STUDIES ON THE STRUCTURE AND FUNCTION OF THE CRAB EYE

SALLY JANE STOWE

A Thesis submitted for the degree of Doctor of Philosophy of the Australian National University.

CANBERRA MAY, 1979

DECLARATION.

I declare all the original work presented in this thesis to be my own.

S. J. Stowe

Chapters IV and V are taken from two published papers, with minor alterations to avoid repetition, and the addition of some extra photographs. These were:

Stowe, S., Ribi, W.A., and Sandeman, D.C. (1977). The organisation of the Lamina ganglionaris of the Crabs Scylla serrata and Leptograpsus variegatus. Cell.Tiss. Res. 178, 517-532

Stowe, S. (1977) The Retina-Lamina Projection in the crab Leptograpsus variegatus. Cell. Tiss. Res. 185, 515-525

A manuscript for publication based on Chapters II and III is in preparation.

One other publication was made during the tenure of my C.S.F.P. Scholarship:

Stowe, S. and Leggett, M. (1978) Retina-Lamina Connectivity and polarisation sensitivity in Crustacea Vision Res., 18 1087.

ACKNOWLEDGEMENTS

I would like to thank Professor Horridge for the opportunity to work in a most stimulating environment, and for his encouragement during a crucial time.

It is a pleasure to thank my supervisors, who were, in order of appearance, Dr David Sandeman, Dr Willi Ribi, Dr Dave Dvorak, Dr David Blest, Prof. G. Adrian Horridge, and Dr Steve Shaw, for their support and willingness to offer help and advice. David Sandeman suggested an anatomical study of the crab optic lobe, and Willi Ribi gave me invaluable advice on the art of Golgi staining.

Many other past and present members of the department have helped me enormously, especially Joachim Erber and Martin Wilson, who taught me something of electrophysiology, and Eldon Ball, who is always willing to give of his time and knowledge. Kevin Downing's ability to smooth out difficulties has always been an important support.

I have benefited greatly from discussions with many people, among them Yasuo Tsukahara, Pete Lillywhite, Barry O'Brien, and Simon Laughlin, to whom I am indebted for his comments on Chapter III, although he is not responsible for any errors remaining.

Bruce Ham braved all weathers to collect crabs, and was responsible for the design and maintenance of the crab tanks.

I would like to thank the staff of the EM Unit, Photography, and the Workshop, who have always been extremely helpful.

All the colour prints are the work of Kerrie Ruth, and I am also grateful to David Sandeman for drawing Fig. 1.1, and to Chris Snoek for Fig. 2.1.

David Blest has helped me with reduced silver staining, by printing the electron microgaphs, by correcting grammar, and in innumerable other things.

This work was done during the tenure of a Commonwealth Scholarships and Fellowships Plan grant, for which I am most grateful.

ABSTRACT

The eyes of crabs are of interest for a number of reasons. Some crabs, including Leptograpsus, are active over a wide range of light intensities, from night to bright sunlight, a change of seven or eight log units in brightness. They are known to be highly sensitive to very slow movements. Since their eyes are of the apposition rather than the superposition type they have much higher visual acuity than most other decapods.

The eyes of the rock crab, Leptograpsus variegatus, and the Queensland mud crab Scylla serrata, have been examined in a number of ways. In Scylla, the structural organisation of the first optic neuropile, the lamina, has been investigated using Golgi and reduced silver staining. It is very similar in structure to the lamina of decapods with superposition eyes, such as crayfish, and contains five types of monopolar neurons and at least three types of large tangential fibres. The seven retinula cells which make up the main part of the fused rhabdom terminate in two layers within the lamina. The eighth cell, which forms only the distal tip of the rhabdom, has an axon that passes through the lamina and ends in the second optic neuropile, the external medulla. By tracing Leptograpsus retinula cell axons through a series of 1 or 2 µm sections, it was found that all the retinula cell axons from one ommatidium go to the same lamina cartridge, and not to several, as has been recently proposed.

An EM study of the structure of the retina of Leptograpsus showed that the distal portion of the rhabdom increases in diameter from c. 2 µm in the day to c. 5 µm at night. Five types of cells in the retina contain pigment that acts as a light screen. The position of these cells and the pigment in them was examined by light microscopy in a variety of

illumination conditions during the day and at night. The spectral transmission of the different types of screening pigment was measured by microspectrophotometry.

The spectral sensitivity of Leptograpsus retinula cells was found by intracellular recording from eyes in situ. The dark adapted spectral sensitivity peaks at c 485nm. Light adaptation causes various changes in spectral sensitivity which can largely be accounted for by movements of the screening pigments. The net effect is to produce a broader but fairly constant spectral sensitivity over a wide range of light levels.

The polarisation sensitivity is high, about 10:1. The angular sensitivity, which alters slightly in response to light and dark adaptation, ranges from about 2° in bright light, to $3\text{-}4^{\circ}$ in the dark, in the forward-looking portion of the eye.

It is concluded that Leptograpsus uses a variety of means to maintain a high acuity and constant spectral sensitivity over a wide range of light levels.

CONTENTS

Chapter I

GENERAL INTRODUCTION

Even to the casual observer, crabs give the impression of being highly visual animals. The often brightly coloured crabs of mudflats have a large repertoire of display movements, the classic example being the threat and courtship signals of the various species of the fiddler crab Uca, reviewed by Crane (1975). Escape from predators is often visually mediated, as can be seen from the speed with which a slight movement will send a platoon of Mictyris many yards away on a flat beach scurrying off in the opposite direction, a foraging Leptograpsus disappearing down a crack in the rocks, or an Ozius caught investigating a shallow pool surreptiously gliding under a clump of weed.

Field and laboratory studies under more controlled conditions bear this out. Uca, for instance, finds the way back to the area of its burrow from some distance by a variety of clues which can include remembered land-marks and the polarisation pattern of the sky (Herrnkind 1972). Recording from the optic nerve of Podophthalmus, Wiersma et al. (1964) demonstrated a varied array of movement detectors in crabs, in fact substantially more than were found in some other decapods in the same series of investigations. By monitoring their optokinetic responses to a revolving drum, crabs have been shown to be capable of following movements down to 0.001 to 0.002^{o}/sec (Sandeman et al. 1975), which is rather slower than the apparent speed of the sun in its passage across the sky. This provides an interesting contrast to the fast-flying insects, which seem to have developed the circuitry of their compound eyes to analyse very rapid changes in the visual field (Snyder et al. 1977). The hoverfly Syritta for example can accurately track a target moving with an angular velocity of 250^{o}/sec. (Collett and Land, 1975).

The ability to perceive very slow movements is one of the main reasons why the optic system of the crab is worth investigation. Another is the capacity of the retinula cells to analyse polarised light. Crustaceans, because of the structure of their rhabdom, are potentially more sensitive to the polarisation plane of light than almost any other group. Yet apart from orientation responses to sky polarisation patterns, which are hardly unique to Crustacea, we do not know for what purpose, and to what extent, crabs use the polarisation information available to them.

The general structure of the decapod retina is well known, and is discussed in the next chapter. The rhabdom is formed by seven main cells with interdigitating orthogonally-oriented microvilli, and a distal eighth cell of varying importance. The properties of the eighth cell are effectively unknown. Apart from the obvious differences in the plane of polarisation sensitivity caused by the orientation of their microvilli, it is not known how differences in the structure of the main retinula cells (size of the rhabdomere, position in relation to the surrounding pigment cells) are reflected in their properties. The effect of the pigment screen has been well studied in crayfish, which have superposition eyes, but not in decapods with apposition eyes, such as crabs. The problem of spectral sensitivity in decapods is still obscure in spite of recent work. The manner in which retinula cells project to the lamina has been the subject of speculation without conclusive evidence, and recently it has become obvious that in studying any visual system it is necessary to determine whether there are any circadian changes in the rhabdom itself. I have tried to answer some of these questions.

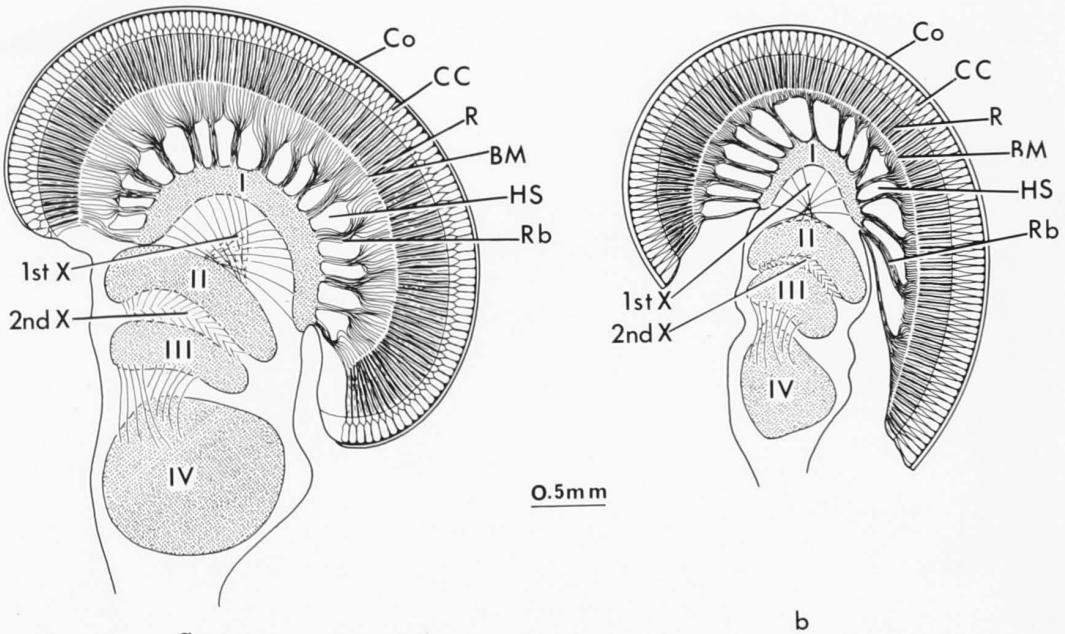
An initial Golgi study of the lamina, the first optic ganglion, was performed using the portunid mud crab Scylla serrata. It was later found that the common grapsid rock crab, Leptograpsus variegatus, is a

much more reliable animal for behavioural and electrophysiological work. Since it is also available locally and has less well developed weaponry, it was used in later work. The basic construction of the eyes of the two crabs is very similar (Fig. 1.1). As discussed in Chapter V, no large differences in the structure of the lamina were revealed by the limited amount of work done on Leptograpsus, and considering the great similarities between the lamina of Scylla and that of other decapods (Hafner, 1973, Nassel, 1975, 1976), it is unlikely that the lamina of another brachyuran crab will be very disparate. The same applies to the gross structure and arrangement of the retinula cells themselves. It is another matter when considering the secondary pigment cells, which are notoriously variable throughout the decapods, and display quite different behaviours in the two crabs.

The pigment systems of crustacean eyes move under a combination of circadian and environmental influences, in a way which must be determined for each species. Obviously, if any work is to be done more centrally in the nervous system of Leptograpsus, it is necessary to have some idea of what factors are causing its eye pigments to move, and their probable state in any experimental situation.

The projection pattern of axons from the retina to the lamina was traced because the "neural superposition" type of connection pattern that had been assumed (e.g. Waterman 1978) seemed most unlikely. It was not well supported by the little anatomical evidence available, and no reasonable theoretical explanation for it had been advanced. A knowledge of the projection pattern is a necessary prerequisite for an examination of the function of the retina, as well as of the lamina and higher order neuropiles.

Fig. 1.1 Horizontal sections through the optic lobes of Scylla (a) and Leptograpsus (b). Co, cornea; CC, crystalline cones; R, retinula cells; BM, basement membrane; HS, haemocoelic sinus; Rb, retinula axon bundles. 1st X, first chiasma; 2nd X, second chiasma; I, lamina; II, external medulla; III internal medulla; IV, terminal medulla.


Co
CC
R
BM
HS
Rb
I
II
III
IV
1st X
2nd X
0.5mm
a
b

Although some measurements were made of polarisation sensitivity and angular sensitivity of retinula cells, the main focus of the electrophysiological study described in Chapter III was spectral sensitivity, since it was thought that it might be profitably studied in conjunction with anatomical and msp work on the screening pigments.

CHAPTER II

Retinal Pigments and their Movements in Leptograpsus

INTRODUCTION

Retinal screening pigments in Crustacea present a bewildering variety of systems, ranging from simple three-pigment eyes to combinations of five or six pigments, some stationary, some moving according to a circadian rhythm, some because of a direct or hormonally-mediated effect of light. Although closely homologous pigment cell types can be recognised across many different species, one of the main lessons of work on pigment migration so far is that generalisation even between the species of one genus is often invalid.

It is necessary to determine the details of the retinal pigment system and its movements for any species whose visual physiology is to be investigated closely. To be most effective, such a study needs to be combined with knowledge of how the various pigments absorb light. This can be rather difficult to obtain, since many pigment cells contain several different types of pigment granules, and it may also be hard to entirely separate classes of pigment cells in fresh preparations. Luckily in Leptograpsus the relationship between the pigment cell types is such that an unfixed eye may be teased apart to yield small clumps containing either one sort of pigment cell, or two easily separable types.

Investigation of pigment movements has been a traditional pastime for crustacean anatomists since Parker (1897). Much of the early work involved species such as the carid prawn Paleamonetes, and the crayfish Procambarus or Astacus (Kleinholz, 1936; Welsh, 1935). The eyes of these animals contain three types of pigment. The dark distal pigment generally forms a collar around the proximal part of the crystalline cone and the

distal retinula cells in the day or light-adapted eye, restricting the amount of non-axial light that can reach the rhabdom. In the night or dark-adapted eye, the pigment moves distally and becomes concentrated within a smaller area, allowing much of the non-axial light to pass on to the rhabdoms. Dark granules of proximal pigment within the retinula cells themselves can move below the basement membrane to expose the rhabdom, or form a sheath around the rhabdom to limit the entrance of light from the side and perhaps attenuate axial light. Reflecting pigment, sometimes called accessory pigment, lies between the ommatidia, sometimes moving back and forth across the basement membrane. When it lies between the ommatidia it should stop light "leaking" from one column of retinula cells to another; when it lies fairly proximally, and is not screened by darker pigments, it acts as a tapetum, reflecting and scattering light back through the rhabdoms.

Recent work, using electron microscopy, has often revealed a more complicated picture. Struwe, Hallberg and Elofsson (1977) made an EM and microspectrophotometric study of the pigment cells of Crangon, a carid shrimp (of a different superfamily from the Palaemonoidae). They found five types of cell containing reflecting or absorbing pigment granules. Schönenberger, (1977), in a detailed EM study of the stomatopod Squilla mantis, also found five types of pigment, including a green reflecting layer. Ball (in prep.) finds five types of pigment in the sergestid shrimp Acetes. Hallberg (1977) finds a basal red pigment cell in several species of mysid shrimp, and this also appears in Oplophorus (Land, 1976) and in the leptostracan Nebalia (Green, 1972). Basal red pigment is a major part of the pigment system of many crabs, including Leptograpsus, Scylla, Ozius, Ocypode, and Mictyris (pers. obs.).

In spite of a long history of research a coherent picture of the hormonal control of pigment movements in Crustacea is only just beginning to emerge (reviewed by Kleinholz, 1976). Even now, much more is known about movements of two pigments, the distal pigment and the retinula cell or proximal pigment, than any others in the eye. The dark distal pigment (at any rate in the crayfish Procambarus) moves in response to hormones: the light-adapting or dark-adapting DRPH (Distal Retinal Pigment Hormones). Its movements take much longer to complete than those of the proximal pigment, have a higher threshold, and do no occur in the isolated eye (reviewed by Aréchiga, 1978). Proximal pigment, as shown by Ludolph et al. (1973) in the crab Callinectes, can move independently in the different retinula cells of one ommatidium. Olivo and Larsen (1978) showed that migration of proximal pigment initiated in an isolated eyestalk by a brief exposure to light is continued in the dark.

This study was undertaken primarily to determine the position of the retinal screening pigments of Leptograpsus under the conditions used in the electrophysiological experiments, in order to gain some idea of their effects on the responses of the retinula cells.

METHODS

Leptograpsus variegatus, the common grapsid rock crab, was collected every three to five weeks near Bateman's Bay, on the New South Wales coast. The crabs were kept in a fibre-glass tank of sea-water containing rocks for shade and shelter. The sea-water was kept circulating through several inches of gravel bed by air pumped in beneath the gravel. The tank was lit by fluorescent lights on a 12:12 light:dark cycle in winter, and a 14:10 cycle in summer, when all the experiments concerning pigment migration were carried out. The crabs were fed on crab meat, either on the remains of experimental animals, or on any of

their fellows foolhardy enough to try to moult in a crowded tank. They were allowed to acclimatise for two-three days at least before being used.

Electron microscopy

For electron microscopy eyes were fixed in 0.1M sodium cacodylate, 0.14M sucrose, 2.5% gluteraldehyde and 2mM calcium chloride at pH 7.3. The crabs used were of almost identical size, of carapace width between 2.8 and 3cm. The eyes of two crabs were fixed in the light adapted condition at midday, and the eyes of two more were fixed when dark-adapted at midnight, being dissected under a red light. They were fixed overnight at 4°C, washed in several changes of the cacodylate buffer (with sucrose), osmicated in 1% osmium in cacodylate buffer for two hours, washed in several changes of buffer, dehydrated through an ethanol series, taken through propylene oxide and embedded in araldite. For examination at low magnifications, pale gold to silver sections were cut on glass knives, mounted on formvar-coated slot grids and stained in uranyl acetate (40 min) and Reynold's lead citrate (20 min). They were examined with a Jeol JEM 100C electron microscope. Measurements were made from photographs. Means and standard deviations were calculated from 40-50 measurements of each type of pigment granule, and 8-10 rhabdoms in each condition.

Pigment Movements

Crabs of 2.5 to 4 cm carapace width were used in these experiments. To produce the conditions under which electrophysiological recordings had been made, the animals were induced to autotomise their legs approximately 1 hour before being placed in the appropriate light regime. Two crabs were fixed in each state investigated, by immersion in hot (70-80°C) fixative for one minute, then the eyes were rapidly cut into cold fixative and the eyestalk cuticle cut as far as the cornea in 4-5 places. Dissection was

completed in 70% alcohol after the eyes had been left overnight in cold fixative. This was 2.27% gluteraldehyde in Millonig's buffer with 0.01% calcium chloride and 15% glucose. "Day" crabs were fixed between 1.30 and 3.30 pm in the following conditions: after 30 minutes at a window in bright sunlight but on a cool substrate; after 60 minutes in "dim light" (10-20 $\mu W/cm^2$); after 30 and 60 minutes dark adaptation; and after 2, 5, 15 and 30 minutes under "bright" (8-10mW/cm^2) light from a 30 watt tungsten microscope lamp. "Night" crabs were fixed between 10 and 12 pm when dark adapted for two hours, 'dim' light adapted for one hour, and 'bright' light adapted for 2, 5, 15 and 30 minutes. The dim and bright light regimes were adopted in order to mimic conditions under which recordings from retinula cells had been made.

After dehydration through an ethanol series (as fast as possible in order to minimise loss of alcohol-soluble pigments) the eyes were taken through propylene oxide to araldite. They were sectioned at 0.5 to 2µm to provide different viewing conditions and mounted unstained under Permount. Measurements were made as a proportion of cone length or retinula cell soma length, to allow for varying crab sizes. The minimum diameter of the "iris" formed by the dark distal pigment around the cone tip was also measured. An eyepiece graticule was used for all measurements of LM material.

Microspectrophotometry

The spectral absorption of the red basal pigment, the retinula cell screening pigment, the dark distal pigment and the light distal pigment was measured using a Zeiss UMSP 1. Fresh eyes were dissected in Carcinus saline (Pantin, 1972), and the retina teased apart to yield pieces containing only one, or two distinguishable, pigment cell types. Measurements were made on squashes of these pieces in saline, with a

measuring diameter of 5 μm in the object plane, from 700 to 350 nm. A section of the slide containing no tissue was used to provide baseline measurements. The red basal pigment formed large oily droplets in the saline, and some of these easily covered the whole area being measured. Clusters of granules were measured for the dark distal and retinula cell screening pigments, and groups of small yellow-brown droplets for the light distal pigment. Quartz slides were used, but glass coverslips. Change in extinction was measured by comparing transmission through pigment and saline at ten or twenty nm intervals. Data for each run were normalised, taking the highest extinction value of each run as unity, then the normalised data were averaged and plotted.

Numbering of Retinula Cells

Two systems of numbering decapod retinula cells are in use (see Table 1). In system A, used by Parker (1897), Rutherford and Horridge (1965), Kunze (1967) and Nässel (1976), an ommatidium from the dorsal half of the eye is numbered in an anti-clockwise direction. In System B, used by Eguchi and his co-workers in an extensive series of papers (Eguchi 1965 onwards), an ommatidium in the ventral half of the eye is numbered in an anti-clockwise direction. Cell 8 is the same in both systems, and cell 7 in system A corresponds to cell 1 in system B. The relationship between the two systems and the orientation of the microvilli in Grapsus according to Eguchi and Waterman (1973) is given in Table 1. System A is used here (see Fig. 2).

Table 1. Numbering of crab retinula cells

System A	1	2	3	4	5	6	7	8
System B	2	3	4	5	6	7	1	8
Orientation of microvilli	V	V	H	H	V	V	H	H and V

(H = Horizontal)

(V = Vertical)

RESULTS

The cornea of Leptograpsus consists of any array of c.40 µm hexagonal facets, with no external demarcation between them. One of the axes of the array runs horizontally when the eye is in the normal position. The corneal cuticle is flexible and thin (c.40 µm), and has no apparent focusing effect when viewed through a hanging drop. Below the cornea of each facet lie two cone producing cells and a crystalline cone 90-120 µm long (Fig. 2.1). The cone tapers to a blunt point above the short rhabdom of retinula cell eight. Four "cone cell roots" continue to the basement membrane as fine threads between retinula cells 1 and 2, 3 and 4, 5, and 6, and 7 and 1. The soma of R8 is divided into four lobes (Fig. 2.2) one of which sends a process proximally which enlarges to contain the nucleus and then continues as an axon besides the soma of R7, eventually joining the group of retinula cell axons that forms below the basement membrane. The soma of R8 contains no pigment granules, and the cytoplasm is pale and full of vacuoles during the day. The most distal parts of R1-7 surround the lobes of R8 (Fig. 2.3). R1-7 form a fused rhabdom of the standard decapod type. Along its 250-350 µm length, two sets of orthogonally oriented microvilli alternate R7, and 4 contribute horizontally oriented microvilli, R1, 2, 5 and 6 have microvilli running vertically when the crab holds its eye in the normal position. The rhabdomere of each cell stretches halfway across the rhabdom; the rhabdomere of R7 covers half the area of the rhabom, the other cells all contribute one quarter each.

During the day, in the light-adapted state, there is little variation in rhabdom diameter from the distal (1.67 $\pm$ 0.12 µm, Fig. 2.4) to the proximal (Fig2.5) end of the rhabdom. The rhabdom is surrounded by a "palisade" of swollen ER cisternae of 4 to 5 µm outer diameter. Between

Fig. 2.1 Semi-schematic diagram of a Leptograpsus ommatidium and associated cells, when light-adapted during the day and dark-adapted at night.

