Photoreceptor Optics

Edited by A. W. Snyder and R. Menzel

Springer-Verlag Berlin Heidelberg New York

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With 259 Figures

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The cover gives a schematic representation of the *Limulus* rhabdom in cross-section redrawn from the famous electron micrographs of W. H. MILLER, J. Biophys. Biochem. Cytol. **3**, 421 – 428 (1957).

ISBN 3-540-07216-0 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-07216-0 Springer-Verlag New York Heidelberg Berlin

 Library of Congress Cataloging in Publication Data. Main entry under title: Photoreceptor optics. "Derived from an international workshop on photoreceptor optics organised by the editors and held in Darmstadt, Germany, during October 1974." Sponsored by the Australian National University and the Deutsche Forschungsgemeinschaft. Bibliography: p. Includes index. 1. Photoreceptors--Congresses. I. Snyder, Allan W., 1940-. II. Menzel, Randolf, 1940. III. Australian National University, Canberra. IV. Deutsche Forschungsgemeinschaft (Founded 1949). QP476.P45. 591.1'823. 75-6700.
Printed in Germany. Offsetprinting: Julius Beltz, Hemsbach/Bergstr. Bookbinding: Brühlsche Universitätsdruckerei, Gießen

Preface

This book derives from an international workshop on photoreceptor optics organized by the editors and held in Darmstadt, Germany, during October 1974. Each participant was invited to review the fundamentals of his field, in addition to presenting recent research results and perspectives. The workshop (and this book) centres around the question of how the properties of photoreceptors - their structure, arrangement, orientation, shape, size, refractive index and membrane properties - influence their absorption of light.

The science of "Photoreceptor Optics" had its origin in the late 1800's with the discovery that the visual photopigment is concentrated within specialized parts of the photoreceptors (e.g. outer segments of vertebrate photoreceptors, rhabdoms in insect photoreceptors). As these special structures have a higher refractive index, they act as light guides, so ensuring that the visual pigment is maximally exposed to the incident light. It is this light-guiding nature of the photoreceptive structure in highly evolved photoreceptors which is the common thread linking the various topics within photoreceptor optics and within this book.

The participants have differing backgrounds. Some are biologists and sensory physiologists whereas others received their biological training after a formal education in the physical sciences. The complexity of the problems facing the visual scientist demands such a union. Visual scientists thus derive their knowledge and tools from diverse disciplines including ultrastructure research, membrane biophysics, electrophysiology, optical systems analysis, electromagnetic theory and quantum mechanics. Photoreceptor optics is a synthesis of these disciplines with the goal of understanding the function of photoreceptors from their structural organization.

The workshop was sponsored by the Australian National University and the Deutsche Forschungsgemeinschaft. We are especially grateful to Professor B.W. NINHAM and the ANU for their willingness to support a meeting outside Australia. This unorthodox procedure made the workshop a reality.

Many of our colleagues contributed to the success of the workshop. We are particularly grateful to Dr. S. LAUGHLIN and Dr. C. PASK. We would also like to thank Margaret Blakers and Mechtild Menzel for their invaluable contribution to the preparation of this book.

January 1975

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Section F. Electrophysiology of Photoreceptors

Introduction to Photoreceptor Optics – An Overview

RANDOLF MENZEL and ALLAN W. SNYDER

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1. Definition and Objectives of Photoreceptor Optics

Photoreceptor optics is the science that investigates how the optical properties of photoreceptors - their arrangement, orientation, shape, size, refractive index and membrane properties - influence their absorption of light and establish many of their specialised functions (MILLER, 1974; SNYDER, 1974).

The science has its origins in the late 1800's with the discovery that the visual photopigment is contained within a specialised part of the photoreceptor and that these parts have a higher refractive index than their surrounds. As a consequence of the higher refractive index, the photoreceptor acts as a light guide, ensuring that the visual pigment is maximally exposed to the incident light. This principle is the common thread linking the various topics within photoreceptor optics.

