591 (nm).

The two beams could be used together in two basic modes: (Fig. A.1).

(1) **mixed beam method**

The adapting beam was mixed with the test beam by means of a half-silvered quartz mirror in the collimated light path. The two beams were then focussed through the same lens onto a common light guide. Test and adapting beams were thus spatially coincident.

(2) **dual beam method**

The adapting and test beams were focussed onto separate light guides. In this mode test and adapting lights may be spatially separate. This mode was mainly used for centre/surround type stimulation. Thus the test beam light guide was mounted axially on the Cardan arm, but at the centre of a broad, diffuse reflecting disc (consisting of aluminium foil covered by lens tissue). The adapting beam light guide was also mounted on the Cardan arm, but light from it was back-projected, by means of a front surface mirror onto the
diffusing screen. The lens tissue had spectrally flat transmission in the range 350-650 nm.

Both beams had independent shutter control. In adapting experiments the adapting beam was usually left on continuously, whilst test flashes were superimposed upon the background. For some experiments however, the two beams were instantaneously substituted for each other by using an alternating shutter arrangement. This second method has the advantage that a variety of intensity decrements, as well as increments may be presented. In addition the adapting beam may be calibrated in terms of the test beam by finding the intensity (continuously variable by use of the wedge) at which the alternating shutter configuration results in a null response from the cell.

6. Calibration

Calibrations of the stimulus intensities in terms of monochromatic quantal flux were performed at frequent intervals using a Hewlett Packard radiant flux meter (Type 8330 A) placed in the same relative position to the light guide as the fly's eye. This instrument is stated by the manufacturers to be "accurate to within ± 5% of full scale on any range, including uncertainty of NBS - traceable calibration standards, transfer calibration, linearity and electronic instrumentation over an ambient temperature range of 0-55° C". As a further check the actual instrument used was checked against a similar instrument, and both instruments gave identical readings.

Over the period of experiments the relative transmission values of the interference and neutral density filters varied
by less than ± 5%, although absolute values deteriorated slightly with the age of the lamp. All calculations of absolute and relative sensitivities were continuously updated by the use of the transmission data from the most recent calibration.

For some experiments, very low intensities were used, at which the radiant flux meter was not sensitive enough to give accurate measurements. In such intensity regions, the calibrations were extended by use of a photomultiplier tube (RCA model No. 8654). Since this gave a relative measure only, this in turn was calibrated with respect to the radiant flux meter, at some arbitrary intensity, so that absolute quantal flux could be deduced, for instance, at the very low levels required to generate discrete quantum bumps.

The spectral transmission of the interference filters was measured using a Spectroradiometer (IL 780, International Light Inc.) to check that the peak wavelength and bandwidth met the manufacturer's specifications.

7. Measuring procedures

Numerous experimental paradigms were employed during the course of the study, and the more specialised ones are described, as they occur, in each chapter. The most widely used measurements, however, were of spectral, angular and polarisation sensitivities. Such relative sensitivities are defined as the reciprocals of the intensities required to generate constant responses as the wavelength, E-vector direction, or angle of stimulation is varied (Table A.1).
Except for the case of the anomalous BR cells (Chapter III), a standard method, which assumes that the shapes and slopes of the V/log I curves are independent of wavelength, E-vector direction and angle, was employed to calculate such sensitivities. If this assumption is true, sensitivity can be calculated by measuring responses to isoquantal flashes as the wavelength E-vector direction or angle are varied, and then referring these responses to one V/log I curve, to determine the effective intensity, and hence the relative sensitivity. As many hundreds of these sensitivity functions had to be calculated over the course of the study, a computer program was used for the calculations (Laughlin, 1974a). The basis of the program was a least squares fit to a third order polynomial for the V/log I curve, the equation for which was then used to calculate sensitivities from the responses.

Particular care was taken in dark-adapted determinations to ensure that the test intensity, duration and interstimulus interval were adjusted so as not to cause any significant adaptation during the actual measurements.