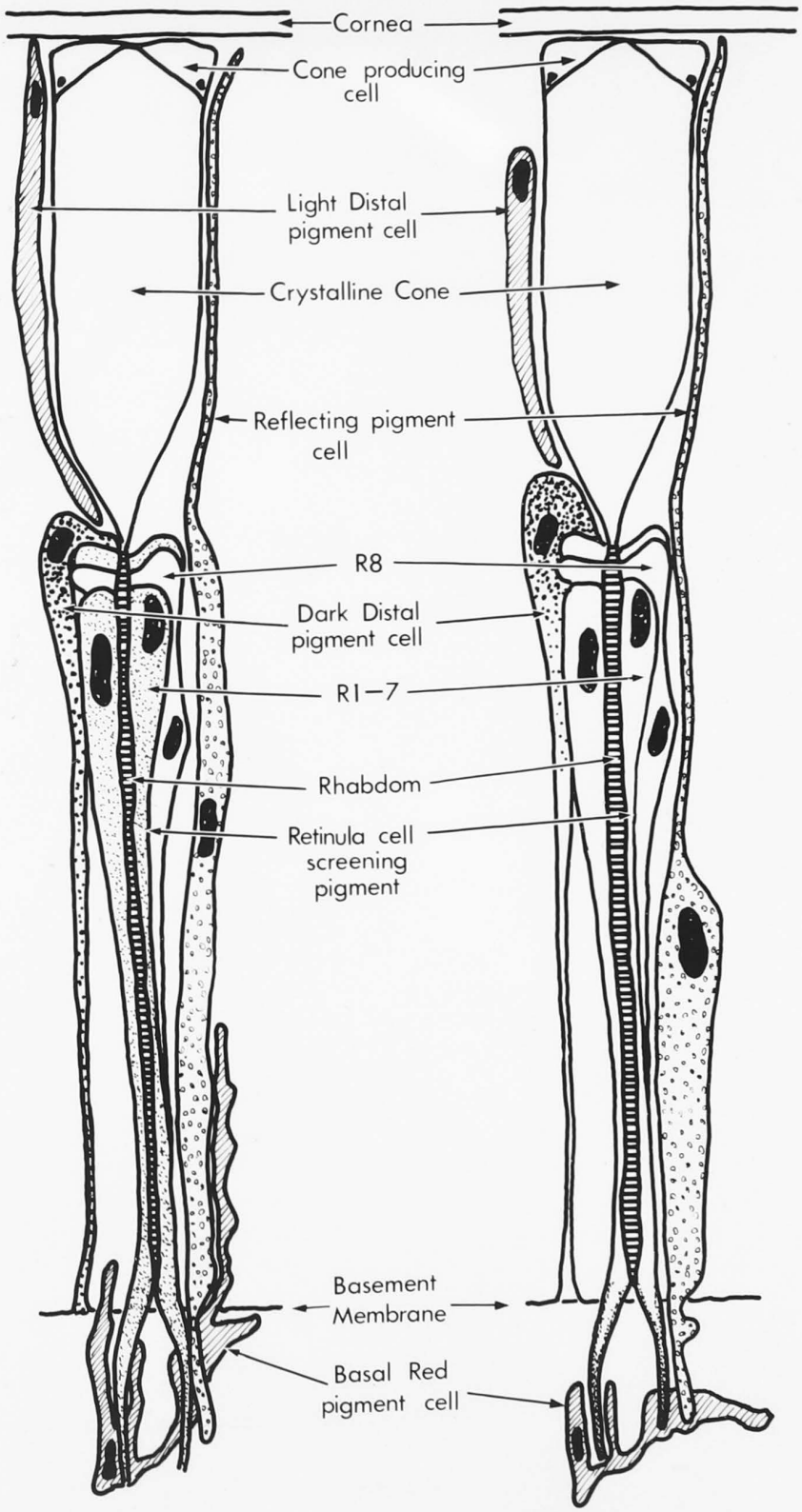
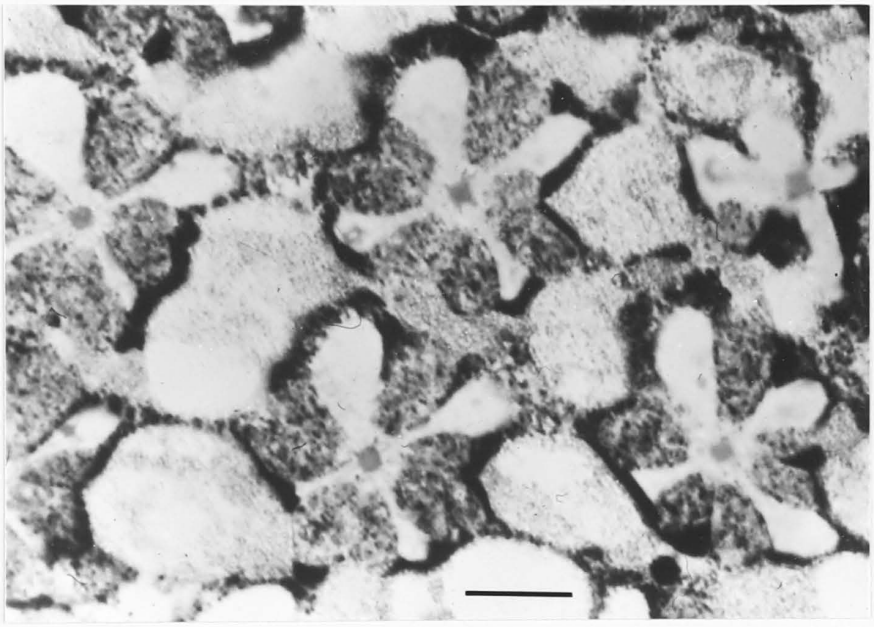
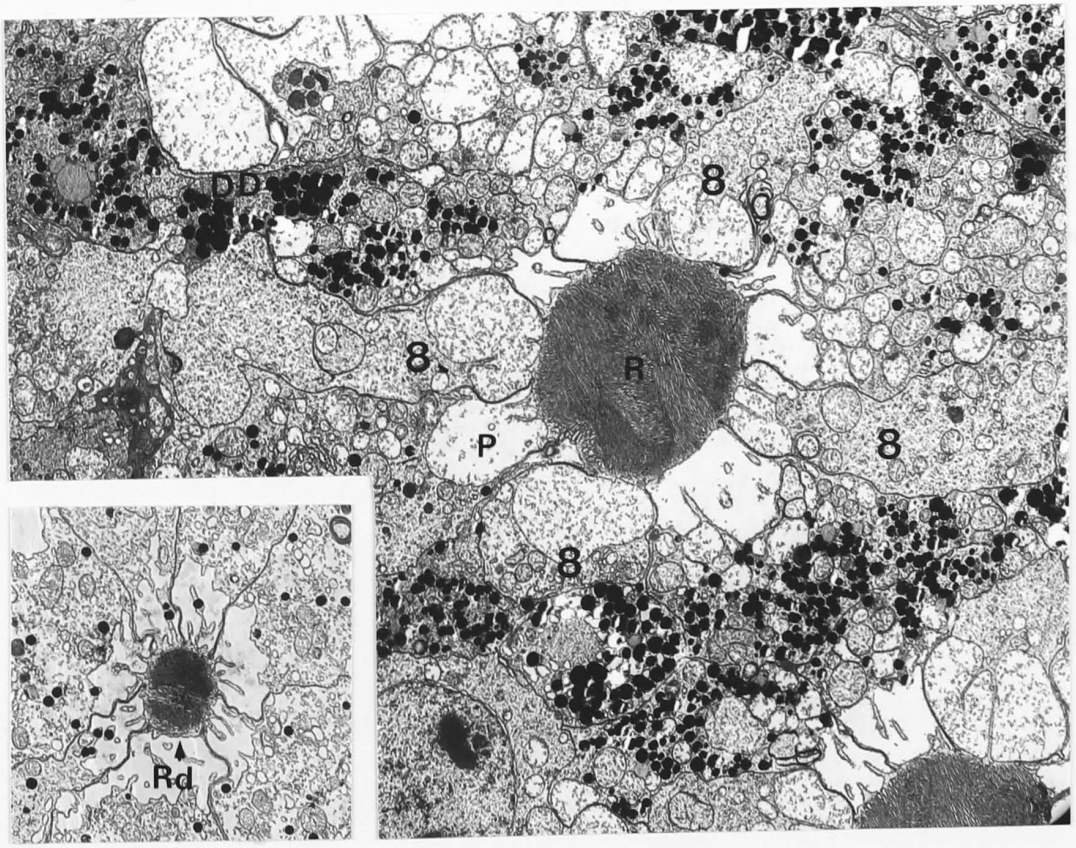

Cornea
Cone producing cell
Light Distal pigment cell
Crystalline Cone
Reflecting pigment cell
R8
Dark Distal pigment cell
R1–7
Rhabdom
Retinula cell screening pigment
Basement Membrane
Basal Red pigment cell
Light Adapted
[bright sunlight]
Dark Adapted
[night]

Fig. 2.2 Cross-section of a light-adapted Leptograpsus eye at the level of the eighth cell. Scale 5 µm.

Fig. 2.3 Distal tip of the rhabdom at night, showing the four lobes of R8 lying between the pigment containing R1-7. Inset, day rhabdom at a level c. 10 µm proximal, and the same magnification. (4,900 X)
DD, dark distal pigment cell; R, rhabdom; P, palisade; 8, Retinula cell 8; Rd, rind of cytoplasm.

Fig




DD
8
8
R
P
8
8
Rd

Fi

Fig. 2.4 Distal part of the retina during the day, showing R1-7 and the regular arrangement of the dark distal pigment cells. (4900 X) N, retinula cell nucleus; DD, dark distal pigment cells; R1 reflecting pigment cell; R7, retinula cell 7

DD
R7
DD
DD
Rf
N

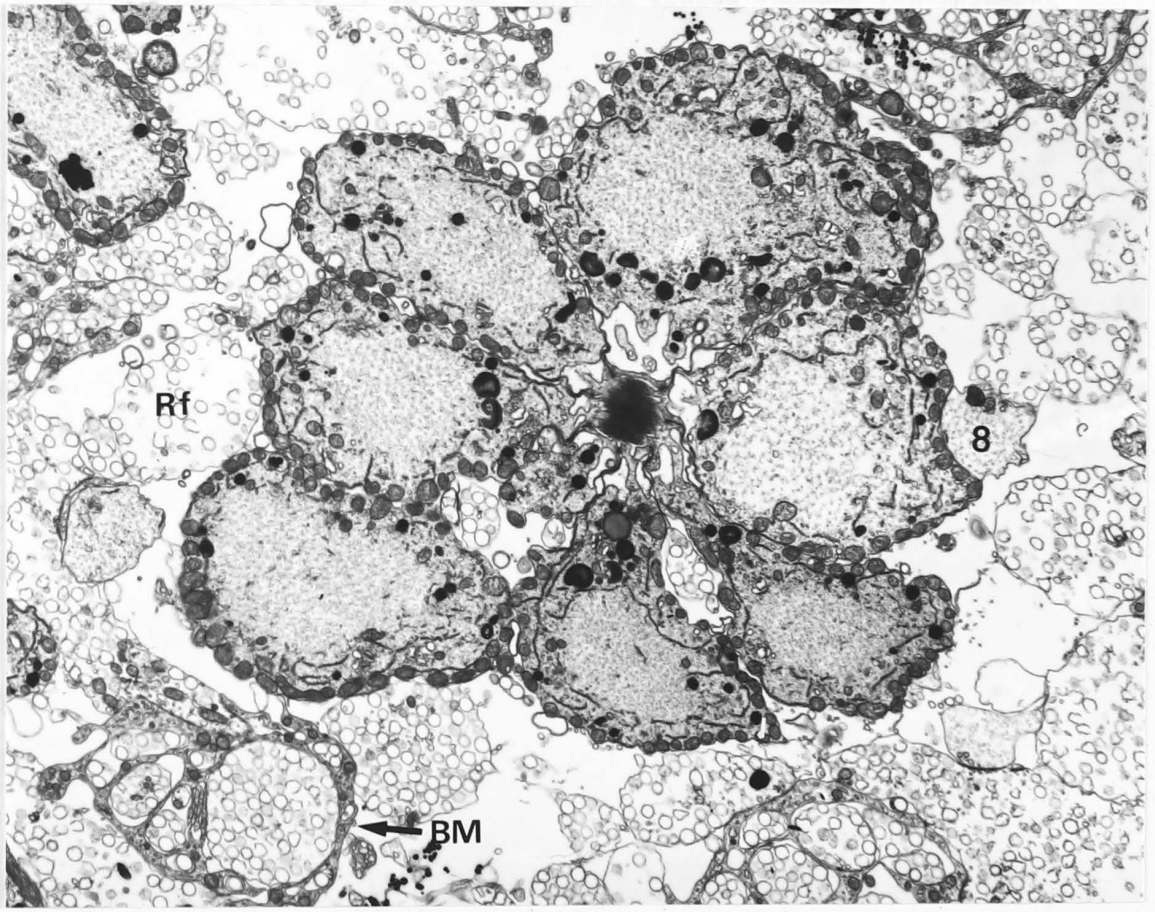


Fig. 2.5 Proximal part of rhabdom in the day, showing clear spaces inside retinula cells, filled with microtubules and surrounded by mitochondria. (6,800 X)

8, axon of R8; BM, basement membrane material; cc, cone cell roots; Rf, reflecting pigment.

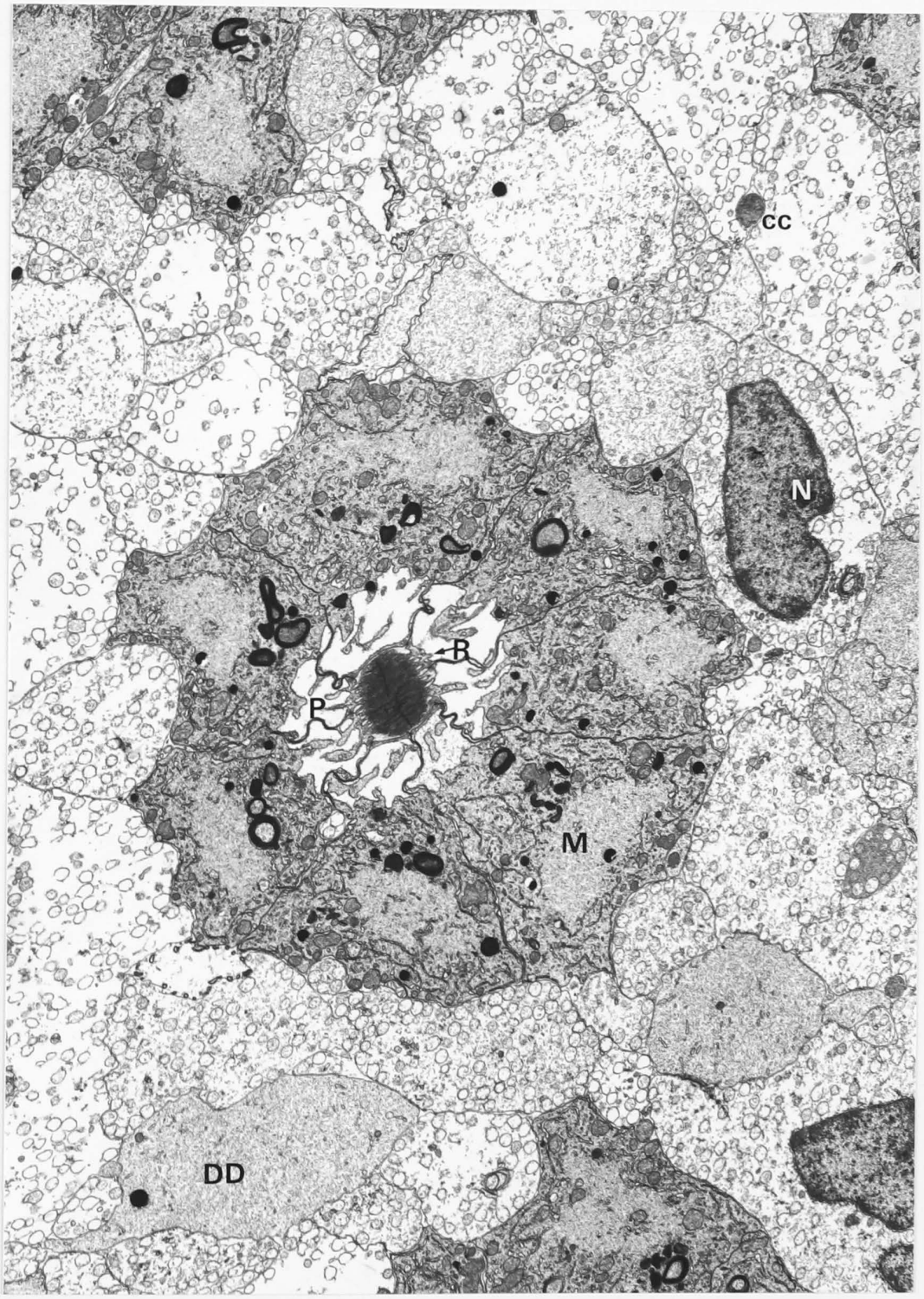
the rhabdom and the palisade is a thin rind of cytoplasm, approximately 0.3 μm thick, from which the microvilli sprout. Within the retinula cells are large clear areas, containing microtubules (Fig. 2.6). Above the basement membrane these areas cover most of the cross-section of the cell, and tend to become smaller as they continue distally as far as the nuclear region.

At night, the diameter of the distal part of the rhabdom increases dramatically to 5.22 ± 0.52 μm (Fig. 2.7), an increase in cross-sectional area by a factor of about 10. The rhabdom tapers towards the basement membrane, where the diameter is 1.93 ± 0.51 μm, a cross-sectional increase of c. 1.8. The "palisade" is 9.44 ± 0.72 μm in outer diameter distally, but decreases towards the basement membrane, where it is hardly visible. The rind of cytoplasm around the rhabdom is not evident at night. The clear areas or microtubule fields are found just above the basement membrane and in the axons below it, and a proportion of them contain apparently non-membrane-bound yellow droplets of varying sizes, the largest filling up almost the whole cross-section of the cell (Fig. 2.8, 2.12c).

Between the crystalline cones lie thin sheets containing the yellow-brown light distal pigment in small, oily droplets 0.2 ± 0.1 μm in diameter (Fig. 2.9a). The nuclei of these cells lie towards the cornea.

Each omma has two dark distal pigment cells containing dense purplish granules 0.3 ± 0.07 μm in diameter. The nucleus is in the distal part of the cell. In most conditions the dark distal pigment cells surround the cone tip, and the pigmented part of each tapers down to about halfway along the length of the retinula cells, with a fine non-pigmented extension continuing as far as the basement membrane. An extension of each cell, sometimes containing pigment granules, reaches between retinula cells to the rhabdom, one on either side of R7.

Fig. 2.6 Halfway down retinula cell column, day eye. (6,900 X). R, rind; P, palisade; M, microtubule-filled space; N, nucleus of reflecting pigment cell; DD, extensions of dark distal pigment cells; cc, cone cell root.


cc
N
R
P
M
DD

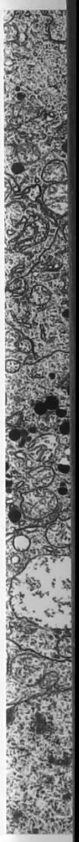
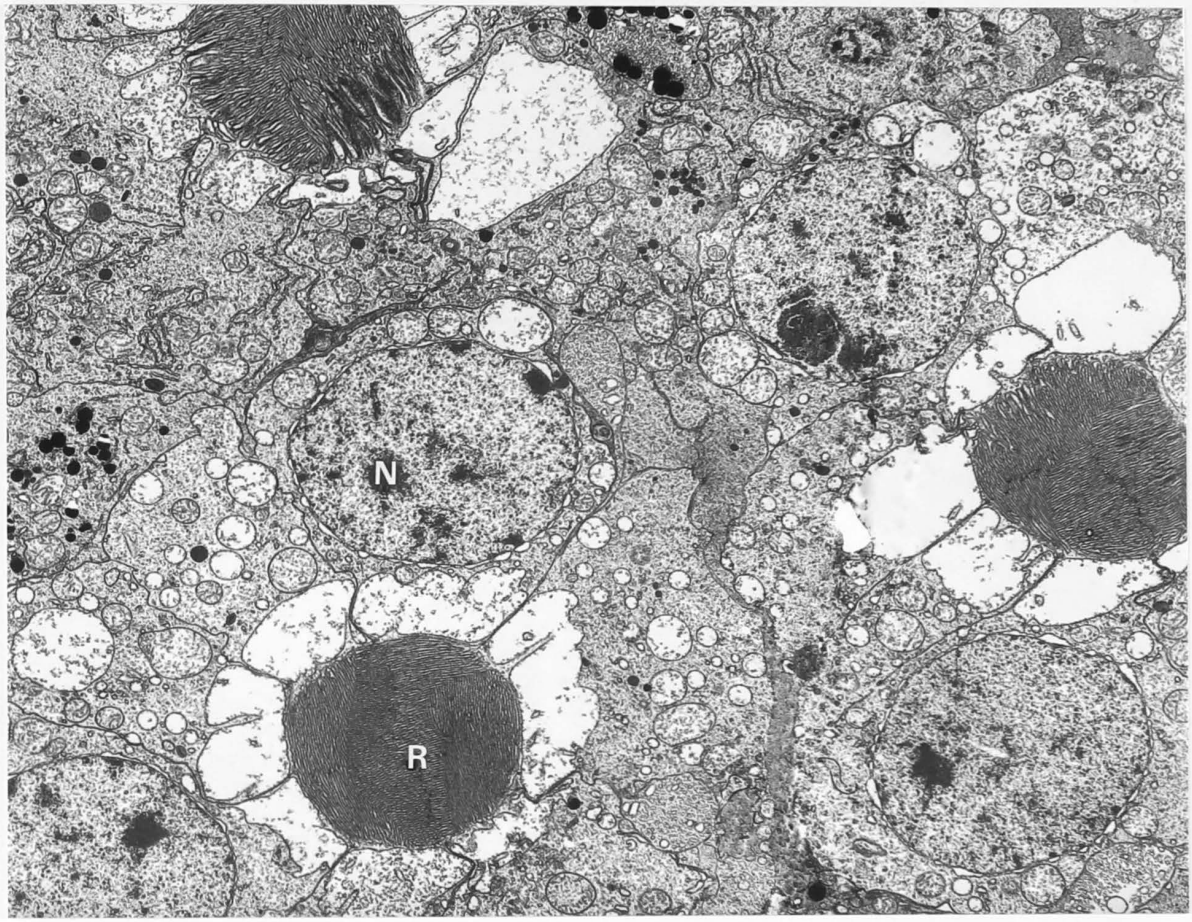
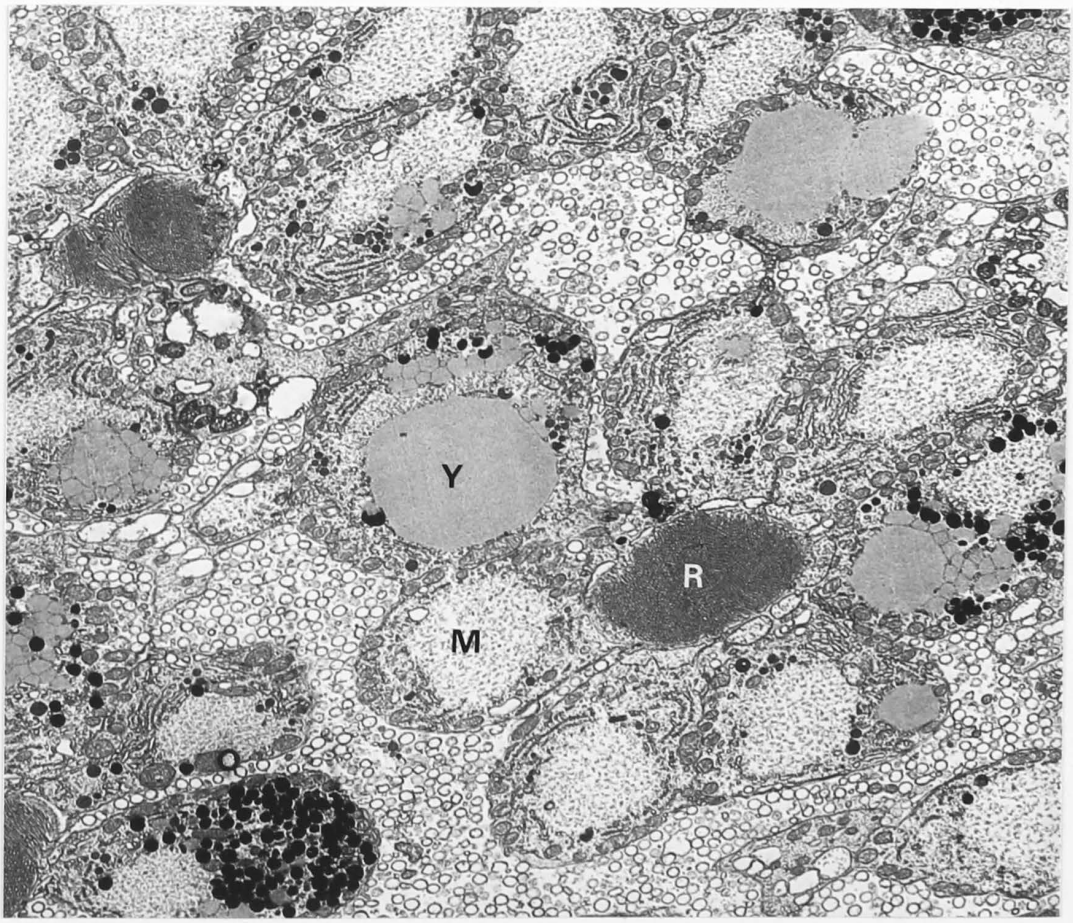


Fig. 2.7 Distal part of night rhabdom. (5,300X) N, retinula cell nuclei; R, rhabdom.

F

Fig. 2.8 Just distal to the basement membrane in a night eye, showing large yellow droplets within retinula cells. (6850X) R, rhabdom; M, microtubule-filled space; Y, yellow droplet.


N
R


Y
R
M

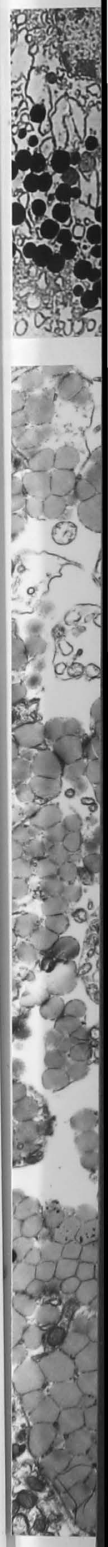


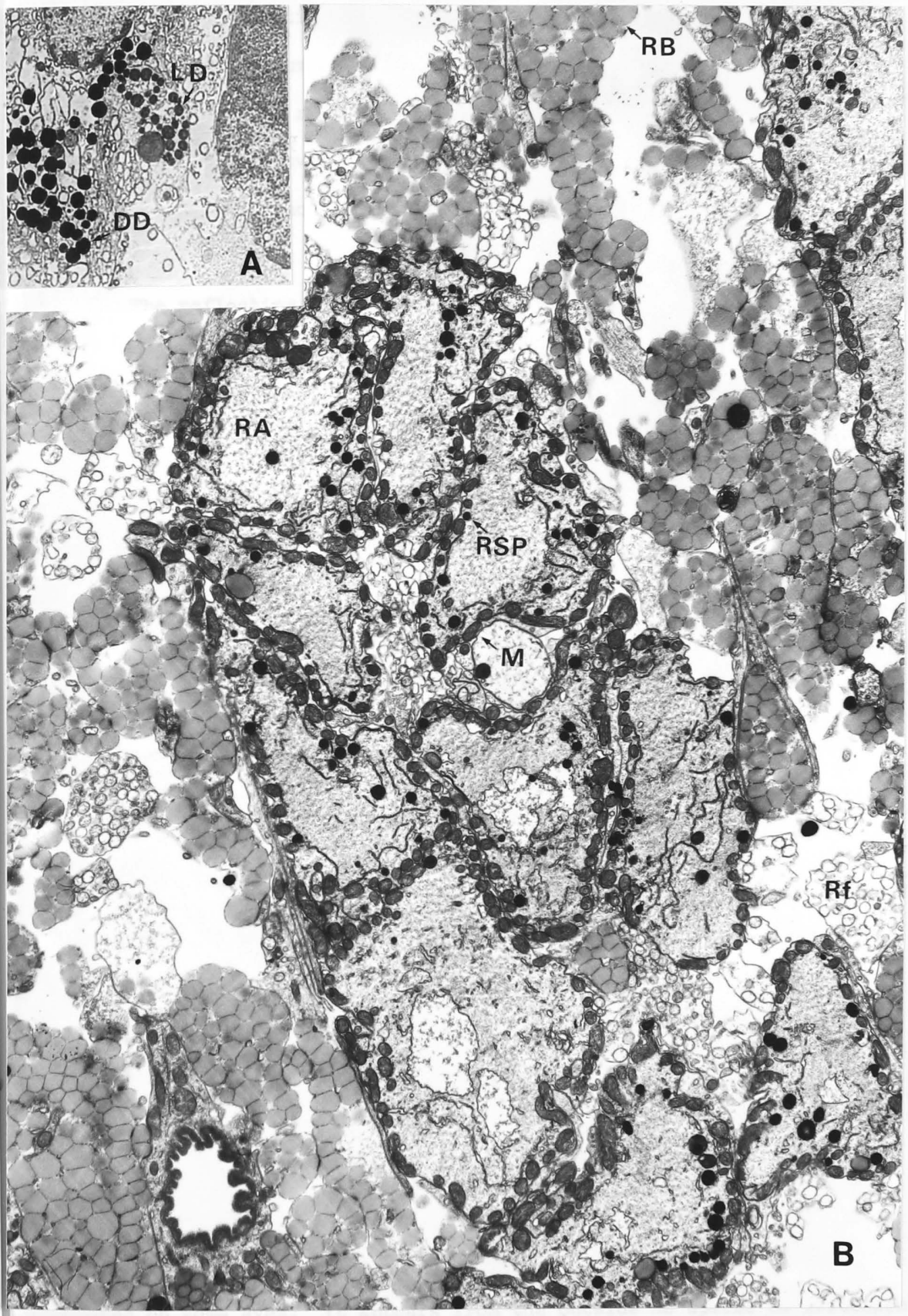
Fig. 2.9a Pigment granules at the level of the crystalline cone (8000X). LD, light distal pigment; DD, dark distal pigment.