The goal of photoreceptor optics is to explain the structural basis of a photoreceptor's absolute, spectral, directional and polarisation sensitivities. We cannot over-emphasize the role of photoreceptor structure, quite apart from its membrane biochemistry, in the determination of these sensitivities. By far the richest explorations here have been with the rhabdomeric photoreceptors, those typical of invertebrates, which through the diversity of their gross structure, in addition to the arrangement and properties of their microvilli membrane, exhibit a myriad of photoreceptor optic principles. We will illustrate some of these principles after introducing some elementary anatomical concepts.

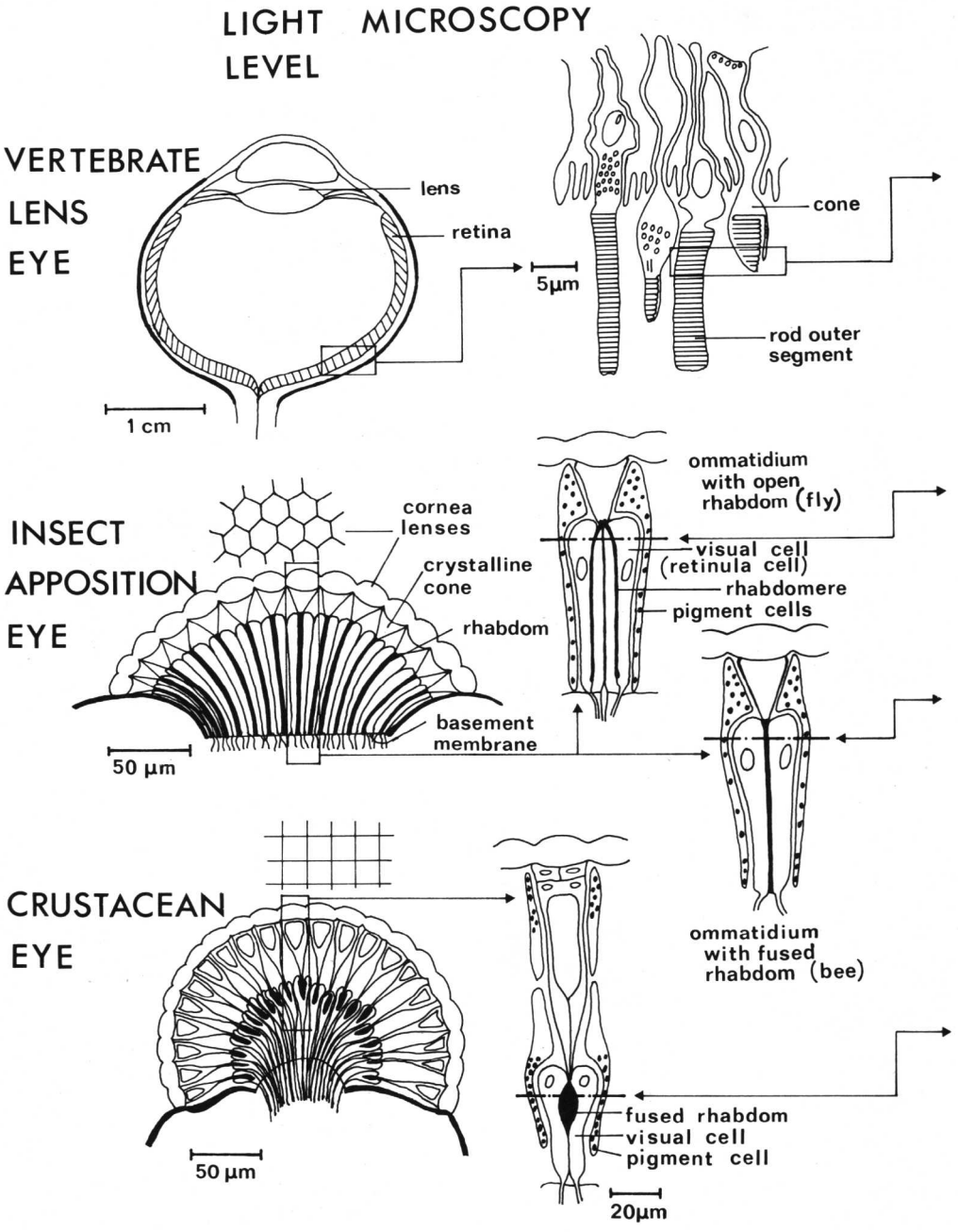
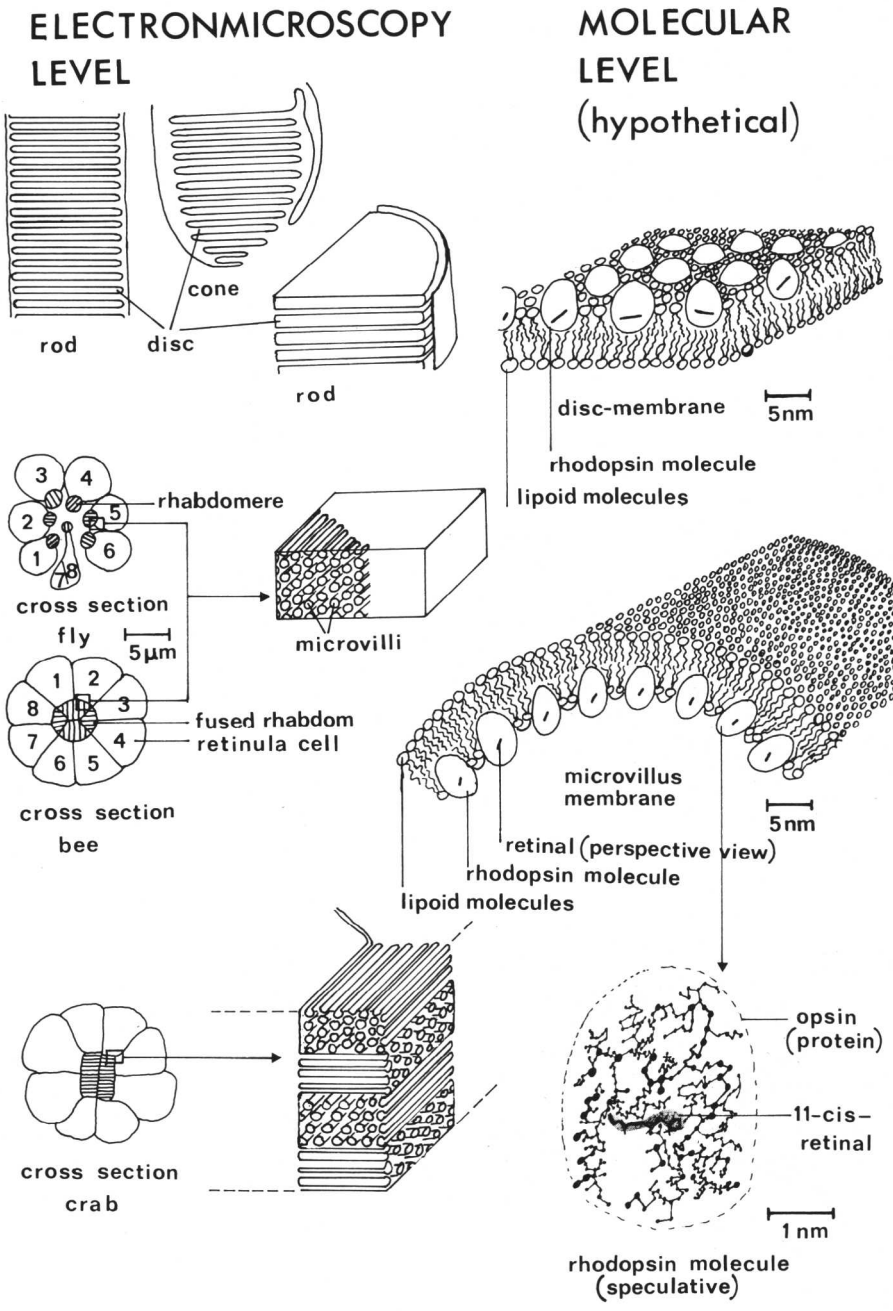