Definitions of standard terms used throughout the thesis are found in Table A.1.
**TABLE A.1**

Definitions of terms and units

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V$</td>
<td>amplitude of response measured as deflection from dark resting potential (mV)</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>saturated (maximum) peak response amplitude of a dark-adapted cell (mV). Normalised responses are expressed as $% V_{\text{max}}$</td>
</tr>
<tr>
<td>$I_a$</td>
<td>the intensity of the background (or adapting illumination) expressed in relative terms.</td>
</tr>
<tr>
<td>$I_t$</td>
<td>the intensity of the test beam expressed in the same units as $I_a$</td>
</tr>
<tr>
<td>intensity/response function</td>
<td>the function which, at any one adaptation state relates the total voltage response ($V$) to total intensity, $I = (I_a + I_t)$</td>
</tr>
<tr>
<td>$V/\log I$ curve</td>
<td>the intensity/response function plotted as $% V_{\text{max}}$ against $\log I_{\text{max}}$</td>
</tr>
<tr>
<td>voltage bandwidth</td>
<td>total voltage response range of a cell at any one adaptation state (mV)</td>
</tr>
<tr>
<td>dynamic range</td>
<td>the range of intensities which at any one adaptation state, corresponds to the voltage bandwidth of a cell. For photoreceptors the dynamic range is defined between the response limits of 5-95% $V_{\text{max}}$</td>
</tr>
<tr>
<td>sensitivity</td>
<td>a general term for responsiveness, to avoid ambiguity, several operational definitions are used:</td>
</tr>
<tr>
<td>detection sensitivity</td>
<td>the reciprocal of stimulus intensity required to produce a criterion signal: noise ratio (see Chapter II).</td>
</tr>
<tr>
<td>range sensitivity</td>
<td>reciprocal of the total intensity required to produce a criterion absolute voltage response (50% $V_{\text{max}}$ in this study). Range sensitivity defines the intensity region within which the dynamic range lies at any one adaptation state. In the dark-adapted state, it may be defined as:</td>
</tr>
</tbody>
</table>
APS\textsubscript{50} the reciprocal of the quantal irradiance required to produce a response of 50\% V\textsubscript{max} when using a parallel axial monochromatic beam of the most effective (peak) wavelength (quant\textsubscript{a} \text{cm}^2 \text{s}^{-1}) (after Laughlin, 1976a)

PAQ\textsubscript{50} (peak axial quanta) the reciprocal of the APS\textsubscript{50} (i.e. the quantal flux required to produce a response of 50\% V\textsubscript{max}) quanta.cm\textsuperscript{-2} .s\textsuperscript{-1})

DAI\textsubscript{50} relative intensity required to produce a response of 50\% V\textsubscript{max} (log units)

relative sensitivity (spectral, angular, polarisation) reciprocal of the intensity required to elicit a constant criterion response as the wavelength, E-vector direction or angular position is varied, normalised to 100\% at the most effective wavelength, E-vector or angle.

polarisation sensitivity ratio between the maximum and minimum relative sensitivity as the E-vector direction of polarised light is rotated through 180\textdegree.

contrast intensity fluctuations normalised relative to mean intensity. The standard definition is used, i.e. contrast = (I_{\text{max}} - I_{\text{min}})/(I_{\text{max}} + I_{\text{min}})

contrast efficiency the slope of the V/log I curve, normalised to %V\textsubscript{max}/log unit.
Many aspects of this study required a confident knowledge of a recorded cell's anatomical identity.

Previous studies (McCann and Arnett, 1972) showed that retinula cells, R1-6, were characterised by a double-peaked spectral sensitivity (peaks at ca. 360 nm and 490 nm). However, the identification of the other two anatomical receptor classes (R7 and R8) had not been satisfactorily made. The available evidence, relating to spectral properties came from e.g. studies in Drosophila (Minke et al., 1975; Harris et al., 1976) and a few equivocal recordings of central cells in Calliphora coupled with dye injection (Smola and Meffert, 1975; Eckert et al., 1976; Järvilehto and Moring, 1976). It was thus felt necessary to perform intracellular recordings coupled with dye injection and subsequent histological identification, so as to establish a scheme by which a cell class could be recognised from its physiological characteristics.