9b Pigment below the basement membrane, (8000X). RB, red basal pigment; RA, retinula cell axons; RSP, retinula cell screening pigment; Rf, reflecting pigment; M, mitochondria

cone

stal

cting



Within the retinula cells themselves are brown screening pigment granules, of 0.3 ± 0.1 μm diameter. These are very mobile, and may be concentrated around the rhabdom or scattered throughout the cell. The retinula cell axons below the basement membrane have pigment granules around their circumference, and in some conditions may be quite densely filled with pigment.

The reflecting pigment is contained within large cells which are usually closely apposed to the retinula cell column over most of its length from the basement membrane to the level of the distal tip of the rhabdom. Processes of the cells extend below the basement membrane in some states (Fig. 2.10). A thin strand of tissue containing reflecting pigment granules extends to the cornea. The nucleus lies about halfway down the retinula cell column. The pigment granules, 0.3 ± 0.05 μm in diameter, are a brilliant white in reflected light, but pale brown in transmitted light. The contents of the granules are usually lost in EM sections.

Beneath the basement membrane the retinula cell axons run through a layer of basal red pigment cells which contain large (from 0.3 to 0.7 μm diam.) oily red droplets, densely packed (Figs. 2.9b, 2.11). They form a thick barrier on the proximal side of the basement membrane, and sometimes send processes through it.

Mainly below this layer, but sometimes extending within it, are a small number of irregularly arranged cells containing dense black pigment. These cells consist largely of widely separated processes wound around the retinula cell bundles from the sub-basement membrane area to the lamina, where they splay out over the distal lamina surface rather in the manner of exposed tree roots.

The seventh and last coloured structure of the retina occurs only in patches on some eyes. It appears from the outside as specks of iridescent green. When dissecting an eye in saline, small platelets are sometimes

Fig. 2.10 Oblique section through the basement membrane in a night eye. (4,900X). RA, retinula cell axons, A', axon passing through basement membrane in a non-standard position. Y, yellow droplets; Rf, reflecting pigment; BM, basement membrane material.

Y
Rf
RA
BM

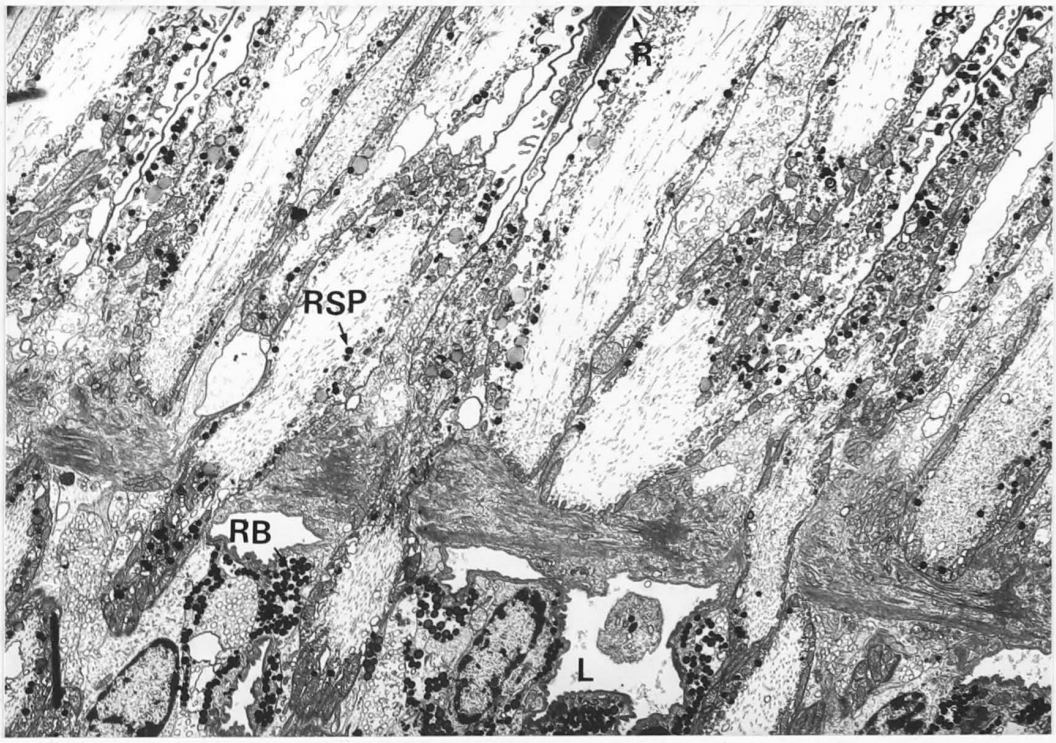


Fig. 2.11 Longtitudinal section through the basement membrane (3,500X) L, sub-basement membrane lacunae; RSP, retinula cell screening pigment; RB, red basal pigment; R, rhabdom.

observed, green on one side only, that have become dislodged from somewhere around the crystalline cone. Nothing corresponding to them has been observed in sectioned material in Leptograpsus. Schönenberger (1977) sees similar structures in the eye of Squilla, and suggests, as seems likely also for Leptograpsus, that they are only related to the superficial colour scheme of the animal.

Pigment movement

The state of the five main pigments and the width of the "iris" formed by the dark distal pigment and/or the retinula cell screening pigment is summarised in Table 1.

Allowing for individual variation and the range of crab sizes used, all that can be said of the iris is that it is approximately twice as large during the night as during the day, and that dark or light adaptation does not cause a marked change within 30 or 60 mins.

The dark distal pigment is largely responsible for the size of the iris, (Fig. 2.12d) but the bulk of the cell changes very little except under very strong illumination, when pigment granules move into the fine strand that extends to the basement membrane. However since this survey has been done using light microscopy almost exclusively, it would not be evident if any change had occurred in those parts of the cells that run in to the rhabdom on either side of R7.

The light distal pigment covers all the cone that is not surrounded by the more proximal darker screening pigments, except at night. When dark adapted at night, the pigment retracts from the distal third or so of the cone. Long exposure to moderate light levels at night causes the pigment to extend distally, and in bright light extension takes 30 minutes.

Table I

Pigment Movements

STATE	IRIS DIAM.	LIGHT DISTAL PIGMENT	DARK DISTAL PIGMENT	RETINULA CELL SCREENING PIGMENT	REFLECTING PIGMENT	BASAL RED PIGMENT
Night DA	13 µm	From 1/3(distally) to 9/10 (proximally) of cone.	Proximal 20% of cone and distal 30% of RC.	Below BM.	Surrounds proximal half of RCs, extends c. 50 µm below BM.	Up to 50 µm below BM.
Night DimLA	10-13 µm	Along cone.	Along distal half of RCs.	Sparse in the distal 30% of RC: and below BM.	Surrounds proximal half of RCs, fine strands along distal half.	Close below BM, some small patches above, for c.15% RC length.
Night 2 min BrightLA	8-9.5 µm	From 1/8 (distally) to 9/10 (proximally) of cone.	Along distal 75% of RCs.	Light but centred around rhabdom, in prox. and dist. 10-20% of RC. Below BM.	As above, distal strands slightly thicker.	Close below BM.
Night 5 min. BrightLA	8 µm	As above.	Along distal half of RCs.	Centred in distal 20%, sparser and scattered in proximal 30% of RC. Below BM.	As above, thick strands.	Very slightly above BM patches.
Night 15 min. BrightLA	10-13 µm	Proximal 95% of cone.	Along distal 60% of RC.	Centred in distal 25% and proximal 10%, scattered throughout. Below BM.	More uniform, but still denser proximally.	Slightly above BM, up to 10% of RC length.
Night 30 min BrightLA	5-7 µm	All along cone.	As above, but up to BM where red pigment movement extensive.	Light centred in distal 25% scattered centrally. Thick and centred in prox. 10%. Below BM.	Uniformly surrounds RCs.	Above BM in large patches up to 50% of RC length.

Table I (cont.)

Pigment Movements

STATE	IRIS DIAM.	LIGHT DISTAL PIGMENT	DARK DISTAL PIGMENT	RETINULA CELL SCREENING PIGMENT	REFLECTING PIGMENT	BASAL RED PIGMENT
Day DimLA	4-5 µm	"	40% RC length, but variable.	Dense between lobes of R8. Throughout RC apart from narrow central band. Below BM.	"	Proximal to BM, but close against it.
Day 30 min. DA	4-5 µm	"	70% RC length.	Distal and Prox. 30% not Centred. Below BM.	"	"
Day 60 min.DA	5-7 µm	"	"	"	"	"
Day 2 min. BrightDA	2.5-4 µm	"	60% of RC length.	Scattered throughout, dense in prox. 30%, and distal tip. Below BM.	As above, also processes extending distally between dark pigment cells.	"
Day 5 min. brightLA	2.5-4 µm	"	", and to BM in places.	"	"	"
Day 15 min. brightLA	5-7 µm	"	60% RC length.	Distal 30% dense, prox 30% more sparse. Below BM.	Surrounds RCs along length, but thicker distally.	"
Day 30 min. BrightLA	4-5 µm	"	"	Throughout RC, denser close to rhabdom in proximal and distal 30%	"	"
Day 30 min Bright sun	4-5 µm	"	Varies from halfway along RC, to BM.	As above, but denser.	Surrounds retinula cells along length, but fragmented appearance proximally.	Up to 30% of RC length.

BM = Basement membrane

RC = Retinula cell

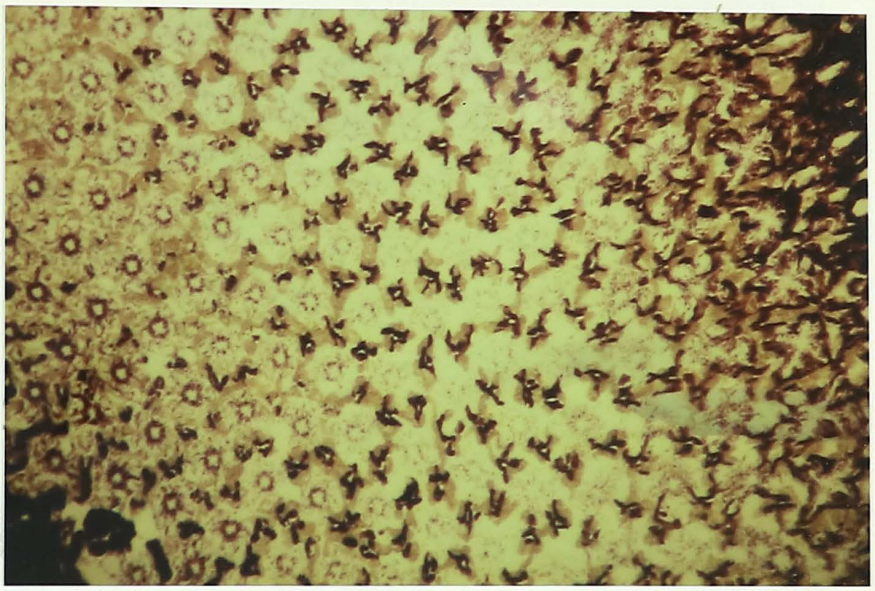
In dark adapted animals at night, the screening pigment granules of the retinula cells are almost entirely below the basement membrane. This is the only state in which the four non-pigmented lobes of R8 are not clearly visible under the light microscope, outlined by the pigment-containing distal tips of R1-7. Under dim light at night, scattered pigment is present in the distal third of the retinula cells. After two minutes of bright light adaptation, this pigment concentrates around the rhabdom and shifts slightly distally. Some granules move up into the proximal part of the soma from below the basement membrane. Eyes fixed after 5, 15 and 30 minutes of bright light adaptation show more granules moving up through the basement membrane to accumulate in the distal and proximal thirds of the cell. Scattered granules appear in the central region from 15 minutes on, but since they do not accumulate there they are presumably moving towards the distal part of the cell, which becomes progressively darker. During the day in dim light, pigment extends through most of the cell apart from the central region, but is more concentrated distally. Dark adaptation causes the pigment to become less dense, keeping the same general distribution but with less concentration about the rhabdom. Under strong light, the granules first concentrate distally and proximally, within two minutes. During the 30 minutes of bright light adaptation used, more granules continued to move up into the soma from below the basement membrane. The pattern is similar to that produced by light adaptation at night but there are always more granules present. After half an hour in bright sunlight, the distribution is similar but even denser, with very few granules remaining below the basement membrane.

At night, the proximal half of each retinula cell column is enveloped by reflecting pigment cells under all conditions examined. However a progressive change takes place in the distal half. In the dark-adapted eye the only reflecting pigment in the distal half of the eye is in the fine threads that run to the cornea, leaving a clear area between retinula cells. In dim light these threads are thickened. After 15 minutes of strong illumination the clear area is no longer evident, and after 30 minutes the reflecting pigment is equally distributed between the proximal and distal halves of the retina. This is also the case during the day, under dim light or up to as much as 60 minutes of dark adaptation. After two minutes of strong light adaptation, the reflecting pigment begins to push up between the dark distal pigment cells. After 15 minutes, there is more reflecting pigment between the retinula cells in the distal half of the retina than the proximal. At thirty minutes, the extreme distal projections of reflecting pigment have been displaced by dark distal pigment. After thirty minutes in bright sunlight (Fig. 2.12a), the reflecting pigment cells extend over both proximal and distal halves of the retina. However, they do not completely envelope the retinula cell columns, but present a rather tattered, fragmented appearance. The reflecting pigment cells also send processes below the basement membrane, but when light adapted in the day, these are masked by the basal red pigment.

When dark adapted at night, the basal red pigment is retracted to some extent (about 20-30 μm) from the basement membrane. Under dim light at night, and dim or bright artificial light during the day, the pigment is below the basement membrane but closely apposed to it (Fig. 2.12b). Under bright sunlight, or bright artificial light at night, the red basal pigment cells send processes through the basement membrane, on the outside of the retinula cell column/reflecting pigment cell complex.

Fig. 2.12a Oblique section through the retina of a crab exposed to bright sunlight for 30 minutes. Osmicated, otherwise unstained. The red basal pigment can be seen lower left, dark distal pigment top right. Retinula cell screening pigment is reddish-brown, and the reflecting pigment light brown. (450X).

12b Longtitudinal section of day eye, dim light adapted, showing red basal pigment below the basement membrane. Osmicated, lightly stained with toluidine blue. (530X)



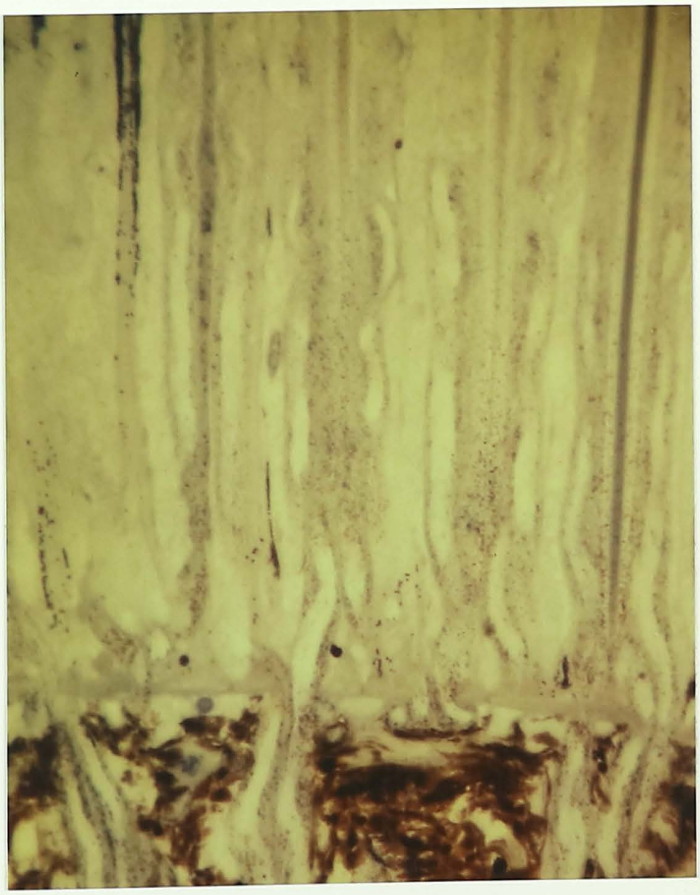
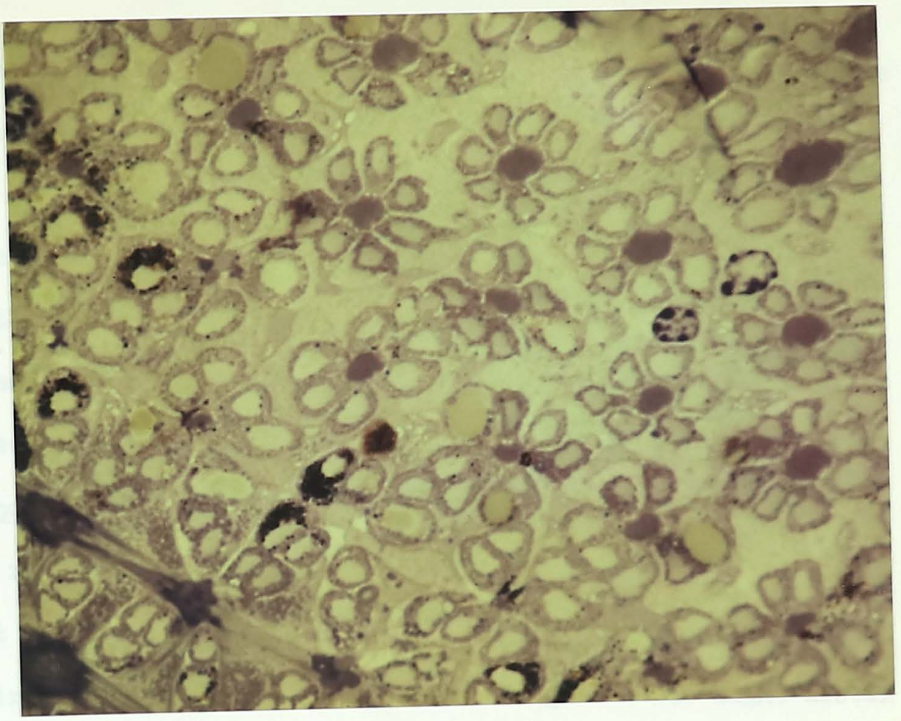
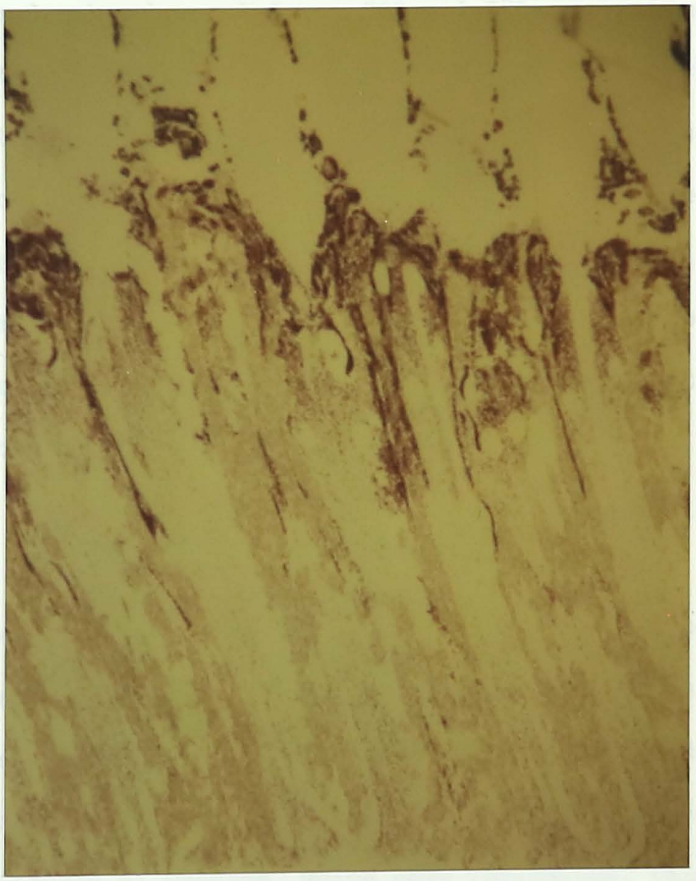


Fig. 2.12c Oblique section of the night eye of Leptograpsus, showing the increase in size of the rhabdom from near the basement membrane (lower left) to the distal retina (top right), and large yellow droplets within the retinula cells. (1100X)

12d Unstained longtitudinal section from crystalline cone (top) to basement membrane (bottom) showing position of the light distal pigment between the cones, the aperture formed by the dark distal pigment (arrow) and the distal concentration of retinula cell screening pigment. (420X)





These processes were seen to extend up to one third of the way along the retinula cells. Two of the eyes adapted to dim light at night showed small patches of red pigment extending up to 15% of the length of the retinula cells. Since the red pigment in unosmicated eyes was largely dissolved by the dehydrating alcohols, the amount of red pigment above the basement membrane is probably under-estimated, although the basement membrane did seem to offer some protection against the alcohol.

Microspectrophotometry

The retinula cell screening pigment (Fig. 13a) and the light distal pigment (Fig. 13b) show a high absorbance in the ultra-violet, increasing fairly smoothly towards longer wavelengths. The light distal pigment has a slightly lower extinction at wavelengths longer than about 500 nm. The dark distal pigment also has its highest extinction in the UV, but there is a rise in absorbance between 500 and 580 nm, peaking at 540 nm (Fig. 13c). Extinction at the red end of the spectrum is markedly higher than for the retinula cell screening pigment or the light distal pigment. The absorbance of the red basal pigment is low in both the ultra-violet and the red, peaking in the green at about 500 nm (Fig. 13d). The extinction curve of the reflecting pigment (not illustrated) shows a similar shape to the retinula cell screening pigment but its reflectivity characteristics were not measured.

DISCUSSION

Most of the screening pigments of the Leptograpsus eye appear comparable to those described and sometimes chemically identified in other Crustacea. The exception is the light distal pigment, which does not seem to occur commonly in Crustacea. The yellow pigment granules described in the chromophores of Crangon, which have inclusions, (Elofsson and Kauri, 1971) are not ultrastructurally similar.

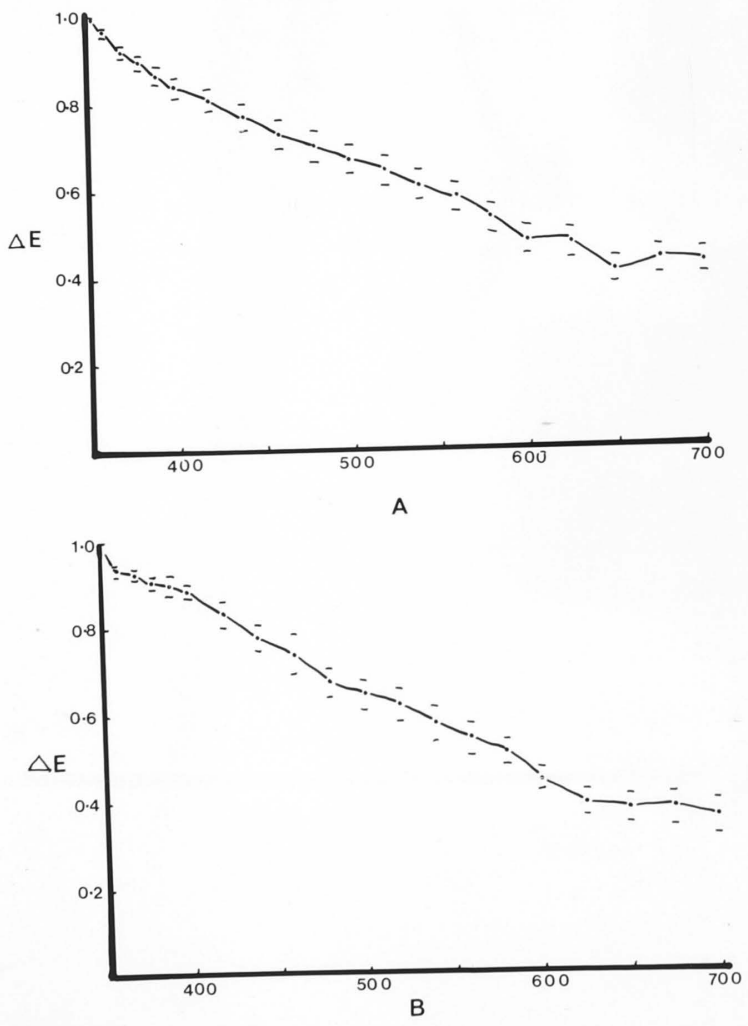
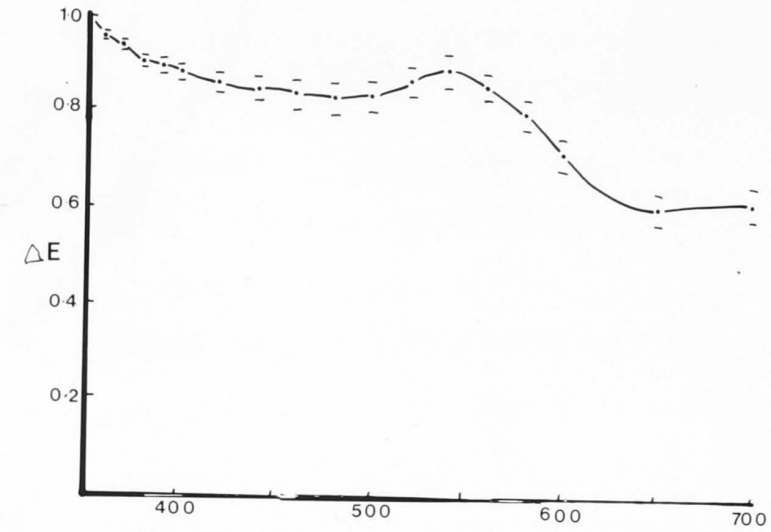


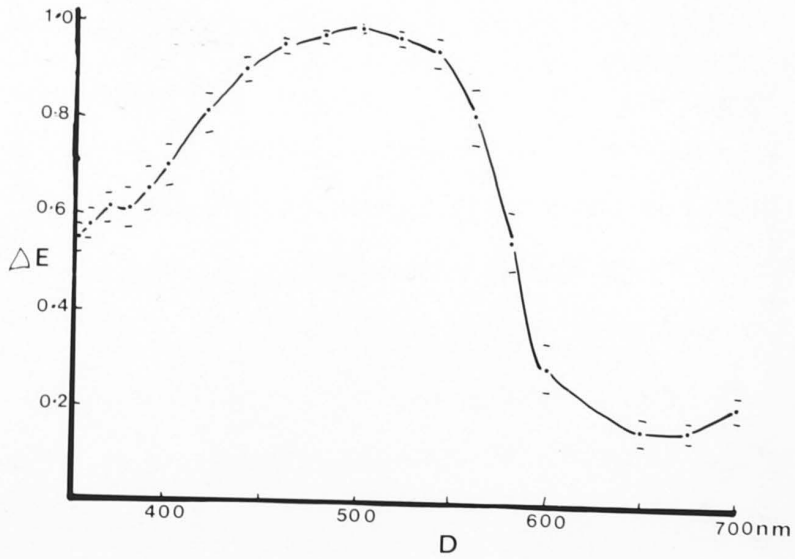
Fig. 2.13 Normalised differences in extinction between retinal screening pigments of Leptograpsus and a saline baseline.