Fig. 1. Elementary morphology of three different types of eyes on three magnification levels, and definitions of some important structural elements. From the vertebrate lens eye (upper third) typical rods and cones, and the membrane structure of the outer segment are given. As examples of the insect apposition eye, the ommatidia of the fly and the bee are shown lengthways and in cross-section. The crustacean



type of compound eye is represented by a typical decapod eye. The molecular organisation of disc and microvillus membrane is hypothetical (see text). The sketch of a rhodopsin molecule in the lower right corner is completely speculative and only demonstrates what we need to know for better understanding of photosensitivity

2. Structural Organisation of Photoreceptors – Elementary Morphology

In order to orientate the reader, we compare some of the anatomical features of vertebrate and invertebrate eyes of those animals which have highly evolved visual systems. Photoreceptors of such highly evolved animal groups as vertebrates, arthropods and cephalopods are specialised, elongated cells, tightly packed together in the retina, onto which the lens focuses an image of the visual world. The spatial resolution of the photoreceptor mosaic increases with the packing density of the light-absorbing structures, but the number of quanta reaching each photoreceptor decreases. The evolution of lens and compound eyes with high spatial resolution was, therefore, necessarily coupled with the development of mechanisms which increase the probability of light absorption in each photoreceptor.

The structural basis for high absorption is the multilayered membrane system of the light-absorbing parts of photoreceptors. In vertebrate rods this is the outer segment, which is made up of hundreds of intracellular membrane envelopes (discs), which carry the rhodopsin molecules. In cones, a smaller number of such discs is produced by infoldings of the cellular membrane (Fig. 1). Arthropod and mollusc visual cells (retinula cells) carry the photopigment in densely packed, tubular membrane protrusions, the microvilli. As a result of the dense membrane packing in discs and microvilli, these photoreceptive structures have a higher refractive index than their surrounds and so act as light guides. In vertebrate photoreceptors the outer segments, and probably also those parts of the inner segments which are densely filled with mitochondria, are light guides.

In most arthropod and in cephalopod eyes, groups of retinula cells join together to form a centrally located, single, light-guide structure. Such a group of cells is called an ommatidium, and their common light-guiding structure, a fused rhabdom. In *Limulus*, for example, a varying number of cells (9–14) form an ommatidium, and the rhabdom is a complicated star-like structure (see cover of this book and MILLER, this vol.). In hymenopterans (e.g. bee, ant) there are always 9 retinula cells in one ommatidium and the rhabdom is a simple rod-like structure (Fig.1). The fused rhabdom of crustaceans is unique in having interdigitating packages of microvilli which belong to different retinula cells of the same ommatidium and which have their microvilli directions perpendicular to each other (Fig. 1).

In contrast, the ommatidium of the fly has an open rhabdom; the rhabdomere of each retinula cell is a separate light guide, although all look through the same lens. As Fig. 1 shows, the fly has 6 peripheral rhabdomeres and one central rhabdomere. This central one is thinner ($d = 1\ \mu m$) than the outer six rhabdomeres ($d = 2\ \mu m$), and is formed from two cells (7 and 8), lying one above the other (see KIRSCHFELD and SNYDER, this vol.; FRANCESCHINI, this vol.; STAVENGA, this vol.).