Methods:

For dye injection electrodes were filled with a 4% (w/v) solution of Procion yellow M4RAN or Procion red P3BN. Best results were obtained with unbevelled electrodes. Although bevelled electrodes had lower resistance and passed current more readily without clogging, they did not reliably penetrate cells R7 and R8.
Recording conditions were as described in Appendix A, except that electrode resistances were higher (typically 500-1000 megohms).

After physiological characterisation of a cell, current was passed directly across the preparation. Negative pulses (ca. 200 ms, 2-3 Hz and 0.5-5 nA) were passed for 1-5 minutes. Current was monitored continuously for indications of clogging, and responses to axial test flashes were monitored frequently during the period of dye injection to check that the electrode was still in the same cell.

Following dye injection it was usually possible to recheck the cell's physiological characteristics. The electrode was then withdrawn from the cell (further advancement was found to greatly reduce the chance of recovery), and identification of the stained cell attempted by one of two methods.

a) conventional histological method.

The fly was kept alive, for one hour to overnight, in a fridge at 4°C. The whole eye and adjoining optic lobes were then dissected out in fixative and prepared for sectioning using standard wax histology techniques. After embedding, the whole eye and lobes were serially sectioned, and the sections examined under a fluorescence microscope (Zeiss epi-illumination) using excitation from a 50 W mercury vapour lamp through a BG 12 filter and emission through an orange barrier filter. Stained cells were identified from characteristic features such as medullary terminations and position of nucleus in the receptor layer (e.g. Fig. B.1).
Figure B.1  Camera lucida drawing of an R8 cell stained by Procion yellow. The inset shows a photomontage 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: 50µm. ret. retina; lam. lamina ganglionaris; ch. chiasm; m.e. medulla externa; m.i. medulla interna.
b) "in vivo recovery"

For identification of receptors Rl-7 in white-eyed mutants (*Musca domestica* and *Calliphora erythrocephala*) a novel technique was employed, developed in collaboration with Dr. N. Franceschini.

The technique stemmed from the recent discovery that individual rhabdomere tips may be visualised, in the living fly, by optically neutralising the cornea (by use of a water immersion objective) and then looking through the facets using epi-illumination fluorescence microscopy (Franceschini, 1977). With this method, each rhabdomere Rl-6 fluoresces faintly red, whilst the central rhabdomere of R7 fluoresces either green, or not at all, depending on the rhabdomere type, 7y or 7p (Franceschini *et al.*, 1978; and see Fig. B.2). The characteristic open rhabdomere pattern (Dietrich, 1909) allows one to readily identify each cell number.

After dye injection of a retinula cell, the fly was mounted at the rotational centre of a goniometer, which is then placed on the stage of the fluorescence microscope. By rotating the fly on the goniometer, one can quickly search the eye for the stained cell. Once found the cell's identity can be immediately ascertained, since the rhabdomere (and to a lesser extent the cell body) of the stained cell fluoresces very brightly, whilst the weaker autofluorescence of the other rhabdomeres in the ommatidium allow one to unequivocally identify cell number (Fig. B.2).
Figure B.2  A single receptor, R3, stained with Procion red. 

The photograph was taken using epi-illumination fluorescence microscopy on a living fly (Musca, white) employing the in vivo recovery technique described in the text. The stained cell fluoresces orange, and its position in the open rhabdomere pattern can be told because the other rhabdomeres show a natural red autofluorescence, except for the central rhabdomere which fluoresces green in this ommatidium. In some of the other ommatidia, the central rhabdomere appears dark: it has been shown that these correspond to rhabdomeres 7p, whilst the green fluorescing central rhabdomeres correspond to 7y (Franceschini et al., submitted for publication).
This novel technique is very quick and efficient. Identification of a stained cell is usually possible within ca. 10 minutes of dye injection (cf. ca. 24 hours by conventional techniques) and the recovery rate is high (90%). The technique also offers extra advantages including ready identification of facet position; identification of microvillar direction for correlation with polarisation sensitivity; and the possibility of identifying physiological subclasses of R7 and R8 with rhabdomere type, 7y or 7p (See Chapter V).