A Light Distal Pigment (N=9)

B Retinula Cell Screening Pigment (N=9)



C



D

C Dark Distal Pigment (N=8)

D Red Basal Pigment (N=11)

The granules of the dark distal pigment cells and the retinula cells are similar in size and structure to those containing ommochromes in other Crustacea (Elofsson and Hallberg, 1973). The absorption spectrum of the dark distal pigment is very similar to that of ommin (Butenandt et al. 1958, quoted from Goldsmith, 1964), which is probably the main constituent of the granules. The retinula cell pigment granules, with a higher transmission at longer wavelengths, probably contain a significant amount of the red and yellow xanthommatins.

The red basal pigment droplets are structurally similar to those found in the eye of Nebalia (Green, 1972 - discussed by Elofsson and Hallberg, 1973) and in several mysids (Hallberg, 1977). These are considered to be formed of carotenoids, as are the red pigment-containing chromatophores of the body, and in fact Briggs (1961) found astaxanthin in extracts of Leptograpsus eyes.

The white reflecting pigment of a number of brachyuran crabs has been examined by Zyznar and Nicol (1971). They found it to consist largely of pteridines, which are fluorescent in long wavelength ultra-violet, and a smaller amount of purines, which quench short wavelength UV. In all six crabs, isoxanthopterin was the most abundant pteridine.

The retinula cell screening pigment, the reflecting pigment, the red basal pigment, and to some extent the dark distal pigment, are responsive to changes in the light level during both day and night. This might be expected, since Leptograpsus' typical feeding and activity pattern is much more dependent on the tide than on the ambient light level. During the day, the animals are found in a variety of light conditions, from deep inside dark crevices to flat rocks fully exposed to the midday sun. However, since there is also a difference in the behaviour of the screening pigment complex (apart from the retinula cell screening pigment) during the night and the day, the position of the pigment is not only determined by the ambient light level, but is under some degree of control by a circadian rhythm.

The change in diameter of the rhabdom between night and day is of the same order of magnitude to that found in some spiders (Blest, 1978) and recently in Grapsus (Nässel and Waterman, in prep.). In Grapsus the diameter increases substantially all down the rhabdom, and it is not at all unlikely that the changes seen at 12 pm in Leptograpsus were not complete.

The yellow oily droplets, found only in night eyes, were presumably moving through the clear, micro-tubule-filled "tunnels" within the retinula cells, and could well be carrying material for incorporation into growing rhabdoms.

CHAPTER III

RECEPTOR RESPONSES AND SPECTRAL SENSITIVITY

INTRODUCTION

The general form of the receptor response has been described for several crabs (Shaw 1966, 1969), among them Leptograpsus (Erber and Sandeman 1976). The response is typical of arthropod photoreceptors (reviewed by Fuortes and O'Bryan 1971), being a depolarisation which consists, at all but response levels below about 5mV, of a transient peak followed by a plateau. The polarisation sensitivity of cells R1-7 is known to be high in decapods (Shaw 1966, 1969). However angular sensitivities have been little studied. Walcott (1974) found very wide angular sensitivities (24^{o}) in the dark-adapted superposition eye of Cherax, a crayfish. Leggett (1978), using Scylla with eyes in situ, studied changes in acceptance angle during dark adaptation, and found rather wide acceptance angles during the day (c. 4^{o}) which became very broad ($10\text{-}11^{o}$) when dark-adapted at night.

Crustacean spectral sensitivity, and in particular colour vision, is a somewhat confused area, with puzzling discrepancies in the evidence from various sources. Only one photopigment has been found by spectroscopic examination of eye extracts or isolated rhabdoms, but some electrophysiological and behavioural evidence implies there is more than one colour-type of retinula cell. This evidence is now reviewed.

Evidence from analysis of extracted pigments.

Measurement of the λ max (peak absorption) of photopigments that have been extracted from the rhabdom with digitonin often yields values up to 20nm shorter than are found by the examination of pigment in intact rhabdoms, or by recording the responses of retinula cells. A similar but smaller shift occurs in vertebrate material (reviewed by Bowmaker, 1973).

The cause is unknown. The size and inconsistency of the effect in crustaceans means that evidence from this source is of little use unless it is supported by observations using other methods.

In the majority of species studied, only one visual pigment has been found. The exceptions are the crayfish Procambarus and Orconectes, in which Wald (1967) found two pigments, one absorbing maximally in the yellow region and the other in the green. However neither of them can be identified with pigments found by other methods. Extracts of eye pigment from brachyuran crabs have yielded green absorbing photopigments; 513nm with a metarhodopsin of λ max 495nm, in Leptograpsus and Hemigrapsus (Briggs 1961), 476nm in Callinectes (Fernandez 1965) and 480nm in Uca (Bruno and Goldsmith 1974).

No trace of a photopigment maximally sensitive in the blue or violet range has been found in pigment extracts.

Evidence from microspectrophotometry

Microspectrophotometry allows measurement of visual pigment "in situ" in the rhabdom, using a measuring beam diameter of as little as 1.5μm. Crab rhabdoms that have been measured with msp have all yielded a visual pigment with a λ max. of around 500nm; 493nm in Libinia (Hays and Goldsmith 1969), 504nm in Callinectes (Goldsmith and Bruno 1973) and 502-506nm in Carcinus (Bruno, Mote and Goldsmith 1973).

Only one visual pigment has been found in the rhabdoms of other crustaceans examined in this way, including crayfish, where Goldsmith (1978b) found a photopigment with λ max at 530nm.

Evidence from ERGs

Spectral sensitivity of a number of species has been tested using the ERG response. Since the shape of the ERG depends on the number and structure of the responding elements, the surrounding tissue, and the position and recording characteristics of the electrodes, as well as the

form of the response of a single photosensitive cell, any conclusions drawn from ERG data are necessarily rather tentative. The ERG of a large proportion of tested species, including euphausiids (Boden et al. 1961) and the hermit crab Eupagarus (Stieve 1960), offers evidence for only one visual pigment. Many crustacea have an ERG peaking around 500nm, very similar to the curve that would be produced by a single unshielded pigment, and substantially unchanged by adaptation to red or blue light, as in the lobster Homarus (Kennedy and Bruno 1961, Kampa et al.1963; Wald 1968, λ max 525nm), the crabs Callinectes (Goldsmith and Fernandez 1968, λ max 505nm), Uca and Sesarma (Scott and Mote 1974, λ max 508nm) an anomuran, Pleuroncodes (Fernandez 1973, λ max 523nm) and the isopod Glyptonotis (Laughlin, pers.comm. 495-500nm). In some species the shape of the spectral sensitivity curve is unchanged by light adaptation, but has a secondary peak at shorter wavelengths, as in Squilla mantis (Schiff 1963), and Porcellio (Goldsmith and Fernandez 1968).

In other animals, the shape of the spectral sensitivity curve of a light-adapted eye is different from that of a dark-adapted eye. The interpretation of these changes is unclear without additional evidence, since they could be caused by either changes in the distribution of screening pigments, or by selective adaptation of one of several types of visual pigment. The effect may be independent of the adapting wavelength, as in Libinia (Wald 1968), where the light-adapted sensitivity is almost flat between 390 and 500nm, while the dark-adapted curve is symmetrical, with a peak at 490nm.

In other cases, the effect depends on whether blue or red light is used to adapt the eye. In the crayfish Procambarus and Orconectes, Wald (1968) and Goldsmith and Fernandez (1968), found that adaptation to

red light suppressed the peak at 570nm and revealed a smaller peak at 425-450nm. Similarly, the shrimp Palaemonetes paludosus has a dark-adapted spectral sensitivity peaking at 550nm with a shoulder at 380nm which is selectively enhanced by red light adaptation (Goldsmith & Fernandez 1968).

The difference in dark-adapted spectral sensitivity peak of crayfish ERG 570nm) and the λ max of the visual pigment measured by msp (530nm) has been explained as the effect of the red-brown screening pigments by Kong and Goldsmith (1978), who examined a white-eyed mutant which lacked them. Goldsmith (1978) extended the argument to cover other decapods with superposition eyes and similar screening pigment. The most drastic shifts so far seen as a result of selective adaptation, occur in the crab Carcinus (Wald 1968). The dark-adapted curve was rather broad, peaking at 500-520nm. Adaptation to red light caused a shift to 425nm. While adaptation to blue light caused a shift in the other direction, to 565nm. However, both light-adapted curves were very broad, maintaining a high sensitivity over most of their range. This work was repeated by Bruno, Mote and Goldsmith (1973), who found the dark-adapted curve was narrower, with a maximum of 493nm making a reasonable fit with the absorbance of the rhodopsin as measured by msp, but the results of their selective adaptation studies were equivocal.

Intracellular recordings from retinula cells

Most published determinations of spectral sensitivity in crustaceans using intracellular recording have been made using excised eyes, as have some of the ERGs, and are therefore not necessarily totally reliable. Studies on dark adapted crab eyes have shown peak spectral sensitivities very close to those found with ERGs (Carcinus, Bruno, Mote and Goldsmith 1973; Callinectes, Scott and Mote 1974). Leggett (1978) using the mud crab Scylla, with eyes in situ, showed that there are sometimes considerable shifts in the spectral sensitivity of light-adapted cells, from a dark-adapted peak of 495nm. An intact Antarctic isopod, Glyptonotis, showed

a dark-adapted peak close to 500nm, (Laughlin, pers. comm.).

The crayfish Procambarus has been relatively closely studied. Nosaki, in 1969, reported two classes of retinula cell, one maximally sensitive to violet light, the other, more common type maximally sensitive to yellow-orange light. This study was repeated on the same equipment by Waterman and Fernandez (1970). The "V" cells were grouped around 440nm, the "Y-O" type averaged 594nm, ranging from 538 to 634nm peak sensitivity.

Micro-anatomical evidence from selective adaptation of retinula cells

Eguchi, Waterman and Akiyama (1973) exposed the eye of Procambarus to long (6 h) periods of yellow or violet light, and counted the number of "lysosome related bodies", supposedly associated with light adaptation in Libinia (Eguchi and Waterman 1967), in cross-sections of the main retinula cells R1-7. Although some bodies were found in all cells under both light regimes, there were more in R1 and R7 in yellow light, and in R4 and R5 in violet light (Parker's numbering system). The remaining cells showed no clear effect. The distribution of retinula cell screening pigment, which has been shown to move independently in the cells of one ommatidium (Ludolph et al. (1973) was not mentioned. Msp measurements comparing the difference spectra of points within rhabdoms of crayfish (Goldsmith 1978b) demonstrated that pigment composition within the rhabdom of R1-7 is spatially uniform. It is therefore unlikely that the results of Eguchi et al. were caused by a division of retinula cells into those with violet-sensitive or yellow-sensitive pigment.

Evidence from interneuron recordings

Perhaps the clearest physiological indication of colour vision comes from interneuron recordings. In 1970 two sets of experiments were reported on the spectral sensitivity of sustaining fibres recorded from the optic tract of Procambarus, Treviño and Larimer found dark-adapted

spectral sensitivity curves with maxima between 570-575nm, narrower than the receptor spectral sensitivity. Red light adaptation exposed an input with a maximum at 445nm. Near the threshold, the set of spikes produced by a single flash could be resolved into two bursts. The shorter latency burst (30-40 msec) was more sensitive in the range 575-650nm, while the sensitivity of the longer latency (120-130msec) burst peaked at 445nm. Woodcock and Goldsmith, in a similar experiment, found that 90% of the units they recorded had peak sensitivity in the yellow-green, between 560-580nm, but about 10% had the peak in the blue, near 460nm. The shape of the curves suggested that each sustaining unit was receiving at least some input from both the yellow-green and the blue systems. During dark adaptation, the shifting of the peak response to shorter wavelengths paralleled the retraction of the screening pigment, and could be reversed by injecting the animal with eyestalk extract, which caused the screening pigment to extend to the light-adapted state.

Evidence from behaviour for colour vision

Colour discrimination is notoriously hard to demonstrate (e.g. Menzel, 1979 in press), and the behavioural evidence from decapods is sparse. While crabs in particular are often brilliantly coloured and apparently make use of this in visual display it is hard to rule out other factors in the behaviour that might be significant. The prime example of this is the display behaviour of Uca where close study has shown that the various species not only have different coloured chelae, but also wave them in different patterns and make different sounds with them (Crane, 1975). Many laboratory studies involving the response to stripes, either in a choice situation (e.g. Eupagarus, Stieve 1960) or using optomotor responses (e.g. Potamon, Bäuerlein 1969) are complicated by the very high ability of

the animals to distinguish between shades of grey. Experiments involving coloured lights are less open to question. Many planktonic crustacea are, under certain circumstances, phototactically repelled by short wavelengths and attracted to longer wavelength light (Daphnia, von Frisch and Kupelwieser 1913; Bosmina, Ceriodaphnia, several copepods, stomatopod larvae, Baylor and Smith 1957). Hyatt (1975) succeeded in demonstrating a discrimination in phototactic behaviour among some combinations of blue, red, white, and UV light over a considerable range of intensities in Uca.

With this background in mind, the following experiments were undertaken in order to determine the spectral sensitivity of the retinula cells of Leptograpsus over a wide range of background intensities. In view of the variety of shielding-pigment states demonstrated in Chapter II, and since none of the pigments, taken in isolation, approaches a neutral density some changes in spectral sensitivity with light adaptation are to be expected. Leggett (1978), working with Scylla, found a variety of spectral classes which he interpreted as being due to a combination of adaptation effects and differences in the properties of the screening pigments from between ommatidia. These differences (for which no other evidence was given) supposedly make colour vision possible with only one photopigment. The theoretical screening effects of the pigments present in Leptograpsus on spectral sensitivity were calculated in order to see if those pigments known to be common to all cells (at least R1-7) could produce the range of spectral sensitivities that were measured.

The polarisation sensitivity and angular sensitivity of some cells was measured to demonstrate the reliability of results with intact eyes.

METHODS

Preparation and recording

Crabs were made to autotomise their legs, and the back of the carapace fixed with quick-setting cyano-acrylate glue to a Perspex holder mounted on a magnetic stand. The eyes were positioned as they would be in an alert animal, secured with Plasticene, and immobilised by filling the eye-cup with "Vertex" dental cement.

The crab was positioned in the recording set-up at the centre of a Cardan arm perimeter device, and the eyes wiped with damp tissue to remove any salt deposit left by drying sea-water. A chip of razor blade was used to cut a triangular hole with sides about 5 facets or 200 μm long in the dorsal cornea, and the electrode was quickly introduced vertically with the aid of a Leitz joystick micromanipulator. Haemolymph drying around the electrode provided some stability, but even so movements of the eye produced by the heart-beat were large enough to prevent stable recording in about 50% of the preparations.

The recording electrodes of resistance 150-200 MΩ, were pulled from fibre-filled glass and filled with 3M potassium acetate. The indifferent electrode was a silver wire pushed into the rear leg stump. The signal was recorded through a Grass P16 amplifier and displayed on an oscilloscope and a chart recorder. All measurements, apart from some resting potentials, were made from the chart recorder. Crabs generally remained in good condition, if they were kept moist, for about a day. Recordings were made from June 1978 to January 1979.

Stimulation

The light source was a 150 watt Xenon arc lamp. The stimulating beam passed through a heat filter, a collimating lens, neutral density filters, interference filters, a focusing lens and a shutter (Uniblitz).

The beam was focused onto a 4mm diam quartz light guide attached to the Cardan arm. The quartz neutral density filters (Balzers) allowed attenuation over 5.6 log units in 0.2 log unit steps. The twenty interference filters (Schott and Corion) ranged from 317 to 621 nm peak transmission. The light guide subtended 1.3° at the crab eye. The set-up was calibrated about once a month using a Hewlett Packard type 8330A radiant flux meter. Apart from the first few weeks after the installation of a new bulb, the measured transmission through the filters varied little. The quantal transmission through the filters from 373 to 621 nm inclusive was adjusted to within 13% of 1.8×10^{13} Q/cm^2/sec., but transmission through the UV filters 317, 345, and 358nm was 20-40% less, and was not adjusted. The correction factors applied to the measured responses, then, were not more than 0.06 log units for filters above 373nm, but up to 0.39 log units for the three shortest-wavelength filters.

During all the experiments described here, a 20 msec flash was given at 10 second intervals, and where possible the N.D. filters were adjusted to keep the response below about 25mV. Brighter or more frequent flashes caused appreciable adaptation, so that the responses to consecutive stimuli were not independent.

When a cell with a stable resting potential of 50mV or over was found, the light guide was centred on the optical axis, using flashes delivered once every second. The experimental run was started after the response to a flash of the same intensity, delivered once every 10 seconds, had stopped increasing. Since cells could not be stimulated to produce their maximum response (50-80mV) until they had been completely dark-adapted for about 30 minutes, and did not fully recover for a further half hour, the

maximum response was generally not determined. V/log I curves were measured by starting near threshold and increasing the intensity in steps of 0.2 log units until the response of the cell had reached about 30mV. White light intensity series were interspersed between experimental runs in all experiments. Monochromatic intensity series were sometimes taken, and found to have the same slope as white light series, but they were not generally used as the light intensity available was often insufficient to produce large responses. Polarisation sensitivity or angular sensitivity was determined for some cells, but most were used only for spectral sensitivity measurements. All cells used were in the central, anteriorly directed part of the retina.

Polarisation sensitivity

Sensitivity to the plane of polarisation of white light was measured by rotating a piece of polarising film (Polaroid type HN38) in front of the light guide through 180° in 10° steps. The maximum and minimum responses during the polarisation run were compared with the V/log I curve to find the PS. Polarisation runs were considered valid if all responses fell within the linear part of the V/log I curve (where measurement error and intrinsic variation in response have least effect), and if the size of the responses at 0 and 180° rotation of the polaroid were equal, implying that the sensitivity of the cell had not changed during the run. All PS measurements were made on dark-adapted cells.

Angular sensitivity

White light was used for angular sensitivity measurements. To provide a point source, the light guide was covered with a metal mask with an aperture that subtended 20' at the eye. After the point had been centred and an intensity series made, the point was moved in a vertical arc

through the point of maximum sensitivity in ¼ or ½° steps. Several runs were made, with the source moved alternately up or down. The position of some cells was determined by shining a microscope lamp onto the eye along the optical axis of the cell, and noting the position of the dark pseudopupil that could be seen facing the light. The mapping system used was that of Sandeman (1978), i.e. the position of a point on the eye is given in relation to a vertical axis through the dorsal "peninsula" of cuticle and a horizontal axis which is the "equator" of the eye in the normal position.

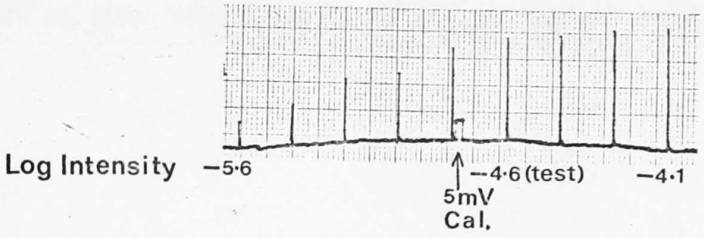
Spectral sensitivity

Spectral sensitivity was calculated from the spectral efficiencies obtained by stimulating with monochomatic flashes of the same quantal content (corrected for the small differences in actual quantal transmission of the filters). It was measured in three "steady states", a) dark adapted for at least 30 minutes, b) in the dim light (10-20 uW/cm^2) of the normal room illumination (a 150 watt pearled tungsten bulb), and c) in the bright light (8-10mW/cm^2) of a 30 watt tungsten microscope lamp focused on the eye from not more than twenty degrees from the axis of the cell being tested. In cells adapted to these conditions (DA, dimLA, bright LA) a white light intensity series was made before and after two spectral runs (ascending and descending), as shown in Fig. 3.1. In transitional states of adaptation, particularly during the first few minutes of bright light adaptation, single spectral runs were alternated with intensity series.

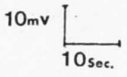
Spectral sensitivities were calculated day by day as data was obtained, with a Nova II computer using a programme introduced by Laughlin (1974), but the results presented here were obtained from curves calculated and plotted by hand.

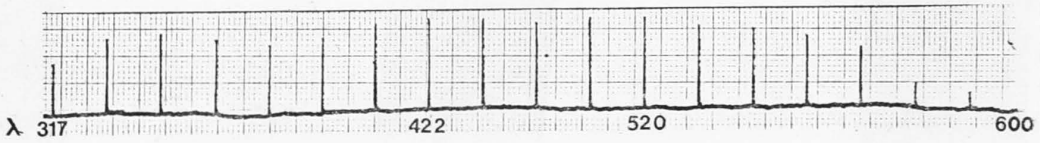
Fig. 3.1 Responses of dark adapted cell (70mV R.P.), consecutive except for short joining segments. The stimulus at 317nm and the two adjacent stimuli are not isoquantal with the remainder. The middle portion of each response has been retouched slightly. The test flash given at the end of the descending spectral series is white light, -4.6 log units, and shows that the sensitivity of the cell is unchanged.

Xeroxed, 3/4 actual size.

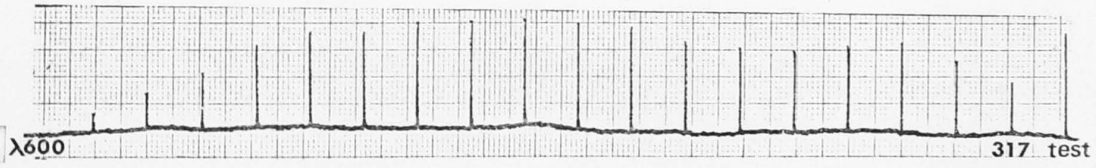


White Light Intensity Series





Ascending Spectral Series



Descending Spectral Series

The effects of the screening pigments were modelled using the data on absorption from Chapter II, and rhodopsin and metarhodopsin absorptions from Dartnall's (1953) nomogram. Calculations were made on a Hewlett-Packard 97 desk calculator.

RESULTS

In good preparations, cells could be held for 30-45 minutes routinely and occasionally for up to three hours. The average resting potential, measured for 42 cells, was 69.8mV, with a standard deviation of 11.8mV.

Polarisation sensitivity

Of 14 cells tested, nine were preferentially sensitive to vertically polarised light and five to horizontally polarised light. The average PS of twelve cells for which quantitative data were obtained was 9.0. However the most common PS (five cells) was between 10 and 11, the distribution being skewed by two cells with very steep V/log I curves, that produced PSs of 2.8 and 2.6, although the response modulation was large. Discarding these cells left the remaining ten with an average PS of 10, ranging from 6.3 to 14.8.

Angular sensitivity

The angular sensitivities of 17 cells, measured as $\Delta\rho$, the width of the angular sensitivity function at the 50% level, were tested under various conditions of light and dark adaptation. The average difference in $\Delta\rho$ between consecutive runs on the same cell in a stable adaptation state was 0.2°.

The average angular sensitivity of eight cells measured under dim light during the day was 2.5°, ranging from 1.5° to 3.2°. The position of six of these was measured, and although the number of cells involved is too small to reach any definite conclusion, those cells with narrower angular

sensitivities were found nearer the most anteriorly directed part of the retina. $\Delta\rho$ of four of these cells were measured during adaptation to bright light. Over times ranging from four to twenty-five minutes, the angular sensitivities of three decreased, from 2.1° to 1.3°, from 3.2° to 1.9°, and from 2.4° to 1.8°, while the fourth was unchanged, at around 3° after four minutes.

The average angular sensitivity of six cells dark adapted at night was 2.7°, ranging from 1.8° to 3.7°. Two cells, initially adapted to dim light, were monitored as they were dark adapting. One, tested during the night, showed an increase in $\Delta\rho$ from 2.4° after 7 minutes DA, to 3.5° after 25 minutes DA. The other, tested during the day, changed from 1.6° to 2.3° to 3.7° over about fifteen minutes DA.

Spectral sensitivity

Thirty-three cells were measured while dark adapted, 21 while adapted to "dim" light, and 16 during adaptation to "bright" light. Most cells were tested in more than one adaptation state.

Dark adapted

The spectral sensitivity of dark adapted cells was almost the same at night (n=8) as in the day (n=7), in spite of an increase of about 1.5 log units in relative sensitivity (measured as the difference in intensity of the standard stimulus needed to produce a constant response) at 500nm (Fig. 3.2). The average λmax of these fifteen cells was 484nm (S.D. 22nm), and the range of λmax was from about 450 to 520nm. (The remaining 18 dark-adapted cells were measured around dusk. Their spectral sensitivities seem identical, but they are not further analysed here).

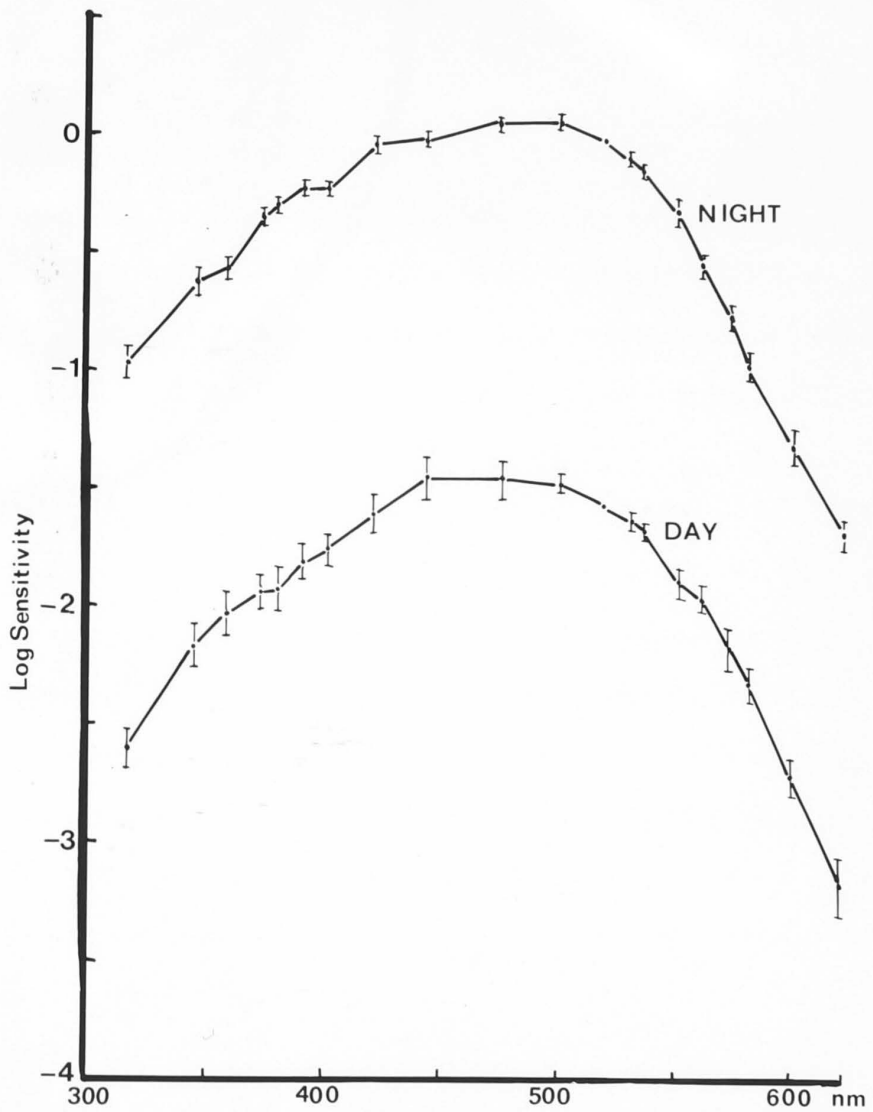


Fig. 3.2 Log spectral sensitivities of dark adapted cells at night (n=8) and during the day (n=7). Each group normalised to the response at 520nm. Bars show S.E.M.