The main aim of receptor optics is to understand the functional consequences of the size and shape of the light-guiding and light-absorbing structures (see below). It has been a well-known fact for 100 years, that cones and rods differ in shape and size in different parts of the human eye (VON GREEFF, 1900). Fish, amphibians and birds frequently have double photoreceptors, and often oil droplets in one or both of the receptors (ref. CRESCITELLI, 1972). The mitochondria-filled part of the inner segment varies enormously in different fish and amphibian photoreceptors. The rhabdoms of arthropod eyes display

an enormous variety of architecture and size (rev. GOLDSMITH and BERNARD, 1974). The length of rhabdomeres or rhabdoms varies from a few microns to nearly a thousand microns (e.g. dragon-fly). The cross-section ranges from 1 µm diameter (e.g. fly retinula cells 7 and 8) to more than 50 µm (e.g. *Limulus*, see MILLER, this vol.). The shape of the cross-section can be circular (bee, ventral eye part), square (bee, dorsal eye part, decapod crustacea), rectangular (primitive insects, PAULUS, 1974) oval (fly and many other insects), a closed ring *(Periplaneta)*, star-like *(Limulus, Ephestia)*, and many other shapes. The variety is increased even more by the fact that the shape of the rhabdom and the cells contributing to it may change over the length of the rhabdom. In addition, the length and shape of rhabdoms and rhabdomeres may alter in response to illumination, dynamically adapting their structure to the functional requirements (WALCOTT, 1974). It is obvious that these are all special adaptations to optimize selected functional parameters. Here receptor optics has an unlimited field for future research. (See also HORRIDGE, this vol.).

In all highly evolved photoreceptors, the photoreceptive membrane multilayer is arranged perpendicular to the light path (Fig. 1, molecular level). This must be of great functional significance, because these receptors have evolved to optimize absorption within the smallest cross-sectional area possible (see above). The reason for such a molecular organisation has recently been worked out for the rod outer segment (see LIEBMAN, this vol.). In essence, the light-absorbing molecule, the chromophore group retinal, is a dipole absorber with greatest absorption when the E-vector of light is parallel to the π-electron cloud of its conjugated double bonds. The E-vector of light is perpendicular to the light path. For unknown reasons retinal is embedded in the protein molecule (opsin) in such a way that the dipole is parallel to the membrane surface. This is proven in rod outer segment (ROS) (see LIEBMAN, this vol.) and in rhabdomeric photoreceptors (TÄUBER, this vol.; see below also). Note, that the molecules responsible for dichroic absorption (retinal in rhodopsin) and for intrinsic birefringence (the fatty acid chains in the membrane lipoid molecules) are arranged perpendicular to each other (see LAUGHLIN et al., this vol. for more details).

3. Functional Organisation of Photoreceptors – General Concepts

In the next several sections we discuss and give examples of some of the possible functional specialisations of photoreceptors.

3.1 Lateral Filters - Rhabdomeres of the Fused Rhabdom

Most invertebrate compound eyes have several photoreceptors fused together to form a common light guide known as the fused rhabdom. A typical example is the worker bee rhabdom illustrated in Fig. 1. GRIBAKIN (1969, 1972) has shown that the rhabdomeres have different spectral absorption characteristics. Since all the rhabdomeres are joined tightly together in a cylindrical light guide, they are optically coupled. The absorptive properties of each rhabdomere influence the light as it passes down the rhabdom. Each rhabdomere acts as if it were an absorptive filter in front of all others, i.e. rhabdomeres of a fused rhabdom function as lateral absorption filters as shown in

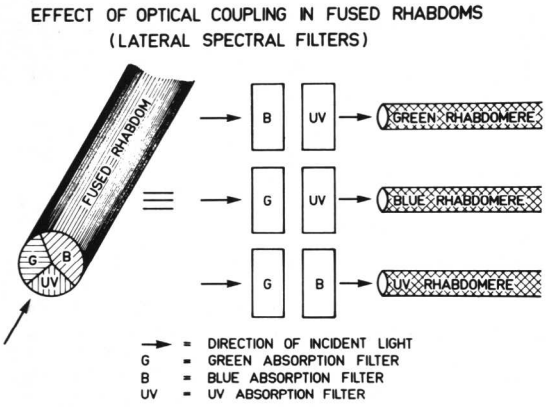


Fig. 2. Illustration showing that in a fused rhabdom, each rhabdomere functions as an absorption filter. For example, the blue and UV-rhabdomeres filter out the blue and UV light from the green rhabdomeres. This sharpens the spectral sensitivity of the green retinula cell. There is no loss in absolute sensitivity, since all the light captured by the rhabdom's cross-section is available to each rhabdomere and each filter is a photoelectric transducer