Results:

1. Photoreceptors

With the conventional histological method, 2 R7 and 3 R8 cells in Calliphora stygia (wild type) were physiologically characterised and histologically identified (Hardie, 1977b). The anatomical criteria for identification were: a) the presence of an axon in the chiasm (distinguishing them from receptors R1-6); b) the extent of the cell body in the receptor layer in the retina - R8 occupies only the proximal half of the retina (Fig. B.1) whilst R7 extends to the top of the ommatidium; c) in addition the nuclei of the two cell types lie at different levels in the retina (R7, distally and R8 proximally).

With the 'in vivo recovery' technique, a further 9 R7 cells were identified in Musca domestica (white), and Calliphora erythrocephala (chalky).
R8 cells were found to have a distinctive spectral sensitivity characterised by a major peak at ca. 540 nm, very low sensitivity (ca. 10%) in the range 400-500 nm, and a second, smaller peak at ca. 360 nm.

R7 cells all had a major peak of sensitivity in the ultraviolet (340-360 nm). The two major spectral classes described in Chapters II and V were both identified as R7.

In addition, both cells R7 and R8 are characterised, by higher noise levels than those seen in R1-6, and by the obvious instability of recordings, when compared to R1-6 (presumably related to their small size).

In addition, over 30 cells R1-6 were stained during the course of the study, all having the typical twin-peaked spectral sensitivity previously reported (McCann and Arnett, 1972).

On the basis of these positive identifications, it was felt unnecessary to stain every cell recorded from to be confident of anatomical identity, and a simple test was designed to rapidly identify spectral class, and hence anatomical identity. This consisted of presenting a pair of flashes (one green, and the other uv) 100 ms apart and repeated continuously, once a second, during electrode advancement. The intensities of the flashes are chosen so that they elicited equal responses in R1-6 cells. Then, on penetration of an R7 cell, only a response to the uv flash was observed, and on penetration of an R8 there is a response to both, but the response to the green flash is much greater. Coupled with the diagnostic feature of noise levels, this
method allows almost instance cell identification, and thus allows one to sample, rapidly, the large number of cells required before penetrating an R7 or R8. Typically, 15-20 R1-6 cells must be sampled and rejected before encountering an R7/8.

2. Lamina neurons

In Chapter IV an account of light-adaptation in receptors and large monopolar cells (LMC's) is presented. During this study a handful of LMC's were dye-injected to confirm that the responses used for the analysis did in fact originate from the LMC's, L1 and L2. In Calliphora stygia (wild type) two cells of each, L1 and L2, were stained with Procion yellow during this period, and subsequently identified. They all had typical responses, as previously described (Zettler and Järvilehto, 1971).

On one occasion in Calliphora stygia (wild type), a neuron was stained and identified as an L4 neuron on the basis of basket-like arborisations arising at the base of the lamina, cell body in the nuclear layer of the lamina and the depth of its medullary termination (Fig. B.3).

This cell gave a tonic discharge of small action potentials superimposed on a maintained depolarisation, in response to a continuous light in a limited receptive field. However, the limited recording time did not allow a more accurate characterisation. Superficially, the responses could be likened to those of tonic units recorded extracellularly in the chiasm between the lamina and medulla by Arnett (1972).
Figure B.3  Cell body, synaptic region and axon of an L4 neuron injected with Procion yellow. The diagnostic feature of this cell type is the basket-like arborisations at the base of the lamina (Strausfeld, 1976). The response of this unit consisted of a train of spikes superimposed upon a small depolarisation. Superficially, at least, it could be likened to the responses of the 'tonic units' described by Arnett (1972) from extracellular recordings in the chiasm.
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BIBLIOGRAPHY


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