Dim light adapted

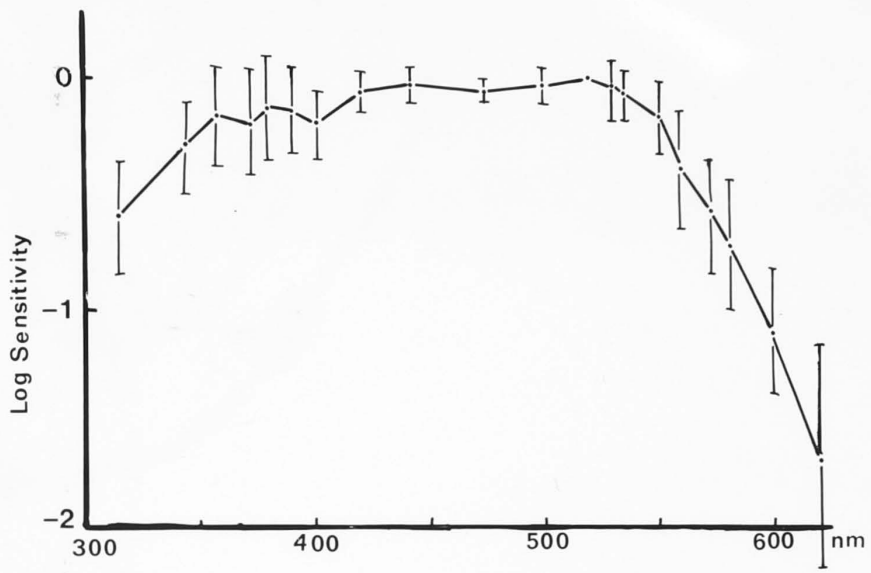
Cells adapted to dim light had broader spectral sensitivites than dark-adapted cells. They were sometimes double-peaked, and had maxima anywhere between 422nm and 537nm. The peak response was, on average, shifted to longer wavelengths than in a dark-adapted cell, to 520nm in the day and 500nm at night. The variation in response of one cell over 12 runs, or about one hour, is shown in Fig. 3.3. The averaged spectral sensitivities of 10 cells recorded during the day and 4 cells measured at night are shown in Fig. 3.4. The shapes of the two log spectral sensitivity curves are not very different, although the day curve is rather flatter. The relative sensitivities are very similar, the day and night groups being less sensitive than cells dark-adapted at night by 2.4 and 2.6 log units respectively. A histogram of the peak responses of 19 cells (Fig. 3.5) shows no evidence of a division into more than one colour type, although the scatter is so large that on this evidence alone such a division cannot be ruled out.

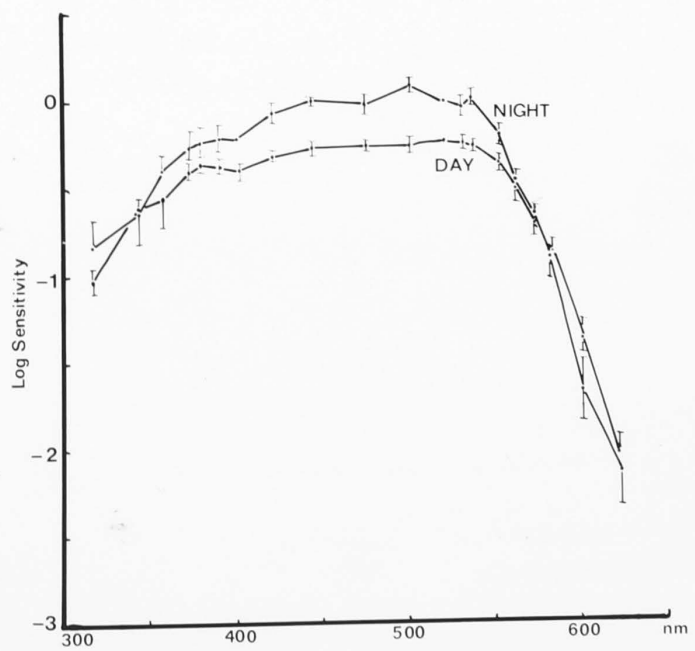
Bright Light Adapted

The general pattern of changes in spectral sensitivity during adaptation to bright light is consistent, but the precise timing and extent of the shifts varies somewhat from cell to cell. During the first 2-5 minutes, the peak sensitivity shifts dramatically to shorter wavelengths, around 360-400nm. The spectral sensitivity curve usually has two maxima at this stage, the smaller at 500-537nm. The relative size of the two peaks changes steadily, the shorter-wavelength peak becoming less pronounced over

Fig. 3.3 Variation in log spectral sensitivity of one cell adapted to dim light, over 12 runs, normalised to 520nm. Bars show S.D.

Fig. 3.4. Mean and S.E.M. of cells adapted to dim light, during the day (n=10) and at night (n=4).


0
−1
−2
Log Sensitivity
300
400
500
600
nm


0
−1
−2
−3
Log Sensitivity
NIGHT
DAY
300
400
500
600
nm

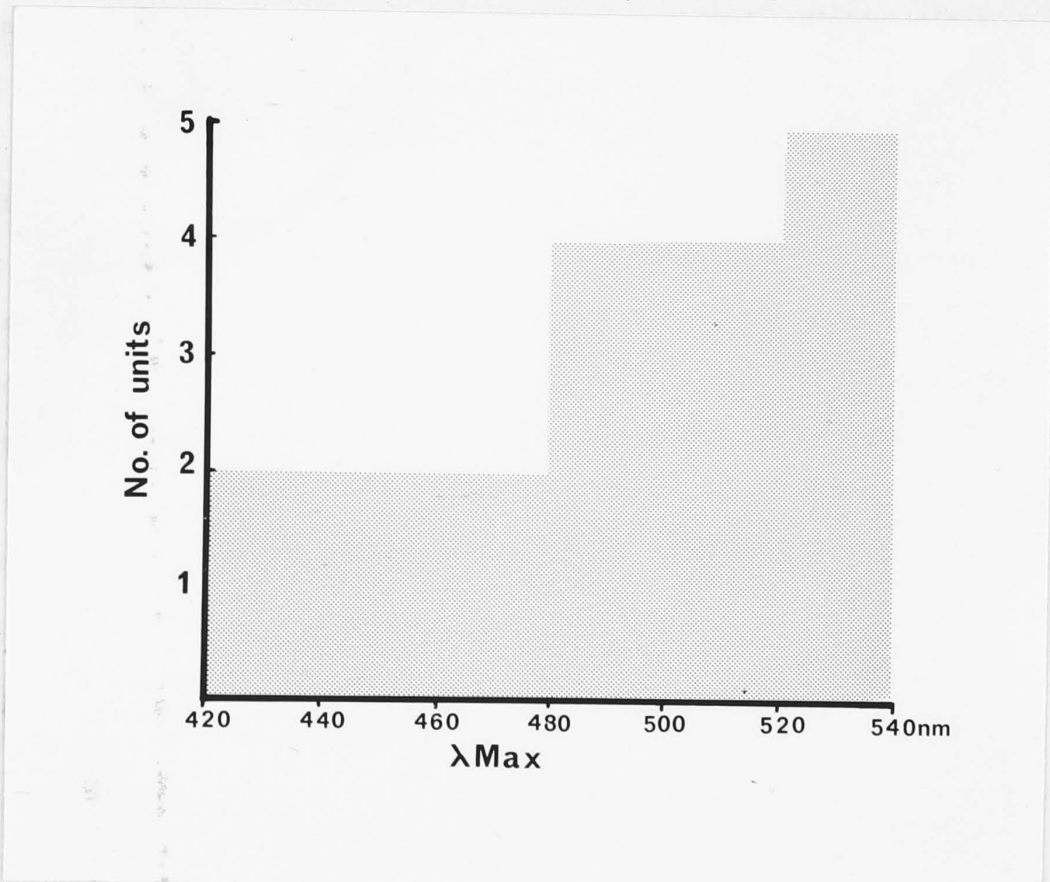


Fig. 3.5 Position of the maximum sensitivity of 19 cells adapted to dim light.

30-40 minutes, until the shape of the spectral sensitivity curve approaches that of a cell adapted to dim light. The pattern is not as well defined in the averaged results (Fig. 3.6) as in individual cells (Fig. 3.7), because of the variation in peak sensitivities and their relative sizes and rates of change from cell to cell.

With the onset of a bright adapting light, sensitivity to white light drops by up to 4 log units. It recovers rapidly for the first few minutes and more slowly over the next half hour or so, to not far below the dim light-adapted level. It was frequently impossible to measure spectral sensitivity until 2-5 minutes after the beginning of the adapting stimulus, because the source did not deliver monochromatic light of sufficient intensity. Changes in sensitivity of a single cell are shown on a log scale in Fig. 3.8. The increase in sensitivity during bright light adaptation is difficult to interpret because the adapting light was not axial. This means that the effect of the various pigments moving into new positions is different for the adapting light and the stimulating light. Very little is known about crab metarhodopsins, and no attempt is made here to analyse the effect of inter-rhabdomic processes in general on sensitivity.

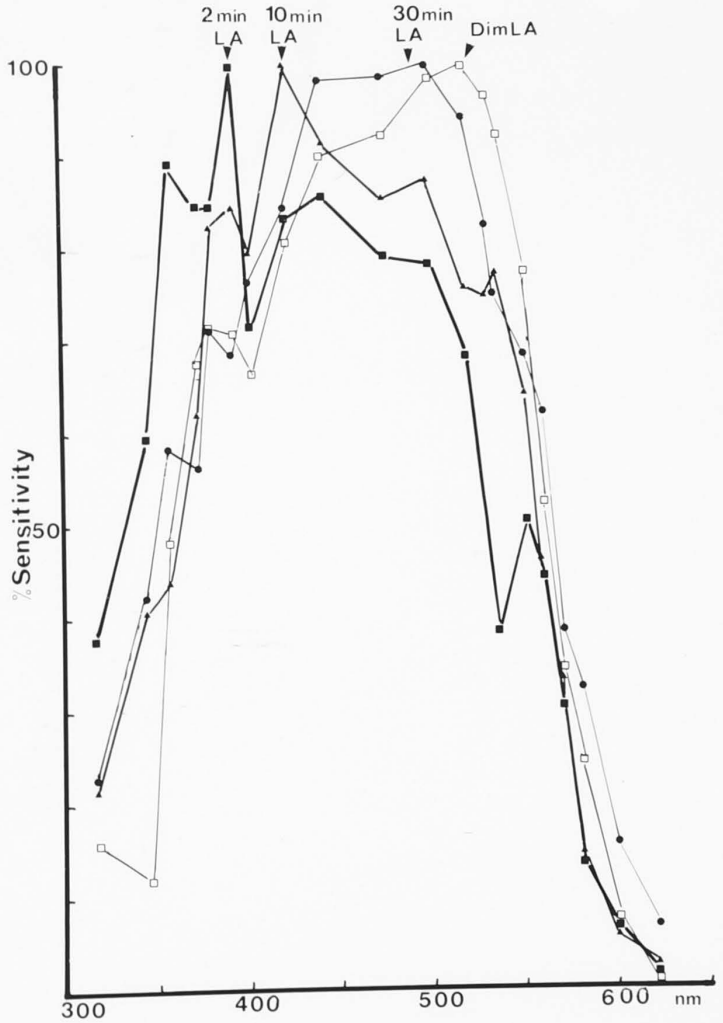


Fig. 3.6 Averaged spectral sensitivites of retinula cells in dim light (n=10), and after two, ten, and thirty minutes adaptation to bright light (n=4,6,5, respectively).

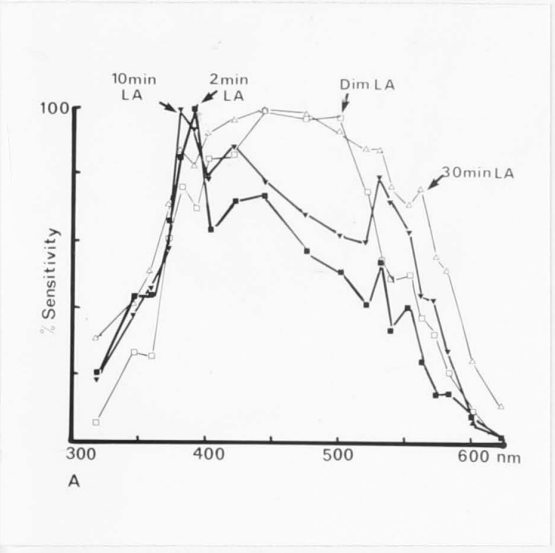
Fig. 3.7 Effects of bright light adaptation on single cells.

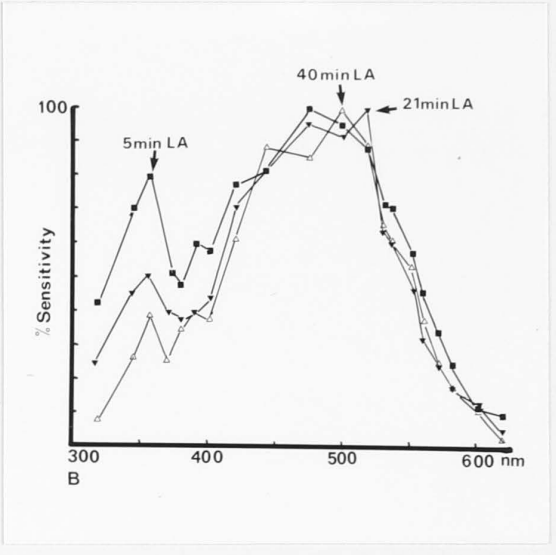
A. dimLA (3 runs), to 2 min. (1 run), 10 min. (2 runs) and 30 min. (2 runs) of bright light adaptation.

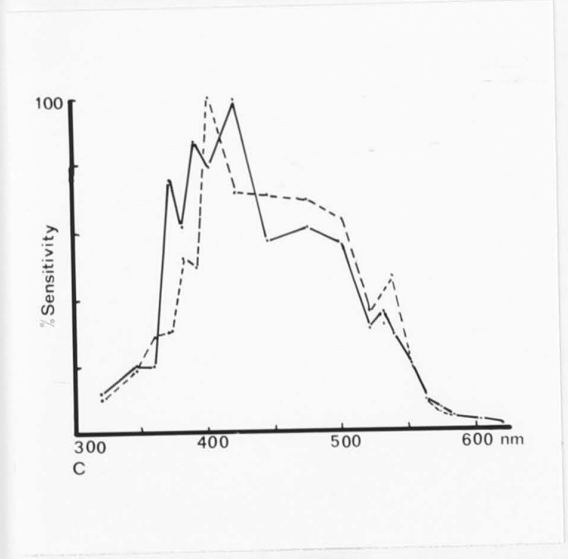
B. 5, 21 and 40 min. of bright light adaptation (2 runs each)

C. Two consecutive runs at 10 min. bright light adaptation.

D. 10 min (2 runs) and 30 min. (1 run) of bright light adaptation.


10min LA
2min LA
Dim LA
30min LA
100
% Sensitivity
300
400
500
600 nm
A


40min LA
21min LA
5min LA
100
% Sensitivity
300
400
500
600 nm
B


100
% Sensitivity
300
400
500
600 nm
C

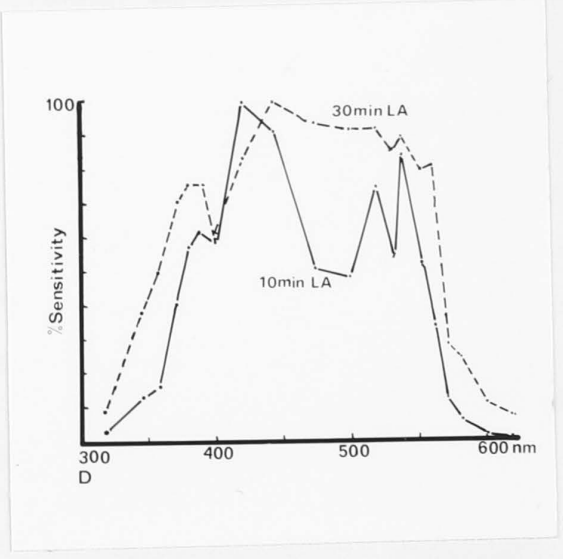
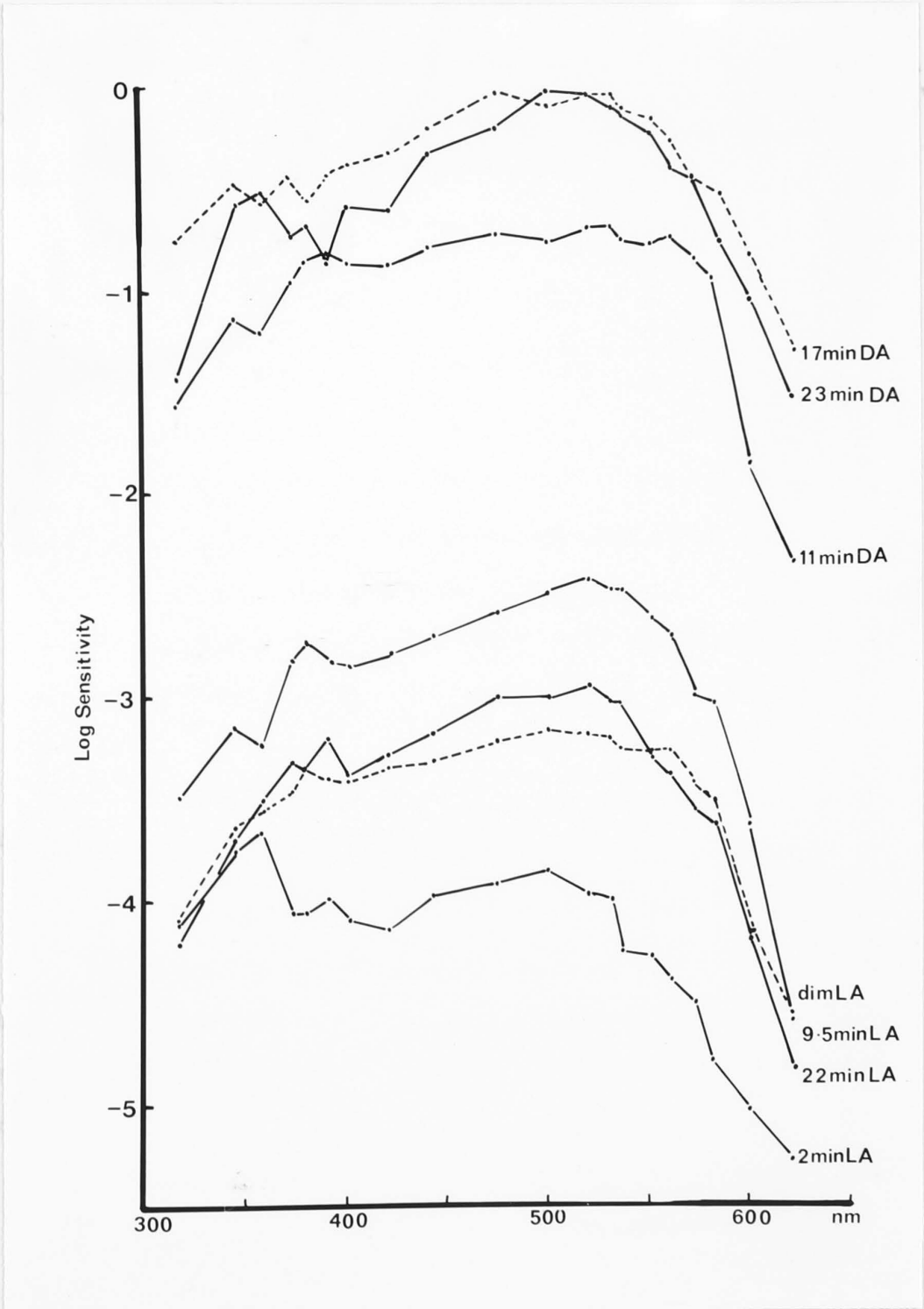

30min LA
10min LA
100
% Sensitivity
300
400
500
600 nm
D

Fig. 3.8 Changes in log spectral sensitivity of a single retinula cell. Starting with the cell adapted to dim light, spectral sensitivity is also shown after 2 min. (1 run), 9 min. (1 run) and 22 min. (2 runs) adaptation to bright light. Dark adaptation was then begun, and the spectral sensitivity tested after 11 min. (1 run), 17 min. (1 run) and 23 min. (2 runs). The U-V subpeak in the 23 min. DA curve is atypical.


0
−1
−2
−3
−4
−5
Log Sensitivity
17min DA
23min DA
11min DA
dim LA
9·5min LA
22min LA
2min LA
300
400
500
600
nm

Effects of Screening Pigments-Theory

The amount of light absorbed by a material is given by the exponential relation

$$\log_e \left\{ \frac{I_i}{I_t} \right\} = \alpha . \ell \qquad (1)$$

where I_i = intensity of incident light

I_t = intensity of transmitted light

ℓ = path length of the light

α = an absorption (extinction) co-efficient that depends on the absorptive power of the material and the wavelength of the light

The absorbance, or optical density, D, is given by

$$D = \log_{10} \left(\frac{I_i}{I_t} \right) \qquad (2)$$

from (1) $$D = \log_{10} \left(e^{\alpha \cdot \ell} \right) \qquad (3)$$

and the absorbance at a given wavelength λ is

$$D(\lambda) = \log_{10} \left(e^{\alpha\lambda . \ell} \right) \qquad (4)$$

The theoretical absorbance of a thin layer of rhodopsin can be found from a nomogram (Dartnall 1953) that uses absorbance values measured for a photopigment of one λmax to generated absorbances for a photopigment of any λmax.

The absorption, A, is the fraction of light absorbed;

$$A = \frac{I_i - I_t}{I_i} \qquad (5)$$

$$= 1 - \frac{I_t}{I_i}$$

and from (2) $$= 1 - 10^{-D} \qquad (6)$$

and $$A(\lambda) = 1-10^{-D(\lambda)} \qquad (7)$$

The normalised function $A(\lambda)/A(\lambda max)$ is the spectral sensitivity.

The theoretical effects of screening pigments on visual pigment absorption have been discussed by Goldstein and Williams (1966), and the treatment applied to some crustacean superposition eyes by Goldsmith (1978a). The effect of the screening pigment can most easily be assessed if it is treated as being in one of two extreme positions, either overlying the visual pigment, or homogeneously distributed throughout it. One of these may be close to the real situation in some cases - all the light distal pigment lies above the rhabdom, for instance, and in some circumstances metarhodopsin may well be uniformly dispersed along the rhabdom. More usually, the situation is somewhere between the two - the retinula cell screening pigment, for instance, may be concentrated just distal to the rhabdom, but it also lies beside the rhabdom along the length of the retinula cell, and in the second position it probably acts as a dispersed screen. However pigment which lies beside the rhabdom will act as an overlying screen for light entering the rhabdom obliquely.

The functions given by Goldsmith (1978a), for a rhodopsin of absorbance $D(\lambda)$, and a pigment screen of absorbance $D'(\lambda)$ are, for an overlyiing pigment screen,

$$A(\lambda) = 10^{-D'(\lambda)}[1-10^{-D(\lambda)}]$$

and for a homogeneously distributed pigment,

$$A(\lambda) = \left[\frac{D(\lambda)}{D(\lambda)+D'(\lambda)}\right]\left[1-10^{-[D(\lambda)+D'(\lambda)]}\right]$$

Using these equations, the effect of various screening pigments in Leptograpsus were calculated, either as if they were an overlying screen, or as if they were homogeneously distributed, for a variety of optical densities of screening pigment, rhodopsin, and metarhodopsin.

Screening pigments

In Chapter II, the screening pigments were measured individually, by microspectrophotometry. The method used yields the relative absorbance spectra, i.e. $D'(\lambda)/D'(\lambda max)$, but not the absolute value of D $D'(\lambda max)$, the absorbance at peak wavelength, since the thickness of the pigment being measured is not known. In the calculations, $D'(\lambda max)$ is therefore varied, within reasonable physiological limits, to find the best fit with the measured spectral sensitivities of the retinula cells.

Rhodopsin

The peak sensitivity of rhodopsin was taken as 485nm, from the measured values in dark-adapted cells. To find the optical density of the rhabdom at this wavelength, it is necessary to know the coefficient of absorption, α (see Eqn. 3). This is not available for Leptograpsus, but Bruno et al. (1973) have measured the coefficient of absorption in isolated rhabdoms of the crab Carcinus as 0.6% per µm at the peak wavelength. This is considerably lower than the absorption coefficient measured in the spider crab Libinia by Hays and Goldsmith (1973) as 1.3%/ µm. The Carcinus coefficient is used here because Carcinus is closer to Leptograpsus phylogenetically, ecologically and morphologically than is Libinia. Taking the average length of the rhabdom as 350 µm, the axial optical density of

the rhabdom can be found by substituting in Eqn. 4:

$$D = \log_{10}(e^{0.006 \times 350})$$

$$= 0.91$$

and the absorption, from Eqn. 6, is

$$1-10^{-0.91}$$

$$= 0.88,$$

i.e. 88% of axial light at peak wavelength is absorbed.

Metarhodopsin

Briggs (1961) found a metarhodopsin of λmax 495nm in Leptograpsus eye extract. Msp measurements on another crab, Libinia (Hays and Goldsmith 1969), revealed a metarhodopsin with maximum absorbance at slightly longer wavelengths than the λmax of the rhodopsin, which was 493nm. It therefore seemed reasonable to use Briggs' value of 495nm as the peak absorbance of the metarhodopsin. The molar extinction co-efficient was assumed to be the same as for rhodopsin.

Self-Screening by Rhodopsin

If no separate screening pigment is involved, absorption by rhodopsin is given by Eqn. 7. Fig. 3.9 shows the absorption of an unscreened 485nm visual pigment taken from Dartnall's (1953) nomogram, the normalised absorption of a 485nm pigment of peak optical density 0.91, corrected for self-screening, and the pooled spectral sensitivity measurements from day and night dark-adapted cells. The experimental data are closely approached by the theoretical curve corrected for self-screening. It can be concluded that the dark-adapted spectral sensitivity is the result of the rhodopsin alone, and that screening pigments do not appreciably alter spectral sensitivity in this state.