Fig. 2 and discussed by SNYDER et al. (1973). As a result of this filtering, the shapes of the spectral sensitivity or absorption curves are relatively insensitive to the amount of light absorbed, i.e. the broadening of curves by self-absorption is prevented without loss of absolute sensitivity. This is one of many examples illustrating the principle that the fused rhabdom cannot be considered as a loose collection of photoreceptors sharing the same dioptric apparatus, but rather it must be viewed as an integrated unit.

3.2 Structures Adapted for Polarisation Sensitivity

Nearly all rhabdomeres show some sensitivity to the direction of the electric vector E of linearly polarised light. The origin of this polarisation sensitivity (PS) is the dichroism of the individual microvillus. Nevertheless, evidence is accumulating in favour of the view that the PS of a retinula cell, or more specifically the dichroism of a microvillus, is a by-product of adaptations to maximise absolute sensitivity (SNYDER and LAUGHLIN, 1975; LAUGHLIN et al., this vol.). When a rhabdomere shows a high level of polarisation sensitivity, it is usually found to be associated with a very specialised structure. The best known example is that of the crustacean rhabdom discussed in section 2 above. A theoretical analysis shows that the PS of each retinula cell is independent of its absolute sensitivity and exactly equal to the dichroic ratio of microvilli (SNYDER, 1973). This result is due to the layered rhabdom of alternating, orthogonal microvilli.

Partitioned or tiered rhabdoms: theoretical studies show that if two rhabdomeres of the fly type are arranged with one above the other, then the upper rhabdomere acts as a polarisation filter for the lower. If the rhabdomeres have their microvilli orthogonal to each other, then the PS of the lower rhabdomere is amplified (SNYDER, 1973). This is in fact the arrangement of rhabdomeres 7 and 8 of fly and the 9th cell of the worker bee which are believed to be highly sensitive PS detectors (KIRSCHFELD, 1973; MENZEL and SNYDER, 1974). Other tiered rhabdoms show a similar effect (LAUGHLIN, this vol.).

Using a theoretical analysis, GRIBAKIN (1973) has shown that retinula cell 8 in fly and the 9th cell of worker bee are designed to optimise the sum of absolute and polarisation sensitivities.

Some vertebrates can detect the direction of linearly polarised light (WATERMAN, this vol.). The mechanism for detection remains an enigma, although the hypothesis of SNYDER (1973a) would appear to be consistent with all known experimental findings.

3.3 Mode Effects

Light intensity is transmitted along photoreceptors as patterns known as waveguide modes (ENOCH, 1963; FRANCESCHINI and KIRSCHFELD, 1971; VARELA and WIITANEN, 1970). The observation of mode patterns is a consequence of the small diameter of the light guide. Mode patterns have no role in vision, although in theory some fused rhabdoms can distinguish between different modes (SNYDER and PASK, 1972; BERNARD, this vol.). Nevertheless, the observation of modes serves to emphasize that the photoreceptor is a dielectric or optical waveguide (KAPANY and BURKE, 1974). Optical waveguides exhibit several interesting properties: (1) Only a fraction of a mode's light energy is within the waveguide, the remainder travels along but outside the waveguide; (2) the light capture area of a waveguide is greater than its geometrical cross-section; (3) light energy is interchanged between parallel waveguides (cross-talk). These three phenomena are strongly wavelength dependent. They are reviewed by SNYDER (this vol.).