Screening by Metarhodopsin

The distribution of metarhodopsin within the rhabdom under conditions of constant illumination depends on the rate of photoregeneration of rhodopsin from metarhodopsin along the rhabdom, and the rate of metabolic regeneration. Both processes are known to occur in decapods, in Homarus for example (Bruno et al. 1977), but the rate constants and the relative importance of the two processes vary widely among arthropods, and there is no data available for grapsid crabs. In insects, the distribution of metarhodopsin along the rhabdom is usually strongly dependent on the wavelength of the stimulating light, (Hamdorf et al. 1973) but in crabs, where the peak absorbance of the rhodopsin and the metarhodopsin are very close, this factor is of much less importance. The effect of metarhodopsin on absorbance was therefore calculated in the two extreme conditions, without supposing that there was any firm justification for either. The actual situation presumably lies somewhere between the two. In both cases the total optical density of rhodopsin and metarhodopsin was held constant at 0.91, while the relative proportions were varied.

A homogeneous mixture of metarhodopsin and rhodopsin produces spectral sensitivity curves with peaks shifted to slightly shorter wavelengths (c. 470nm) than the pure rhodopsin absorbance. There was little change in the shape of the normalised spectral sensitivity curve for metarhodopsin optical densities ranging from 0.4 to 0.9099, i.e. well beyond the physiological limits. If metarhodopsin is treated as an overlying pigment (Fig. 3. 10) the spectral sensitivity curve becomes broader, because of relatively increased absorption at short wavelengths, and the peak is shifted to c. 440nm, developing a shoulder at about 520nm.

If the accompanying reduction in absolute absorption is taken into account, the spectral sensitivity curves generated by rhodopsin-metarhodopsin mixtures do not resemble any obtained by recording from cells.

Screening by Retinula Cell Screening Pigment

Since that part of the retinula cell screening pigment which lies alongside the rhabdom is competing for light with rhodopsin all along the light path, it was treated as a homogeneously distributed pigment. That part which lies in the extreme distal part of the retinula cells, around R8, can more properly be considered as an overlying screen.

As a homogeneously distributed filter, with O.D. up to 3.0, the effect of this pigment is to make the spectral sensitivity slightly narrower than that of the pure rhodopsin, without appreciably altering the position of the peak absorption. As an overlying screen (Fig. 3.11) the pigment causes the spectral sensitivity to become narrower, and the peak shifts to longer wavelengths. An increase in pigment O.D. from 0.01 to 3.0 produces a shift in λmax from 485 to 520nm, with a decrease in absorbance of about 2 log units at 500nm.

Screening by the Dark Distal Pigment

As an overlying pigment, the dark distal pigment at optical densities up to 3.0 produces a slight narrowing of the spectral sensitivity curve, with negligible shift in the position of peak absorption. The effect of the pigment as a homogeneously distributed filter was not tested.

Screening by the Red Basal Pigment

Since the red basal pigment lies at the proximal end of the rhabdom, and is fairly far removed from the rhabdom even when it extends distally between ommatidia (Fig. 2. 12a), it is rather difficult to determine its position in the light path, and hence the appropriate way to analyse its

effects. This is most unfortunate, since it is the only screening pigment in the eye with a high transmittance in the UV, and seems to be the only factor which could be causing the transient UV- violet peak that appears in the spectral sensitivity of cells adapting to bright light. The effect of the red pigment was calculated as an overlying, screen and as a homogeneously distributed filter, with a rhodopsin of optical density 0.4, since some bleaching might be expected under these conditions. Treated as a homogeneously distributed pigment, with an optical density of 1.0, the red basal pigment produces a slight upwards kink in the spectral sensitivity curve at about 380nm. As an overlying pigment, it produces a sharp peak at 380nm, increasing in prominence with increasing optical density of the pigment. Comparison of Fig. 3.12 with the figures showing changes in the spectral sensitivity of individual cells during bright light adaptation shows the similarity of the effects.

Screening effects of the light distal pigment and the reflecting pigment.

The shapes of the absorption curves of these pigments are very similar to that of the retinula cell screening pigment, differing only slightly in slope (see Chapter II). Their effect has not been calculated separately, since the light distal pigment is not in a position to have much effect on axial light, and the reflecting pigment probably transmits a negligible amount of light.

Screening effects of combination of pigments

It is unlikely that the screening pigments in an eye such as the crab's normally act in isolation, and given the variety of effects produced by single pigments, it should be possible to combine them to produce almost any spectral sensitivity curve. Conversely, most spectral

Fig. 3.9 Broken line; absorbance of a 485nm rhodopsin, from the nomogram.

Solid line; Rhodopsin of optical density 0.91, showing the effect of self-screening.

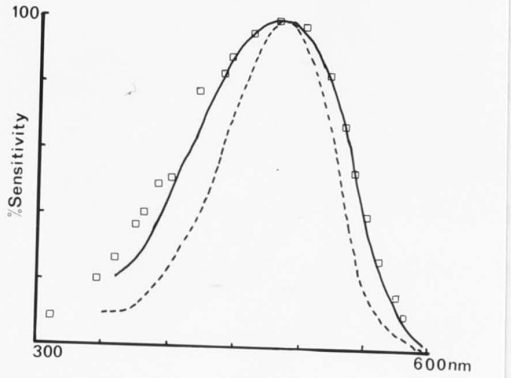
Squares; averaged spectral sensitivity of 15 day and night dark-adapted cells.

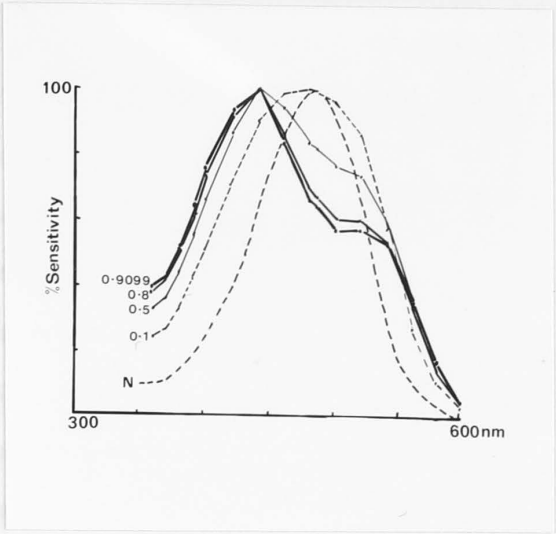
Fig. 3.10 Calculated effects of varying optical densities of a metarhodopsin of λmax 495nm overlying a rhodopsin of λmax 485nm. Total optical density of metarhodopsin and rhodopsin is 0.91, values shown are metarhodopsin optical densities. N; 485nm nomogram.

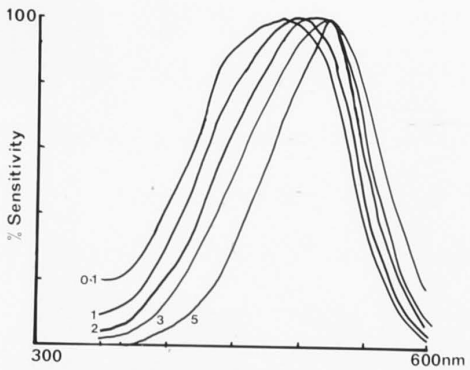
Fig. 3.11 Calculated effects of varying optical densities of retinula cell screening pigment overlying rhodopsin of optical density 0.91.

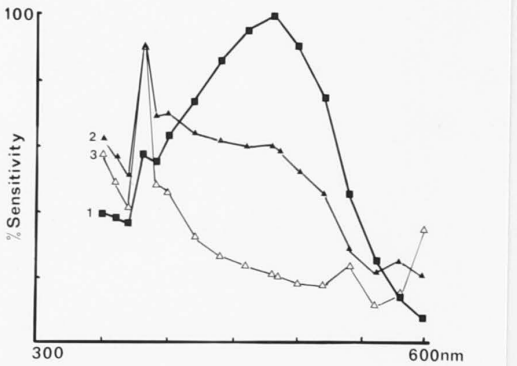
Fig. 3.12 Calculated effects of varying optical densities of red basal pigment overlying rhodopsin of optical density 0.4.

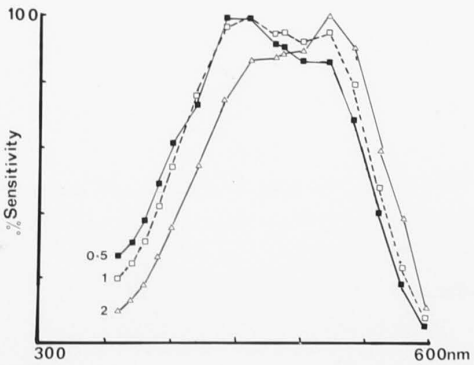
Fig. 3.13 Calculated effects of a combination of varying optical densities of retinula cell screening pigment and metarhodopsin of optical density 0.5 overlying rhodopsin of 0.4.


100
%Sensitivity
300
600nm


100
%Sensitivity
0·9099
0·8
0·5
0·1
N
300
600nm


100
% Sensitivity
0·1
1
2
3
5
300
600nm


100
%Sensitivity
2
3
1
300
600nm


100
%Sensitivity
0·5
1
2
300
600nm

sensitivity curves could probably be produced in several ways, so juggling combinations of pigments might seem to be a rather pointless exercise.

Nevertheless one combination which should reflect a real situation is the effect of increasing optical densities of retinula cell screening pigment on a mixture of rhodopsin and metarhodopsin (Fig. 3.13). With a metarhodopsin optical density of 0.5, the curve produced is much broader than that of either pigment acting in isolation. It has broad maxima at around 440 and 520nm, which change in relative height as the optical density of the retinula cell screening pigment is increased from 0.5 to 2.0. These curves are very similar to the spectral sensitivity curves recorded from cells adapted to dim light. This is the only combination of pigments which has been modelled so far, and it is not necessarily the one naturally operative, but it does offer one possible explanation for spectral sensitivities measured in dim light, which are broader than the dark-adapted spectral sensitivities and show a range of peak sensitivites.

DISCUSSION

Polarisation sensitivity

The most commonly found PS values, between 10 and 11, are similar to those found by Shaw (1969) in *Carcinus* and *Ovalipes*. Theoretical analysis of fused rhabdoms by Shaw (1969) and Snyder (1973) has demonstrated that a high PS is only to be expected, since selective absorption in different rhabdomeres is sufficient to counter-act the effect of self-screening, however long the rhabdom. The PS in such a rhabdom should be

equal to the dichroism of the photopigment in the membrane (Snyder, (1973). Part of the variation in measured values could come from distortions in the alignment of the rhabdom produced by the process of recording, although this can only decrease the PS.

Angular sensitivity

The angular sensitivities of light-adapted crab eyes in the central region of the eye are very similar to those found in many fast diurnal insects, e.g. Musca 2.5° (Scholes 1969), Calliphora R1-6, 1.5° (Hardie 1979), dragonfly 1.3-1.5°, (Laughlin 1975), bee, 2.7° (Laughlin and Horridge 1971). There was little if any difference between dark-adapted angular sensitivities during the day and at night. Light and dark adaptation during the day produced narrowed (to less than 2°) or widened (to 3-4°) angular sensitivities respectively. One cell monitored during dark adaptation at night showed an increasing acceptance angle. It seems fairly safe to conclude that the angular sensitivity changes in response to light levels during both night and day, but, at least in the central area of the retina, the changes take place over a fairly narrow range. No stable cells had an acceptance angle greater than 4° under any conditions. This may imply that Leptograpsus can maintain acuity of vision to a greater extent at night than say, Scylla, in which Leggett (1979) found a change in dark-adapted acceptance angles from 4° in the day to 10° at night. Both crabs have much smaller dark adapted acceptance angles than the crayfish Cherax (Walcott 1974), in which the acceptance angle changes from 3° when light-adapted to 24° when dark-adapted.

The wider angular sensitivities measured in dark adapted eyes could be caused both by an increase in the acceptance angle of the rhabdom tip, and by a decrease in the optical isolation of the retinula cell columns, allowing scattered light to enter the rhabdom. Three pigments are in a position to affect the first factor; the light distal, dark distal, and retinula cell screening pigments. In Chapter II it was found that there is little variation in the position of the light distal pigment under any conditions. The dark distal pigment forms an aperture above the rhabdom tip that is larger at night than in the day, but there was no evidence that the size of this aperture varied in response to light intensity. The retinula cell screening pigment, on the other hand, moves rapidly both radially and longitudinally in response to light, particularly that part of it which is concentrated in the distal part of the cell. In the light adapted state, much of this pigment lies distal to the tip of the rhabdom. There is evidence of its role in the regulation of angular sensitivity in other crustacea (de Bruin and Crisp 1957, Walcott 1974) as well as in insects (Kolb and Autrum 1972). The amount of light scattered within the retina, and the amount of leakage from the retinula cell columns, seems from the anatomy of the eye to be primarily controlled by the reflecting pigment. In the light-adapted eye, this pigment forms a close sleeve around the retinula cell columns, while in a dark-adapted eye at night, the pigment is largely retracted from the distal part of the retina.

The increase in absolute sensitivity (as measured by the amount of light from a 1.4° source needed to produce a constant response) of about 1.5 log units between night and day dark adapted eyes is produced

with very little change in a angular sensitivity. For this reason, although the retraction of the reflecting pigment and the reduced amount of distal retinula cell screening pigment must be considered as causal factors, their effect is unlikely to be a large one. The other possible causes are the increase in size of the distal rhabdom, and the increase in diameter of the aperture formed by the dark distal pigment, as well as the reduction of screening pigment in the bulk of the retinula cell. If the tip of the rhabdom is exposed to a spatially uniform light flux, the amount of light entering the rhabdom will be directly proportional to its cross-sectional area. An increase in rhabdom diameter from 1.7 to 5.2 μm would increase sensitivity by a factor of 9.4, or about one log unit. The actual distribution of light at the cone tip is unknown, so this figure should be treated as a maximum that is unlikely to be achieved. There still remains at least 0.5 log units of sensitivity increase to be accounted for by other means.

Spectral sensitivity

The dark-adapted spectral sensitivity of Leptograpsus can be adequately explained as the product of an unshielded rhodopsin of λmax 485nm. This peak sensitivity is to slightly shorter wavelengths than the 493-508nm range that has been found in the six other crab species in which spectral sensitivity has been tested electrophysiologically.

The measured spectral sensitivities under various adaptation conditions are interpreted here as being due to the interaction of rhodopsin, metarhodopsin, and various screening pigments. However, other factors which might under some circumstances affect the measured spectral sensitivity must be considered.

Perhaps the most obvious of these additional factors is absorption in the cornea. The spectral transmission of some crustacean corneas has been examined by Goldsmith and Fernandez (1968). All corneas showed an absorption band at around 280nm, probably due to tryptophan and tyrosine residues in the cuticle protein. The absorption per micron was similar for all the corneas they examined, the presence or absence of a distinct shoulder at around 350nm depending on the thickness of the cuticle. Leptograpsus, with a cornea c. 40 µm thick in a large crab, whose 43 µm cornea has a very low absorption before 350nm, where it is about 30%, rising slowly towards 300nm and then more steeply. There is therefore likely to be at least some increase in absorption by the cornea below about 350nm in Leptograpsus, which will depress UV sensitivity under all adaptation conditions.

The effect of absorption by the reflecting pigment has not been considered. This pigment looks brown in transmitted light, and its absorption increases steadily towards shorter wavelengths. Kong and Goldsmith (1978) suggest that in the white-eyed crayfish the equivalent pigment can absorb light when it is within a few µm of the rhabdom, shifting the peak sensitivity about 30 µm towards longer wavelengths. In Leptograpsus the reflecting pigment is always well removed from the rhabdom.

Waveguide effects (Snyder and Miller 1972, Snyder and Pask 1973) have not been considered, since even the day rhabdom is large enough to render them relatively unimportant.

Since frequent checks were made to ensure that the stimulus was on-axis, there should not have been any error due to non-axial stimulation, as described by Eguchi (1971) and Snyder and Pask (1972).

The rhabdom of R8 lies over that of R1-7, and it must be exercising some filtering effect. However since it is something less than 5 μm long in Leptograpsus, this should not be at all significant.

The changes in spectral sensitivity that occur in Leptograpsus under the various conditions of light and dark adaptation during the day and night seem to be explicable, at least in broad outline, by the effects of pigment movements, absorption by metarhodopsin, and self-screening, on a rhodopsin of peak absorbance 485nm. These factors can combine to maintain a constant spectral sensitivity over a wide range of ambient light intensities, although it may take about 30 minutes to stabilise after a sudden, large increase in illumination. The light-adapted spectral sensitivity curve is broader than the dark-adapted curve, and the peak sensitivity is shifted, on average, to longer wavelengths, although there is a wide variation between cells. The shift in λmax is similar to, but less pronounced than, the effect produced by the "red-brown screening pigments" in light-adapted crayfish (Kong and Goldsmith 1978, Goldsmith 1978a). In Leptograpsus, it has been demonstrated that the shift is produced by the retinula cell screening pigment.

Broadening of the spectral sensitivity curve, and the maxima at shorter wavelengths, could be achieved by contributions from several other pigments. The effect of metarhodopsin acting as an overlying filter, as it would do to some extent if it was unequally distributed along the rhabdom, was modelled, but no other pigment has yet been examined in combination with the retinular cell screening pigment. The dark distal pigment, with its secondary peak in transmission at around 480nm, could also be a factor,

as could the red basal pigment under fairly high illumination conditions. More analysis is necessary, both of the effect of combinations of pigments, and of the metarhodopsin and its regeneration mechanisms and distribution within the rhabdom.

From the data available on cells adapted to dim light, one cannot say whether the observed variation in spectral sensitivity is always present, or if most of the cells are in the same state at any one time. It is also quite possible that there is less variation in a completely undamaged eye. Although no evidence of two distinct colour types was found in the dim light adapted eye, it must be remembered that the dark distal pigment cells are not symmetrically arranged in the ommatidium, and may introduce a bias in cell R7, to which they are closest.

The only pigment found to have increased transmission in the UV range is the red basal pigment. Calculation of its effects on absorption by the rhodopsin indicates a small increase in sensitivity at 380nm when the pigment is treated as if it were homogeneously mixed with the photopigment, and a very pronounced peak in the same place if it is treated as an overlying screen. It is likely, therefore, to be the cause of the sharp, transient peak around 380nm that accompanies adaptation to bright light. The retinula cell screening pigment could then cause the progressive attenuation of the 380nm peak.

The evidence for the involvement of the red basal pigment, although quite strong, is purely circumstantial. It has the right absorbance characteristics, it moves into a more prominent position in the retina under the appropriate conditions, and no other possible cause presents itself.

However, the position of the red basal pigment, if it really is as described in Chapter II, is still rather far removed from the rhabdom, and it is hard to see, assuming conventional ideas about light paths within the eye, how the effect produced is so large. Freeze-substitution or freeze-etch techniques should now be used to determine the position of pigments in these various states with more certainty.

In spite of this, it is reasonable to conclude that the optical properties of the pigments, and the response of pigment-containing cells to light, can in principle provide sufficient explanation for the observed variation in spectral sensitivity of Leptograpsus retinula cells R1-7. It can also be concluded that this variation cannot reasonably be supposed to provide the input necessary for colour vision.

CHAPTER IV

THE RETINA-LAMINA PROJECTION IN THE CRAB LEPTOGRAPSUS VARIEGATUS

... where silver-haired, bald-headed gentlemen ...
move among spectroscopic experiments ... and apparatus
for slicing into fractional millimetres the left eye
of the female mosquito.

-"Kim"

SUMMARY

The projection of retinula cell axons to the lamina was investigated by tracing them through a series of semi-thin sections. Forty-four such axons were traced from a single group of ommatidia as far as the distal layers of the lamina. The eight receptor axons of one ommatidium project to a single lamina cartridge. Therefore, because the crab has a fused rhabdom, angular information is conserved in vision, and the outside world is projected literally onto the lamina, just as it is in the standard non-dipteran pattern of insects. The belief of previous workers that other decapod eyes show neural superposition was an inference based primarily on the patterns of penetration of the basement membrane by receptor axons, and on degeneration experiments. This evidence is reviewed, shown to be inadequate and discussed in the light of the projection now demonstrated for Leptograpsus.

INTRODUCTION

In insects with fused rhabdoms all 7-9 receptor axons within one ommatidium have a common optical axis, and their axons enter a single lamina cartridge. In Diptera, which have open rhabdoms, each ommatidium contains

retinula cells with diverging optical axes, and the receptor axons from cells sharing a given optical axis converge from their different ommatidia to enter the same lamina cartridge (Kirschfeld 1967). Benacus and Notonecta (Hemiptera), also with open rhabdoms, are the only non-dipterans in which the receptor axons are known to disperse as they enter the lamina, although the retina-lamina projection has not been determined in detail (Meinertzhagen, 1976). Our ignorance of decapod retina-lamina projections contrasts with the extensive work in this area in insects (e.g. Trujillo-Cenóz and Melamed, 1966; Braitenburg, 1967; Horridge and Meinertzhagen, 1970; Ribi, 1975; Sommer and Wehner, 1975; Meinertzhagen 1976).

Since research on decapod vision has reached the stage where the synaptology of the lamina is being examined (Hámori and Horridge, 1966b; Hafner, 1974; Nässel and Waterman, 1977), similar information is greatly needed. The elucidation of the decapod projection pattern has lagged behind mainly because of morphological peculiarities which hamper axon tracing. The axons are long (0.5 to 1mm) and in the lamina are less than two microns in diameter. After penetrating the basement membrane, retinula cell axons from up to thirty ommatidia form large bundles of fibres which cross a wide haemocoelic sinus (upwards of 0.5mm in Leptograpsus), before diverging to to enter the lamina cartridges in groups of eight. Proximal to the basement membrane and again in the distal part of the lamina, the receptor axons bend and twist irregularly.

Three previous interpretations of the retina-lamina projection in decapods have suggested that receptor axons from one ommatidium enter several lamina cartridges. Hámori and Horridge (1966a, c) argued from degeneration experiments and from crossing of receptor axons visible in the distal lamina of Homarus. The reports of Meyer-Rochow (1975, Panulirus) and Nässel (1976,

Pacifastacus) were based on the penetration pattern of axons through the basement membrane and their arrangement immediately below it, together with the work of Hámori and Horridge. In contrast to the situation in the fly, no satisfactory explanation has been proposed for the supposed neural superposition in the decapod Crustacea.

In this study, retinula cell axons of the common shore crab, Leptograpsus variegatus, have been traced through a series of semi-thin serial sections as far as the distal layers of the first optic ganglion, the lamina.

METHODS

Optic lobes were dissected out and fixed with the cornea still attached, to keep the large fibre bundles as straight as possible. The tissue was fixed in 2.5% gluteraldehyde in 50% sea-water for 2-3 hours, post-fixed in 2% osmium tetroxide in 50% sea-water for 3 hours, dehydrated in an alcohol series, and embedded in soft Araldite. The method of Ribi (1976) was used, first sectioning in the horizontal or vertical plane at 200µm, removing radial segments extending from the retina as far as the lamina in which complete fibre bundles could be seen, then re-embedding in hard Araldite. Tangential sections were then cut with glass knives at 1-2µm thickness and stained with toluidine blue. Series which appeared on first inspection to contain a minimum of axons undergoing sudden lateral movements, or buckling of the whole fibre bundle, were photographed using a Zeiss photoscope with Kodak Panatomic X film. Tracing was done on the photographs by numbering the axons with coloured inks. The loss of one section in a series was often enough to prevent further tracing, and fifteen series were made before one was found in which a sufficiently large number of axons could be followed into the lamina.

RESULTS

Leptograpsus is a large, common shore crab which reaches up to about 200 gms in weight, and whose eyes are 2-4mm in diameter. Each ommatidium contains seven large retinula cells which contribute to a fused, banded rhabdom some 250μm long and a smaller eighth cell which forms the distal tip of the rhabdom (Fig. 4.1A). With one exception, this arrangement is common to all decapods so far examined (Parker, 1897; Eguchi, 1965; Kunze, 1967; Nässel, 1976); in Panulirus, however, the proximal part of the adult rhabdom is anomalous (Eguchi and Waterman, 1966; Meyer-Rochow, 1975). Receptor axons from the seven large retinula cells (R1-7) end within the lamina, while the axon of R8 ends in the external medulla (Stowe et al. 1977). The retinula cells have a constant arrangement within the ommatidia, which is mirror-symmetrical about the equatorial line. The cells may be identified by reference to the positions of the processes of the crystalline cone cells (Fig. 4.1B). In light microscope sections these processes are not usually visible, but the retinula cells can still be readily identified, if the position above or below the equatorial line is known, from the location of R7 along the axis of the horizontal rows of ommatidia (Fig. 4.2). Retinula cells pass through the basement membrane in the pattern described for Astacus (Parker 1897) Ocypode (Kunze 1967), and Pacifastacus (Nässel 1976): axons from one ommatidium go through four holes in the basement membrane, sharing each hole with axons from a neighbouring ommatidium (Figs. 4.1C, 4.2). The pattern in Panulirus is slightly different in that R7 penetrates the basement membrane separately.

Directly below the basement membrane, axons may come together for a short distance in fascicles of eight, formed by the convergence of axons from horizontally adjacent holes (Figs. 4.1D, 4.2). This is unlike the situation in Pacifastacus (Nässel, 1976) where vertically adjacent groups come together (Fig. 4.2). The fascicles are formed from three ommatidia in

Fig. 4.1a Schematic diagram of the path of retinula cell axons in Leptograpsus. Co cornea; CC crystalline cone; R1-7, R8 Retinula cells; Rh rhabdom; BM basement membrane; LF large fibre bundle; CBL cell body layers of the lamina; PL plexiform (cartridge) layer of the lamina.