A quantitative study of photoreceptors requires knowledge of its characteristic waveguide parameter V.

$$V = \pi d \, (n_1^2 - n_2^2)^{1/2}/\lambda \qquad (1)$$

where d is the photoreceptor diameter, n_1, n_2 are the refractive indices of the photoreceptor and its surround respectively, and λ is the wavelength in a vacuum.

It is nearly impossible to determine V from Eq. (1) because of the inability to obtain sufficiently accurate values of n_1, n_2 representative of in situ conditions. Instead, indirect methods of finding V are necessary. Such a method has been developed for fly photoreceptors (KIRSCHFELD and SNYDER, this vol.).

We next consider several possible physiological consequences of mode effects.

3.3.1 Spectral Sensitivity of a Photoreceptor

The effect of containing photopigment within a rhabdom of small diameter is (a) to shift the visible absorption peak to lower wavelengths and (b) to increase the UV peak absorption relative to the visible. The effect is significant only when $V < 2$ throughout most of the wavelength region of interest. Accordingly, the small diameter of fly rhabdomeres 7 and 8 may explain their different spectral absorption from that of the larger diameter rhabdomeres 1 to 6 (SNYDER and MILLER, 1972; SNYDER and PASK, 1973; KIRSCHFELD and SNYDER, this vol.).

3.3.2 Intrinsic Directionality of a Photoreceptor

A photoreceptor has an intrinsic directional sensitivity, not to be confused with the angular sensitivity of the photoreceptor-lens systems

discussed below. The well-known measurements of STILES and CRAWFORD (1933) on the directionality of the human eye are believed to be a direct measure of the intrinsic directionality of our photoreceptors. Although there have been many attempts to provide a quantitative explanation of the Stiles-Crawford results, only those that include waveguide-mode effects exhibit the correct variation with wavelength (SNYDER and PASK, 1973). More recent studies of the colour change associated with the Stiles-Crawford effect are available (PASK and SNYDER, this vol.; WIJNGAARD and KRUYSBERGEN, this vol.).

3.3.3 Angular Sensitivity of the Lens-Photoreceptor System

Our most complete knowledge of the angular sensitivity of a photoreceptor system comes from intracellular recordings of the retinula cells of Arthropods (LAUGHLIN, this vol.). As the angle of illumination is changed, an Airy disc diffraction pattern moves across the distal tips of the rhabdomeres (KUIPER, 1966). Due to the fact that the capture area of a photoreceptor is greater than its physical cross-section, waveguide effects play a significant role in the angular sensitivity of the photoreceptor system. Theoretical analysis shows that the sharpest angular sensitivity occurs when the photoreceptor has a characteristic waveguide parameter V = 2.4 (PASK and SNYDER, this vol.).

3.4 Explanation for the Shape and Length of Photoreceptors

It has been known for more than 100 years that the shape and packing pattern of human photoreceptor cells depends on the retinal location, e.g. cones become progressively fatter, shorter, more tapered, with decreasing refractive index going from the fovea towards the ora serrata. Using optical waveguide theory, MILLER and SNYDER (1973) have provided an explanation for some of these changes. They conclude that the physiological function of human peripheral cones is to serve a dual role, enhancing the sensitivity of the rod system at threshold for scotopic vision while mediating colour vision at photopic intensities.

The length of photoreceptors that are closely packed must be limited to avoid optical cross-talk. It would be interesting to determine if the cones of the human fovea conform to this length criterion in order to avoid the downgrading of their resolution.

4. The Role of Membrane in Photoreceptor Optics

The photoreceptor membrane discussed in section 2 is anisotropic, exhibiting both dichroism and birefringence. Dichroism is the dependence of absorption on the direction of the electric vector $\underline{E}$ of linearly polarised light and indicates the degree of alignment of the absorbing dipoles within the membrane. Birefringence is the dependence of the refractive index on the direction of $\underline{E}$ and indicates the degree of alignment of the membrane substructure, i.e. its crystallinity.