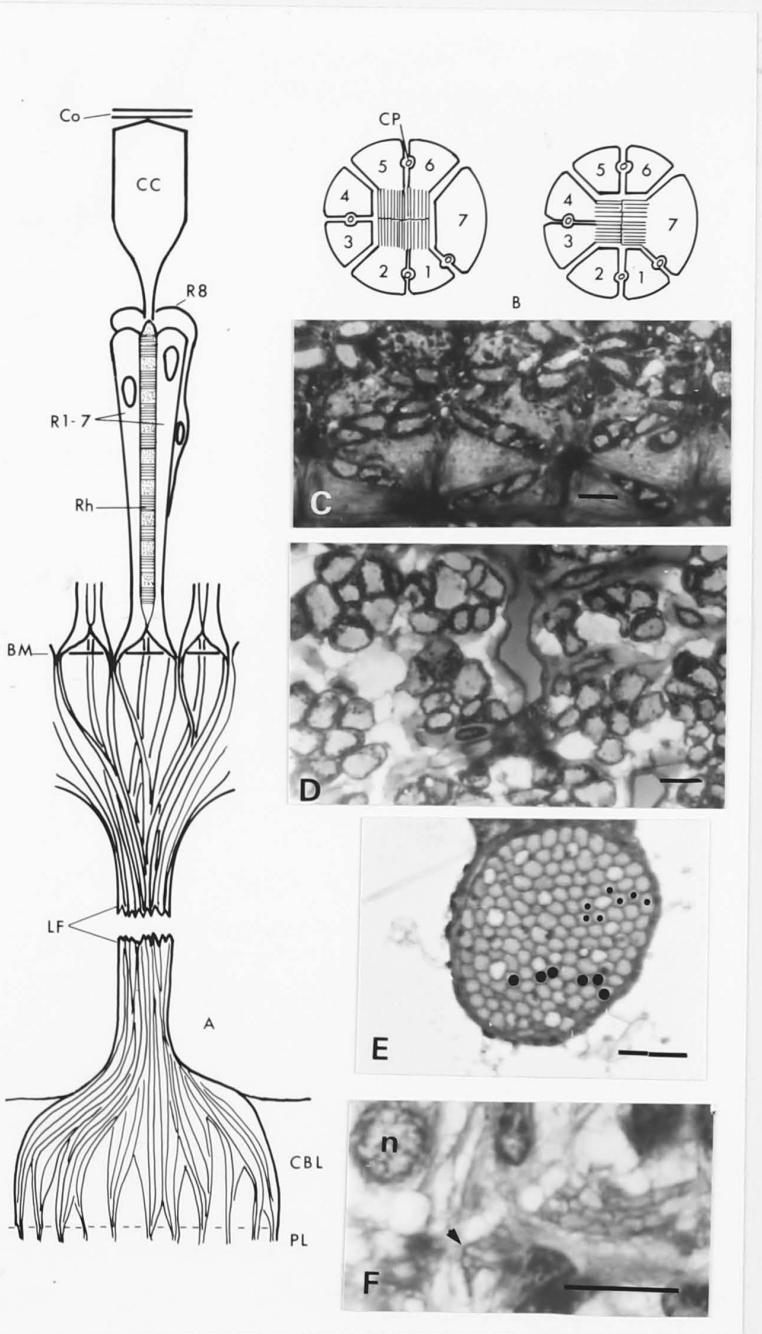
1b Cross-sections of an ommatidium at two levels to show the orientation of the microvilli contributed by R1-7. CP, crystalline cone cell processes.

1c Cross-section at the level of the basement membrane showing the pattern of receptor axons.

1d Fascicles of eight axons formed below the basement membrane.

1e Section through a large fibre bundle showing axons from one ommatidium grouped together (small dots) and from another, scattered (large dots).

1f Section of the lamina distal to the plexiform layer, showing a group of eight axons (arrows) which has diverged from the larger group above and right. This group contains axons from the five black cells shown in Fig. 2. On the left (η) is the nucleus of a lamina monopolar cell. Scale in C-F, 10μm.


Co
CC
R8
R1-7
Rh
BM
LF
A
CBL
PL
CP
5
6
4
7
3
2
1
B
C
D
E
n
F

each case, but in Leptograpsus the distribution is generally R1 from one ommatidium, R2/R3 from another, and R4/R5/R6/R7/R8 from a third, while in Pascifastacus it is (R2/R3), (R4/R5), (R6/R7/R8/R1). In Leptograpsus the fascicles of eight are often not distinct, and within 6-8μm form loosely associated horizontal rows separated by lacunae. Axons from up to 30 ommatidia then form large bundles which cross the haemocoelic sinus to the lamina. Near the edges of the "catchment areas" of the bundles below the basement membrane, axons often twist abruptly to rejoin others of their own ommatidium, although this may mean that they are separated from some of the axons they had been associated with in the fascicles of eight. Within the large fibre bundles, axons from one ommatidium show some tendency to remain together, but may also drift, say, half-way across the bundle before regrouping (Fig. 4.1E). The R8 axon at this stage has approximately the same diameter as the axons of R1-7 (mainly 3-4μm, but ranging from 2 to 6μm). Each axon varies to some extent in diameter and intensity of staining over its course, perhaps because of erratic penetration of the fixative. The axon of R7 is often slightly larger, but this is by no means consistent. After entering the lamina, the fibre bundles divide four or five times successively until groups of eight axons are formed. In this region axons are 0.5 to 1.5μm in diameter.

From one group of fifty ommatidia, of 400 axons, forty-four axons were traced into the lamina almost to the cartridge layer, five as far as a group of eight axons, the remaining thirty-nine to four small groups of axons a few microns more distal. The great majority of axons were lost either in the region proximal to the basement membrane as they enter the large fibre bundles or in the lamina as the fibre bundles dispersed. The forty-four axons came from nine neighbouring ommatidia, and the observed

destinations of these fibres should be reliable, since a) differences in diameter and in intensity of staining aided identification of axons from one photograph to another, b) a mistake in identifying one axon was soon obvious because the relationships with its neighbours appeared disturbed, c) the first 100μm of this series was traced twice, using two series of photographs with 30 ommatidia or 240 axons in common, and only two axons were differently labelled, d) in case of doubt, an axon was abandoned.

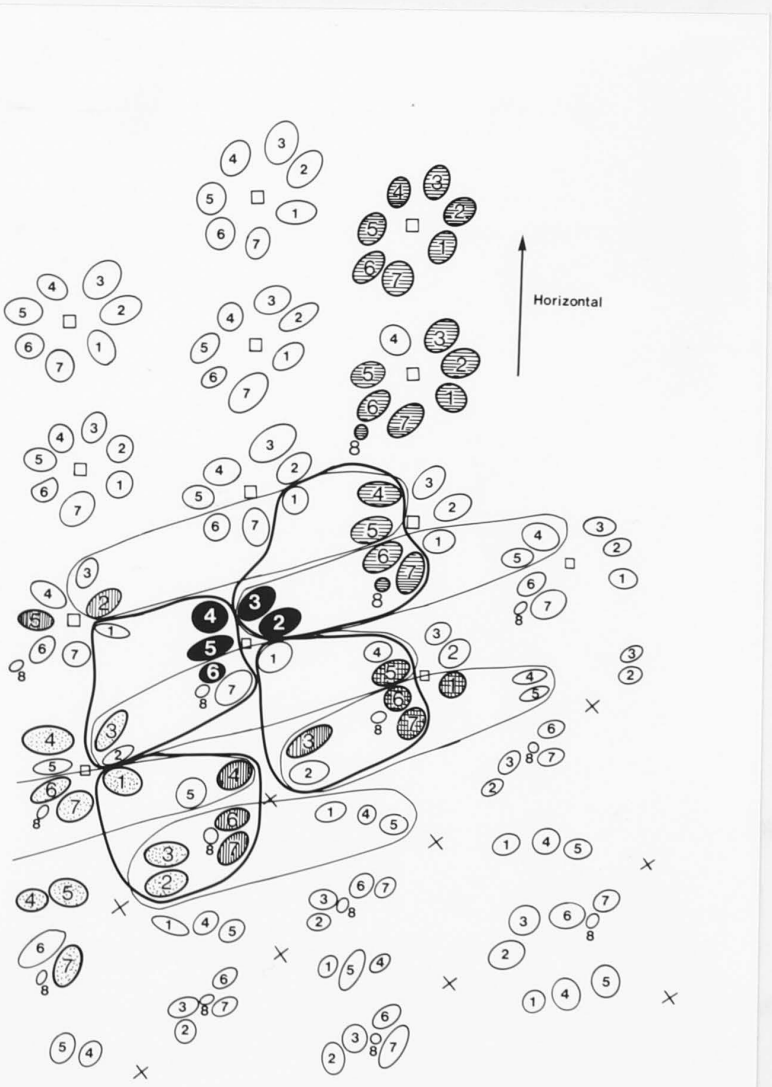
Fig. 4 shows the final groupings of the receptor axons superimposed on their position in the ommatidia above the basement membrane, together with the distribution immediately below the basement membrane. Also indicated are the groupings which should appear according to the scheme proposed for the crayfish (Nässel, 1976). It will be seen that the observed distribution is incompatible with both these schemes. On the other hand, in no case does an axon appear in a different group from others of its parent ommatidium.

Because of the difficulty of tracing receptor axons in the decapod visual system this result lacks the completeness and clarity of some of the demonstrations of the insect projection. Nevertheless, the evidence is sufficient to show that in the crab, receptor axons from one ommatidium project to a single lamina cartridge.

Irregularities

Along the equatorial line separating the upper and lower halves of the retina, a few rows of ommatidia are smaller, and frequently only five or six of the usual seven large retinula cells are present. In these circumstances the individual retinula cells could not be identified, although they penetrated the basement membrane through the usual four holes. Their subsequent path is not known, since the only series in which they appeared was abandoned before the lamina was reached. Kunze (1968) also describes irregularities occurring in the same region of transition in Ocypode.

Position of retinula cell axons in a slightly oblique section including the bases of the ommatidia, towards the top, and axons diverging as they go through the basement membrane at the bottom. The axons which were traced into the lamina are shown. Solid black, horizontal and vertical stripes, hatching and stippling denote the groups to which these axons were traced in the lamina. , Rhabdom; X, projection of the rhabdom position. Thick lines show fascicles formed below the basement membrane in the crab. Thin lines surround the fascicles which would be found according to the pattern in the crayfish.


Horizontal

Apart from this region, only one irregularity was found in tracing many hundreds of ommatidia across the basement membrane. In this case, the axon of R7 passed through the same hole as R1, instead of remaining with R6. R8, which normally passes through with R6 and R7, was not identified. R7 remained close to R1, and when all the axons of that ommatidium drew closer together as the large fibre bundles were formed, the pattern could not be distinguished from that of the surrounding axons.

In the series which was traced well into the lamina, the large fibre bundle split into two as it crossed the haemocoelic sinus. The fibres being traced were concentrated in one half of the large bundle and remained in one bundle after the split, with the exception of one R7 which was some distance from the others of its ommatidium at the time and remained in the other bundle. This bundle entered the lamina very near to the one being traced. As the two bundles diverged they interdigitated in one area, and some of the smaller bundles from each were seen to fuse. They were not followed far enough into the lamina to tell if axons from different bundles entered the same cartridge. However this can sometimes be seen in reduced silver sections of both Leptograpsus and Scylla (Stowe et al. 1977). A large bundle splitting, and axons merging from different bundles (rather than simply interdigitating) are both infrequent events, and their appearance in the same series suggests that the crossings in the lamina were correcting errors made when the large bundle split.

DISCUSSION

Threshold measurements of the optokinetic response of Leptograpsus to a moving sinusoidal grating have shown that a spatial wavelength of 3 to 4 degrees can be resolved (Dvorak and Sandeman, unpublished results). This is approximately twice the interommatidial angle (Sandeman 1978, implying

that the optokinetic system must have access to information from individual ommatidia. The organisation of the crab lamina (Stowe et. al. 1977), suggests that neural superposition of the type which has been proposed for other decapods would not preclude the abstraction of input from single ommatidia, either from the eighth retinula cell or by a lamina monopolar cell summing only those inputs to a cartridge which come from one ommatidium. But acuity measurements are at least compatible with the direct pattern of projection demonstrated here.

Given that the retina-lamina projection in the crab is from one ommatidium to one lamina cartridge, and that the pattern of axons below the basement membrane is very similar to that of other decapods, two questions are raised. Firstly, can this result be extended to the eyes of other decapods and secondly, what is the reason for the regular and widespread arrangement of the retinula cells as they traverse the basement membrane?

Regarding the projection in other decapods (Hámori and Horridge 1966a, c). first suggested that neural superposition was present in the lobster Homarus. They observed crossing of receptor axons in the outer layers of the lamina and also performed degeneration experiments. Crossing of receptor axons in the cell body layers of the lamina is sometimes seen in crabs. As we have seen, it is mostly caused by the interdigitation of the fields of the large fibre bundles, the occasional convergence of fibres from two bundles into one group probably being due to the rectification of errors made more distally. Crossing of fibres in itself, without knowledge of the source of the axons involved, provides little relevant evidence. Degeneration experiments, on the other hand, could provide cogent evidence for neural superposition. In the experiments of Hámori and Horridge (1966c) a large area of the retina was sliced off above the basement membrane. Degenerating retinula cell terminals were found in the lamina. Around the

edges of the affected area lamina cartridges were seen which contained both normal and degenerating retinula cell terminals. For neural superposition to be deduced from this evidence alone, partially destroyed ommatidia would have to be completely absent. This point is critical to the argument and was not demonstrated; from the nature of the experiment it is unlikely to be true.

Neural superposition has been suggested in the crayfish Pacifastacus (Nässel 1976) on the basis of the distribution of the retinula cell axons just below the basement membrane, together with the lack of any subsequent crossing of axons. However, the re-arrangement of axons from the superposition to the one-to-one pattern would not involve any crossing of axons, any more than does the initial formation of the sub-basement membrane pattern. In crabs, fascicles of eight axons are also formed below the basement membrane, but the axons return to single ommatidium groups before entering the lamina cartridges. Over most of the distance between the basement membrane and the lamina, neither type of grouping can be easily recognised. While the crab results have no direct bearing on the type of retinula-lamina wiring present in the crayfish, they do demonstrate that the pattern at the level of the lamina cartridges cannot be inferred from that at the basement membrane. Although crabs have apposition eyes while crayfish, like most other decapods, have superposition eyes, an optical superposition eye is not correlated with neural superposition in insects. In insects neural superposition is found in those species with an open rhabdom where retinula cells with the same optical axis, though they are from different ommatidia, converge on to the same lamina cartridge. The advantage of distributing axons sharing a fused rhabdom, that is with the same optical axis, over several lamina cartridges is not at all clear. The distribution suggested by Nässel does not group the lamina inputs according to shared properties, since cells one to eight would still be present in each cartridge.

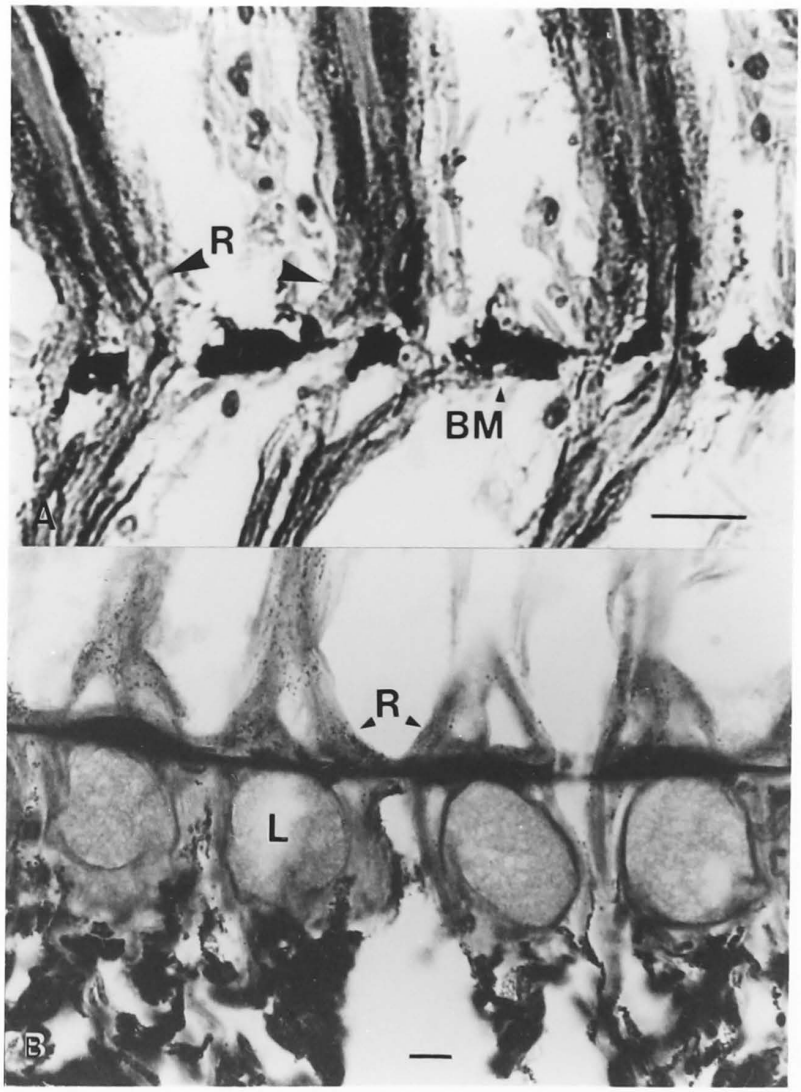
If crabs had a very different type of retina-lamina projection from other decapods, it might be expected that large differences would also be seen in the structure of the lamina. In fact, the types of lamina neurons present in those species so far examined (crayfish Procambarus Hafner, 1973; prawn Pandalus Nässel, 1975; crayfish Pacifastacus, lobster Nephrops Nässel 1977; crab Scylla, Stowe et al. 1977) are extraordinarily similar.

This forces us to consider other possible explanations for the divergence of receptor axons at the basement membrane. In some Crustacea, such as the isopod Ligia (Edwards 1969), and the stomatopod Squilla mantis (Schiff and Gervasio, 1969; Schönenberger, 1977), receptor axons from one ommatidium come together and penetrate the basement membrane through the same hold. In Squilla woodmasonii however, axons from one ommatidium can be seen to diverge and go through several holes in the basement membrane, although they do not share them with axons from neighbouring ommatidia (pers. comm. G. Kelly, Fig. 4.3A). This suggests that the reason for the divergence at the basement membrane is unrelated to the subsequent course of the axons.

The most detailed account available of the basement membrane in decapods is given by Krebs (1972) for the crayfish Astacus. It is shown to be a complex system of "foot cells", fibril-synthesizing cells, the fibrous layer itself, and haemocyanin-filled lacunae lined by thin wall-cells. Two foot cells are found proximal to the rhabdom of each ommatidium. They bear worm-shaped processes interdigitating with the bases of the retinula cells and form numerous gap-like junctions with them. Krebs suggests that the foot cells are glial, and that their close relationship with the retinula cells and the haemocyanin-infiltrated fabrillar layer may imply a respiratory and nutritive function. Since the foot cells are directly beneath the rhabdom they may force the retinula cells to diverge. The appearance of fascicles of eight axons from three ommatidia may then be due to the haemocyanin-filled

Fig. 4.3a Retinula cell axons passing through the basement membrane in Squilla woodmasonii (Photo courtesy G. Kelly) R, Retinula cells; BM Basement membrane. Scale 10μm.

3b Retinula cell axons passing through the basement membrane in Ocypode ceratophthalma. R, Retinula cells; L, haemocyanin-filled lacunae. Scale 10μm.


R
BM
A
R
L
B

lacunae preventing axons from one ommatidium regrouping immediately. The disruption caused by these lacunae is very obvious in Ocypode ceratophthalma where they are particularly large (Fig. 4.3B).

The basement membrane penetration pattern in decapods appears constant, apart from the minor variation shown by Panulirus. Axons from R1, R2/R3, R4/R5 and R6/R7/R8 go through separate holes. This pattern coincides with the distribution of the crystalline cone cell processes, which are among the earliest components of the retina to differentiate (Eloffson, 1969). They reach from the crystalline cone to the basement membrane and their function is unknown, although it has been suggested that they provide structural support for the retinula cells (Boschek 1971). The maintenance of e-vector sensitivity across the retina (Shaw, 1966; Leggett, 1976) requires precise alignment of the rhabdomeres, and this may prove to be achieved by the cone cell processes acting as a guide for the developing retinula cells. If this is so, it is perhaps not surprising that, as the retinula cells diverge at the basement membrane, they retain the grouping into which they have already been divided by the crystalline cone cell processes.

CHAPTER V

THE ORGANISATION OF THE LAMINA GANGLIONARIS OF THE CRABS SCYLLA SERRATA AND LEPTOGRAPSUS VARIEGATUS

SUMMARY

The gross structure and neuronal elements of the first optic ganglion of two crabs, Scylla serrata and Leptograpsus variegatus, are described on the basis of Golgi (selective silver) and reduced silver preparations. Of the eight retinula cells of each ommatidium, seven end within the lamina, while the eighth cell sends a long fibre to the external medulla. Five types of monopolar neurons are described, three types of large tangential fibres, and one fibre which may be centrifugal.

The marked stratification of the lamina is produced by several features. The main synaptic region, the plexiform layer, is divided by a band of tangential fibres; the short retinula fibres end at two levels in the plexiform layer; and two types of monopolar cells have arborisations confined to the distal or proximal parts of the plexiform layer. [The lamina structure of Scylla is remarkably similar to that of other decapods that have been examined.]

INTRODUCTION

Studies on the visual system of the crab are rapidly reaching the stage where the electrophysiological and behavioural results are inadequately supported by anatomical knowledge. Electrophysiological work has been done on the retina, by Goldsmith and Fernandez (1968), Scott and Mote (1974), Shaw (1966, 1969), etc. The output in the optic nerve of decapods has been relatively extensively studied, mainly in crayfish (e.g. Wiersma and Yamaguchi, 1966; Arechiga and Wiersma, 1969; Wiersma and York, 1972),

sometimes in conjunction with the observation of behaviour (Glantz, 1974). Between these extremes, in the four optic ganglia where much processing of visual and other information takes place, very few electrophysiological studies have been done (in crabs, Sandeman, Erber and Kien, 1975; Erber and Sandeman, 1976; Leggett, 1976).

The general morphology of the optic lobes of several decapods has been described by Viallanes (1891), Parker (1897), and others. Hanström (1924) made Golgi studies of many Crustacea. Retinal structure was studied by the early workers and more recently by, for instance, Eguchi and Waterman (1966), Krebs (1972), Kunze (1968) and Rutherford and Horridge (1965).

In the last few years there have been light and electron microscope studies of the first optic ganglion, the lamina, of several decapods, including Homarus (Hamori and Horridge, 1966), Orconectes (Shivers 1967) and Procambarus (Hafner 1973, 1974). Hafner, and also Nässel (Pandalus 1975, Pacifastacus, Nephrops 1976) used Golgi techniques.

Among decapods the crab offers one of the best preparations in which to study visually evoked behaviour, and much is known about its eye movements, which makes it an obvious choice for an anatomical study. This paper describes the neuron types and organisation of the lamina of two species of crab, Scylla serrata and Leptograpsus variegatus, both of which are currently being used for experimental studies.

Materials and Methods

Specimens of the Queensland mud crab Scylla serrata were supplied by the Kamerunga Biological Laboratories, Cairns. Leptograpsus variegatus were collected near Bateman's Bay, on the South-East coast of New South Wales. The optic lobes of over 100 Scylla and 40 Leptograpsus were stained by a variety of selective silver techniques. The methods of Colonnier (1964), Kenyon-Kopsch (from Hanström 1924) and Butler (1971) were used with success, as was Strausfeld and Blest's (1970) modification, using pre-fixation in

Karnovsky's at various pHs between 6.8 and 7.4. The Golgi-Cox method of Ramón-Moliner (1970) was used on Scylla with little success, although this method did stain the rhabdomeres of individual retinula cells. In all cases the tissue was dehydrated in alcohol, and embedded in Araldite. Sections were cut at 50-150μm in the horizontal, vertical, and tangential planes, relative to the centre-line of the eye stalk (Fig. 5.).

The reduced silver methods tried for paraffin sections, were Rowell's (1963) procedure, a modification of the Holmes-Blest method, and Blest's 1976 urea bath technique with a cobalt mordant. Fixation was in aged alcoholic Bouin's or Blest and Davie's (1977) FBP fix. A few series were also stained with Pyronine-Malachite Green (Baker and Williams 1965) and Halmi's aldehyde-fuchsin (from Drury and Wallington, 1967).

Golgi-stained neurons were drawn with the aid of Leitz and Zeiss drawing-tubes, and photographs taken on a Zeiss photoscope with Kodak Panatomic X film.

RESULTS

General Morphology

The surfaces of compound eyes of Scylla and Leptograpsus consist of an array of hexagonal facets with inner circle diameter about 40μm over most of the eye. One of the axes of the array runs horizontally. Below the cornea of each facet lies a crystalline cone 90-120μm long, which in Scylla continues as a crystalline thread 20-30μm long, and in Leptograpsus tapers to a blunt point. Beneath the crystalline cone lies cell 8 with its short rhabdomere. The main part of the rhabdom is made up of the layered rhabdomeres of retinula cells 1-7. The rhabdom ends just above the basement membrane, and the retinula cell axons continue through gaps in the basement membrane to form bundles of 50-200 fibres. These bundles cross a

haemocoelic sinus up to 500μm wide, diverge in the outer layers of the lamina and enter the lamina cartridges in groups of eight fibres.

The lamina is shaped like a shallow canoe, about 200μm deep, with the radius of curvature less in the horizontal than in the vertical plane (Fig. 5.1). The structure of the lamina of Leptograpsus is very similar to that of Scylla, which is described below and pictured in figure 5.2 (A and B).

A fibrous glial sheath surrounds the lamina. It is 10-15μm thick on the distal surface and thinner and less distinct on the proximal surface. Below the distal sheath are two layers of cell bodies: the distal cell body layer which is 20-30μm deep and the proximal cell body layer which is 10-20μm deep. The proximal cell body layer is more regularly arranged, a pair of cell bodies lying above each cartridge. The cartridges of the plexiform layer are 12-15μm apart, 45μm deep, and more distinct in Leptograpsus than in Scylla. A thin layer of tangential fibres, the distal tangential layer, divides the plexiform layer into proximal and distal portions. Below the plexiform layer lies a 40-50μm thick layer of cell bodies (probably mainly glial), blood sinuses, glial cell processes, and tangential fibres, some of which may be up to 10μm thick. Proximal to this is a band of fine tangential fibres and the proximal glial sheath. Axons from a few neighbouring cartridges emerge through the proximal glial sheath in small fascicles which run to the external medulla, crossing in the horizontal plane to form the first optic chiasma.

The two species of crabs reacted very differently to reduced silver techniques. With Fraser Rowell's method the best results were obtained by incubating at 40-50°C, pH 7.2 for Scylla, and 55-60°C, pH 8.6, for Leptograpsus. Leptograpsus gave better results with a 48 hour Holmes-Blest method. However the most successful preparations were of a third species, the xanthid crab Ozius truncatus. (Figs. 5.3E, F).

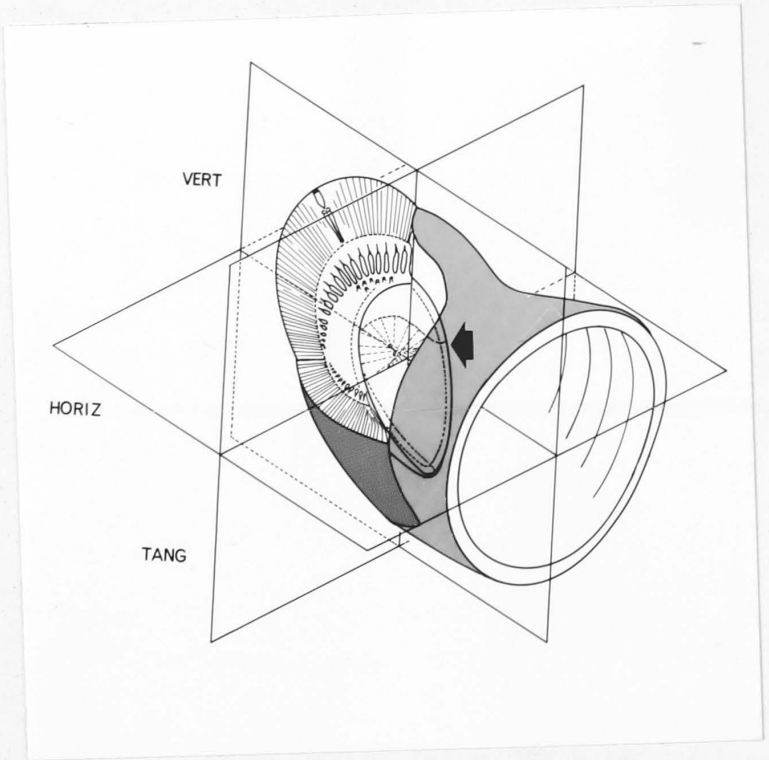


Fig. 5.1 Diagram of the left eye of the Crab Scylla serrata showing the position of the lamina ganglionaris (arrow) within the eyestalk, and the planes in which sections were made, Horiz, horizontal plane; Tang, tangential plane; Vert, vertical plane.

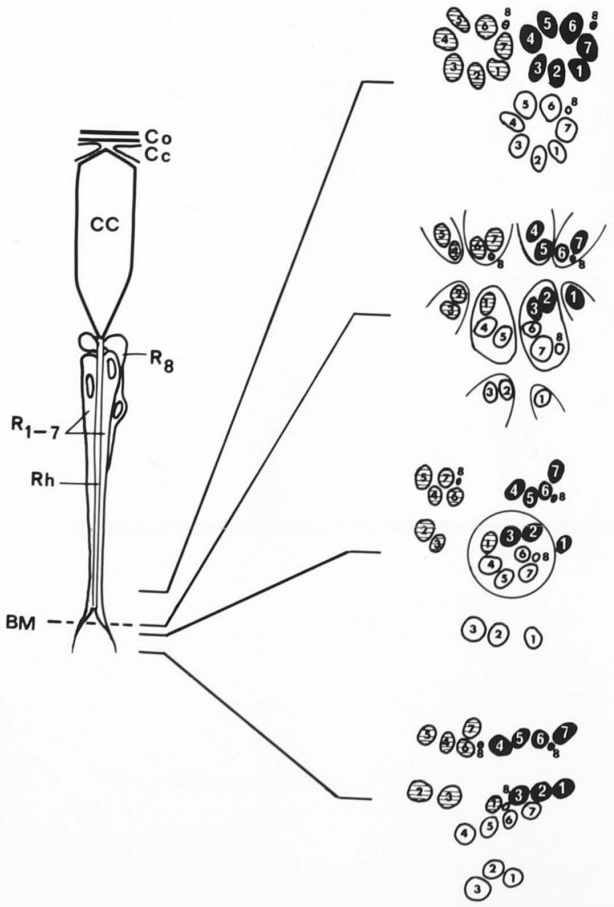
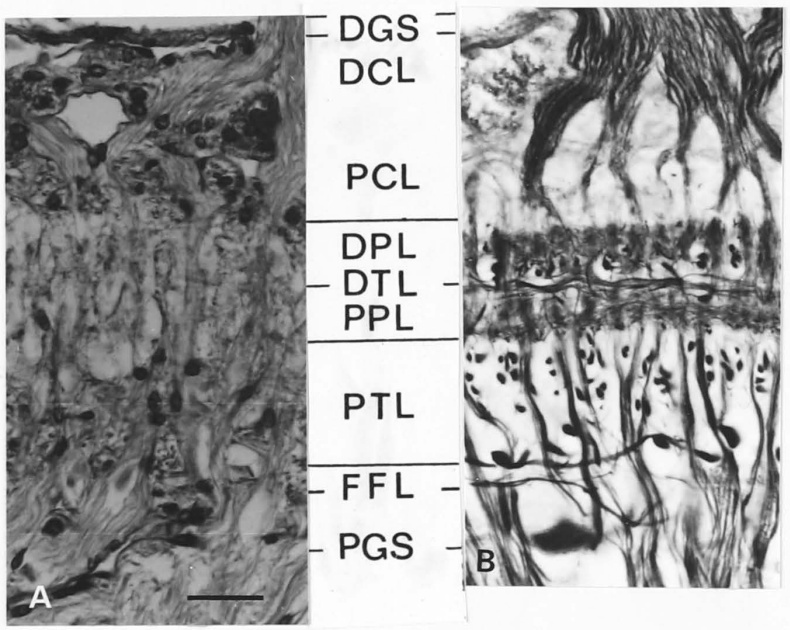


Fig. 5

Fig. 5.2 An ommatidium of Leptograpsus and the arrangement of retinula cells and their axons from three ommatidia at various levels near the basement membrane. The enclosed cluster of cells at the third level (just below the basement membrane) represents the "fascicle of sight" discussed in the text.


DGS
DCL
PCL
DPL
DTL
PPL
PTL
FFL
PGS
A
B

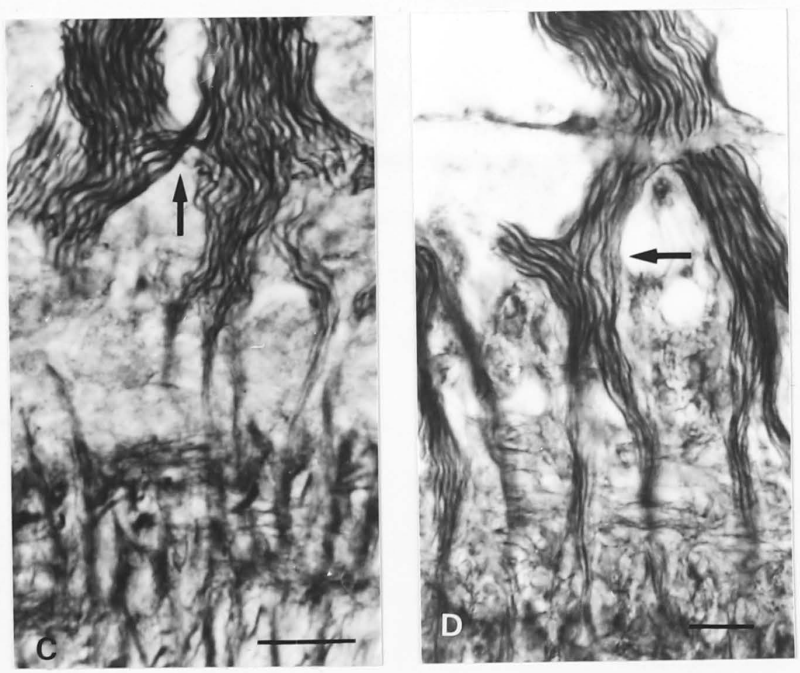

C
D

Fig. 5.3 A and B Horizontal sections of the lamina of Scylla

(A) Halmi's aldehyde-fuchsin stain. Scale 25μm.

(B) Rowell's reduced silver stain. Scale as in A.

DGS, distal glial sheath; DCL, distal cell body layer; PCL, proximal cell body layer; DPL, distal plexiform layer; DTL, distal tangential layer; PPL, proximal plexiform layer; PTL, proximal tangential layer; FFL, fine fibre layer; PGS, proximal glial sheath.

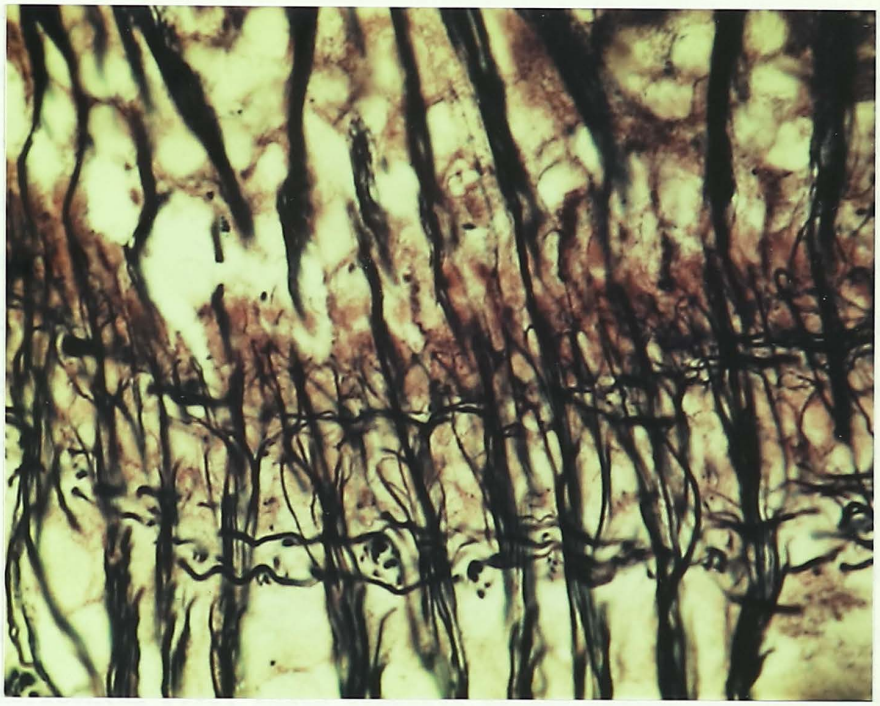
C and D Retinula fibres crossing (arrows) in the lamina of Scylla (C) and Leptograpsus (D). Rowell's reduced silver stain. Scale 15μm in C. 20μm in D.

(next page)

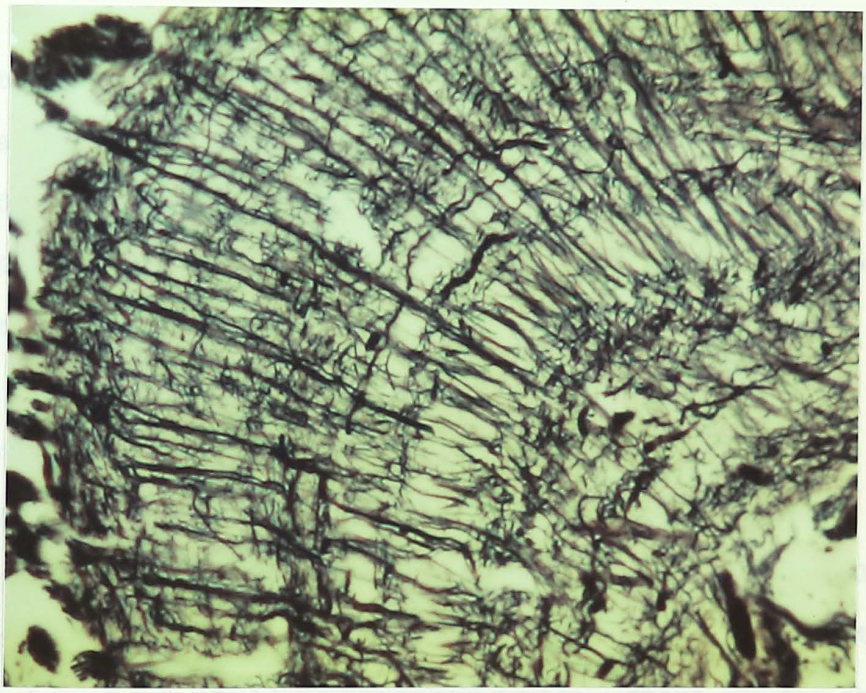
E The lamina of Ozius truncatus, Holmes-Blest method 1100x.

F Ozius external medulla, Holmes-Blest method 670x.

E.



F.



Halmi's stain shows numerous small cell bodies beneath the lamina, interspersed among the fibres of the chiasma.

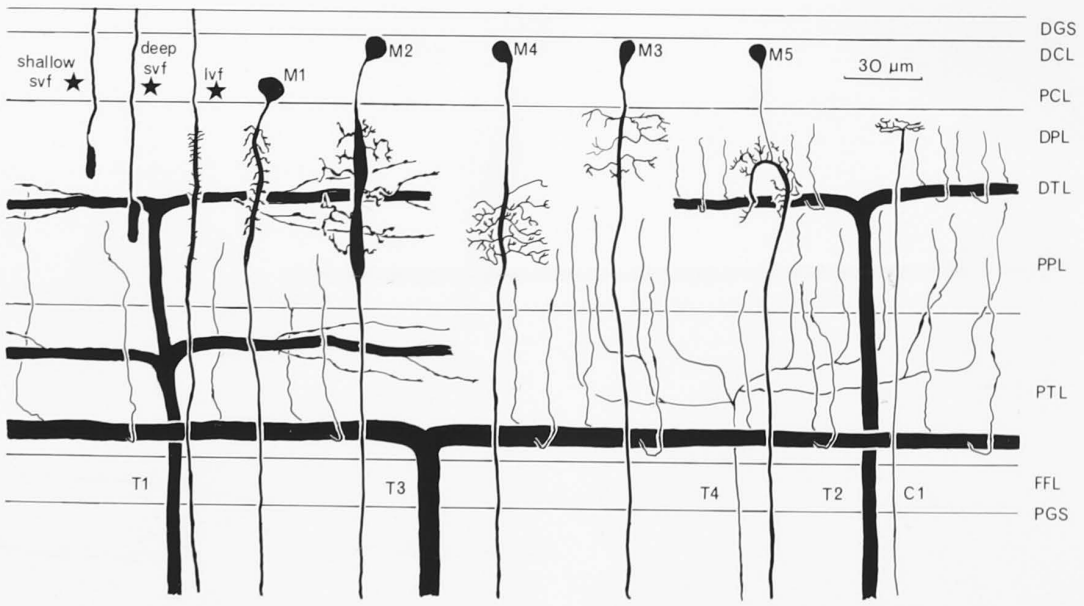
The Pattern of Retinula Cell Axons at the Basement Membrane.

The penetration pattern of axons through the basement membrane in Scylla and Leptograpsus resembles that reported for several decapods (Astacus, Parker, 1897, Krebs 1972; Palinurus, Viallanes, 1892; Ocypode, Kunze, 1967; Pacifastacus, Nässel, 1976), although this does not seem to be the general pattern for all Crustacea (cf. Ligia, Edwards 1969; Squilla, Schiff and Gervasio, 1969). Axons from one ommatidium go through four holes in the basement membrane, each hole enclosing axons from two ommatidia. Mirror-image patterns are found in the dorsal and ventral portions of the retina, as described by Kunze (1968) in Ocypode, with cell 1 always towards the mid-line. Just below the basement membrane eight axons from three ommatidia appear briefly in fascicles. Below this, blood sinuses separate the axons into double horizontal rows, which coalesce as the fibres draw together to form large bundles of up to 200 axons, within which no arrangement is discernible.

In both Scylla and Leptograpsus, reduced silver staining sometimes shows retinula cell fibres crossing as the bundles diverge to enter the lamina cartridges (Fig. 5.3C and D). During tracing of Leptograpsus retinula cell fibres with the aid of 2µm toluidine blue sections, fascicles of eight fibres below the basement membrane were twice seen to diverge, so that their fibres joined different large bundles. Axons from one ommatidium were always seen to run in the same large bundles.

Retinula cells in the lamina

In reduced silver preparations of both animals, eight retinula cell axons can be seen entering each cartridge. Golgi staining shows these to be of three types, two of which end in the lamina. These are the shallow short visual fibres in the distal plexiform layer, and the deep short visual


shallow svf
deep svf
lvf
M1
M2
M4
M3
M5
30 µm
DGS
DCL
PCL
DPL
DTL
PPL
PTL
FFL
PGS
T1
T3
T4
T2
C1

Fig

Fig. 5.4 Retinula fibres and lamina cell types from Leptograpsus(*) and Scylla.

lvf, long visual fibres; svf, short visual fibres; M1-5, monopolar neurons; T1-4, tangential neurons; C1, centrifugal neuron; DGS, distal glial sheath; DCL, distal cell body layer; PCL, proximal cell body layer; DPL, distal plexiform layer; DTL, distal tangential layer; PPL, proximal plexiform layer; PTL, proximal tangential layer; FFL, fine fibre layer; PGS, proximal glial sheath.

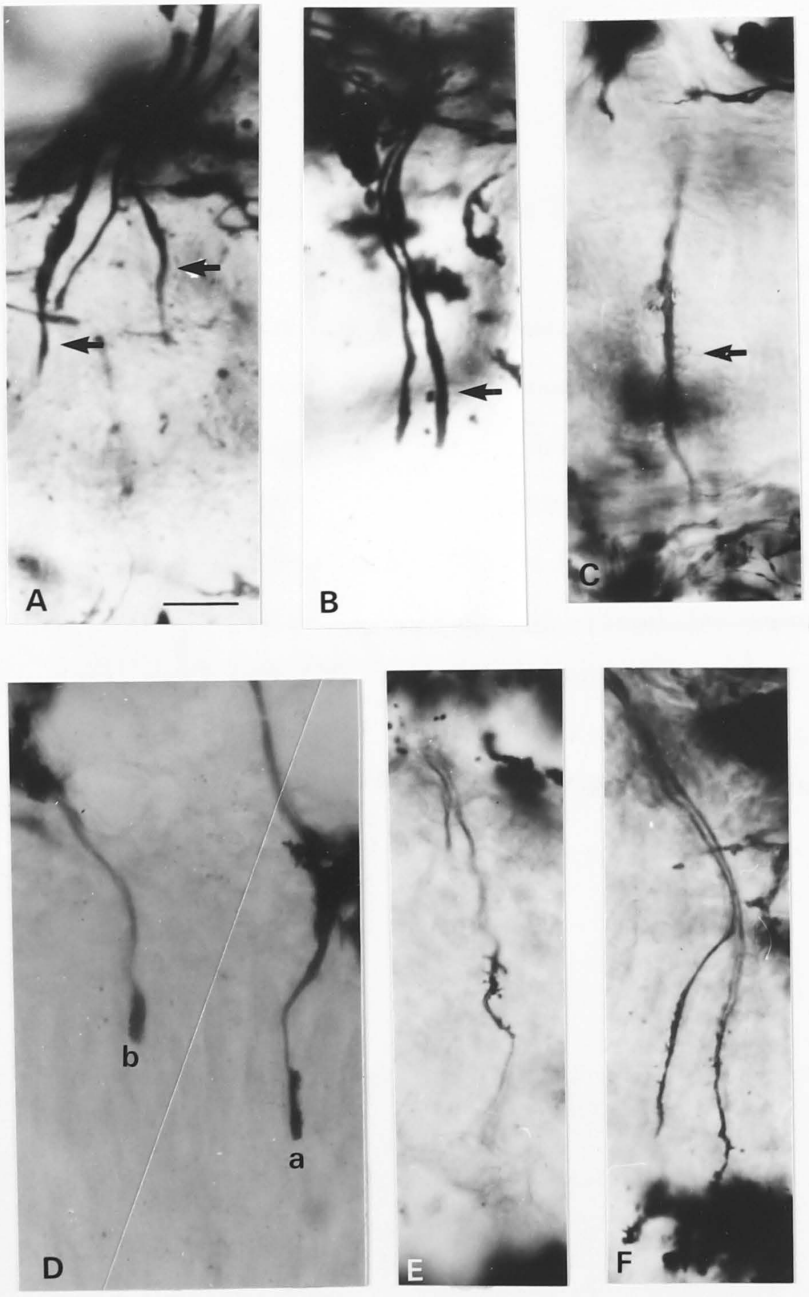


Fig. 5.5

Fig. 5.5 Retinula cell fibres in the lamina of Leptograpsus and Scylla. Golgi preparations. Scale 20µm shown in (A).

A Shallow short visual fibres (arrows) of Scylla.

B Deep short visual fibres (arrow) of Scylla.

C Long visual fibres of Scylla. Arrow indicates the processes in the proximal plexiform layer.

D Deep (a) and shallow (b) short visual fibres of Leptograpsus.

E, F Long visual fibres of Leptograpsus.

fibres in the proximal plexiform layer. The third type, the long visual fibres, passed through the lamina to end in the external medulla (Figs. 5.4 and 5.5). The short visual fibres are the extensions of retinula cells 1-7, while the long visual fibre is from cell 8. In Scylla Golgi material, short visual fibres appear to taper to a blunt point from a diameter of 2-3µm (Figs5.5A and B). The long visual fibres of Scylla are 2-3µm thick in the lamina and have lateral processes of up to 5µm, mostly in the proximal plexiform layer (Fig. 5.5C). In Leptograpsus the short visual fibres are about 2µm in diameter, narrow to 1µm in the plexiform layer and end in sac-like terminals up to 5µm in diameter (Fig.5.5D). The long visual fibre of Leptograpsus is thin (1.5µm) and bears small processes, 1-2µm long, in the plexiform layer (Figs5.7E and F). It has a slightly enlarged, club-like ending in the external medulla.

Lamina Monopolar Cells

Five types of monopolar cells could be distinguished in Scylla (Figs. 5.4 and 5.6), of which four correspond fairly closely with those described in Pandalus, Pacifastacus, and Nephrops (Nässel, 1975, 1976).

Monopolar neuron M1 has a cell body, 8-10µm in diameter, in the proximal cell body layer. A thin neck (ca 1.5µm) extends for about 8µm from the soma. This expands to 3µm in diameter as it enters the plexiform layer, where it gives off short branching processes up to 5µm long.

Monopolar neuron M2 has a cell body in the distal cell body layer. The central fibre is very often swollen to 3-5µm in the plexiform layer. Branched, varicose dendrites extend radially up to 10-15µm throughout the whole depth of the plexiform layer.

M3 and M4 monopolar neurons both have somata in the distal cell body layer and diffuse ramifications extending up to 15µm, the former in the distal, the latter in the proximal, plexiform layer.

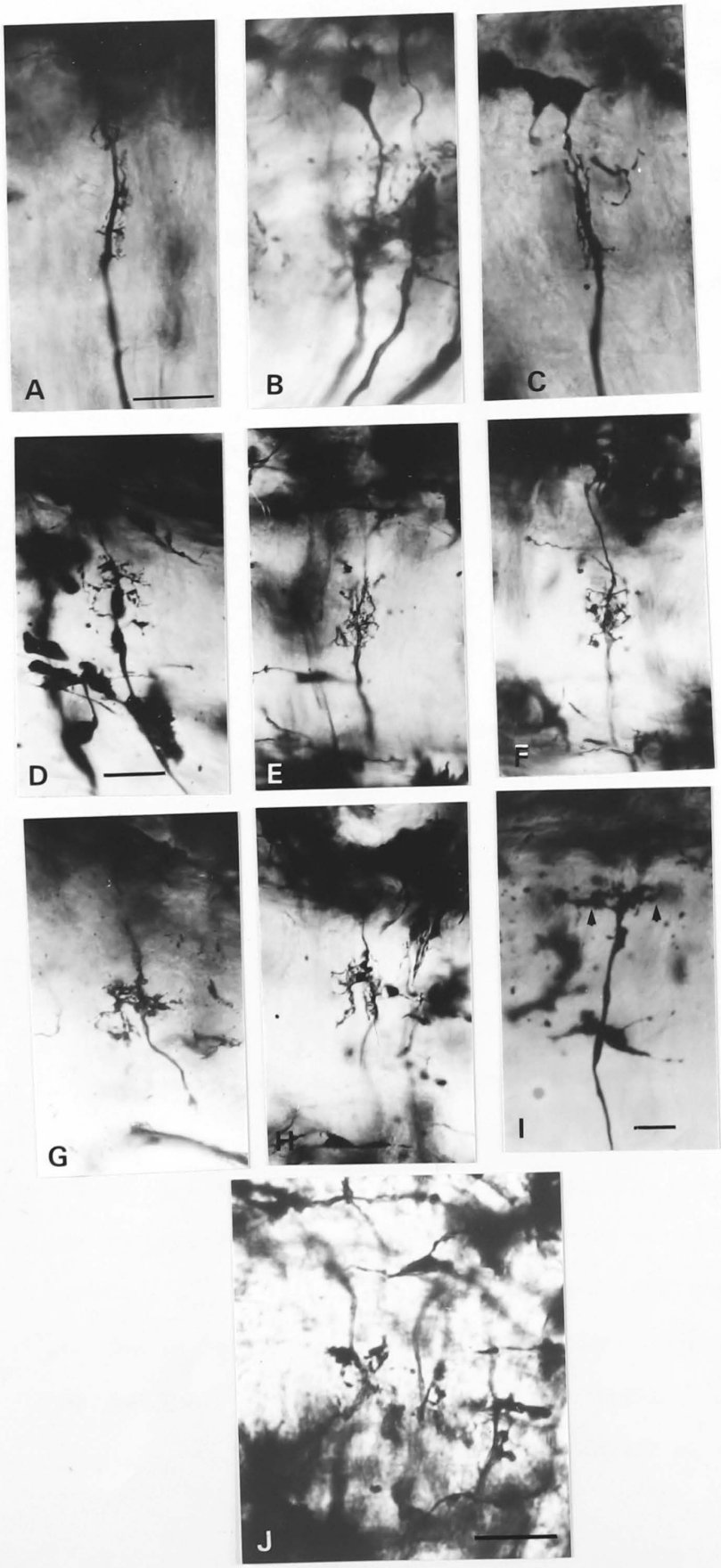

A
B
C
D
E
F
G
H
I
J

Fig. 5.6 Monopolar and centrifugal cells in the lamina of Scylla. Golgi preparations.

A,B,C M1 monopolar neurons. Scale, shown in A, 20µm.

D M2 monopolar Neuron. Scale 20µm.

E,F M3 monopolar neurons, branching in the distal plexiform layer. Scale as in D.

G,H M5 monopolar neurons. Scale as in D.

I C1 centrifugal neuron. Arrows show lateral extent of arborisation. Scale 10µm.

J M4 monopolar neurons, branching in the proximal plexiform layer. Scale 20µm.

M5 monopolar neuron does not resemble any described by Nässel (1975, 1976), or Hafner (1973). It has the appearance of a shepherd's crook. A thin fibre runs to the cell body from the top of the loop, which bears dendrites 5-10μm long. The ascending and descending limbs of the "crook" are about 7μm apart.

The axons of all the monopolar cells join the first chiasma and enter the external medulla.

Centrifugal neurons

In Scylla, a neuron is sometimes seen with an axon entering the lamina from the first chiasma, and mapped by a flat, disc-shaped arborisation. This extends for 20-30μm in the extreme distal portion of the plexiform layer (Fig.5.5 I). It has been classified as centrifugal (C1) only on anatomical grounds on the basis of its similarity to "centrifugal" neurons described in the lamina of insects, (reviewed by Strausfeld 1970).

Tangential neurons

There are two main layers of tangential neurons in the lamina (Figs. 5.4 and 5.7). The distal one lies in the middle of the plexiform layer, dividing it into proximal and distal parts. The proximal tangential layer forms a broad diffuse band between the plexiform layer and the glial sheath, with the largest fibres mainly on the proximal edge of the band. The large fibres (5-10μm diameter) run vertically or horizontally (Fig. 5.7A). Three types of large tangential fibres have been identified in Scylla.

T1 neurons (Fig. 5.7D) have 5-7μm thick primary branches in both the proximal and distal tangential layers. The secondary branches tend to form acute angles with the direction of the primary fibres.

T2 neurons (Fig. 5.7, E and F), also with primary branches 5-7μm thick, run in the distal tangential layer. The secondary branches are given off initially at right angles, then twist, usually in a distal direction, to run parallel to the cartridges.

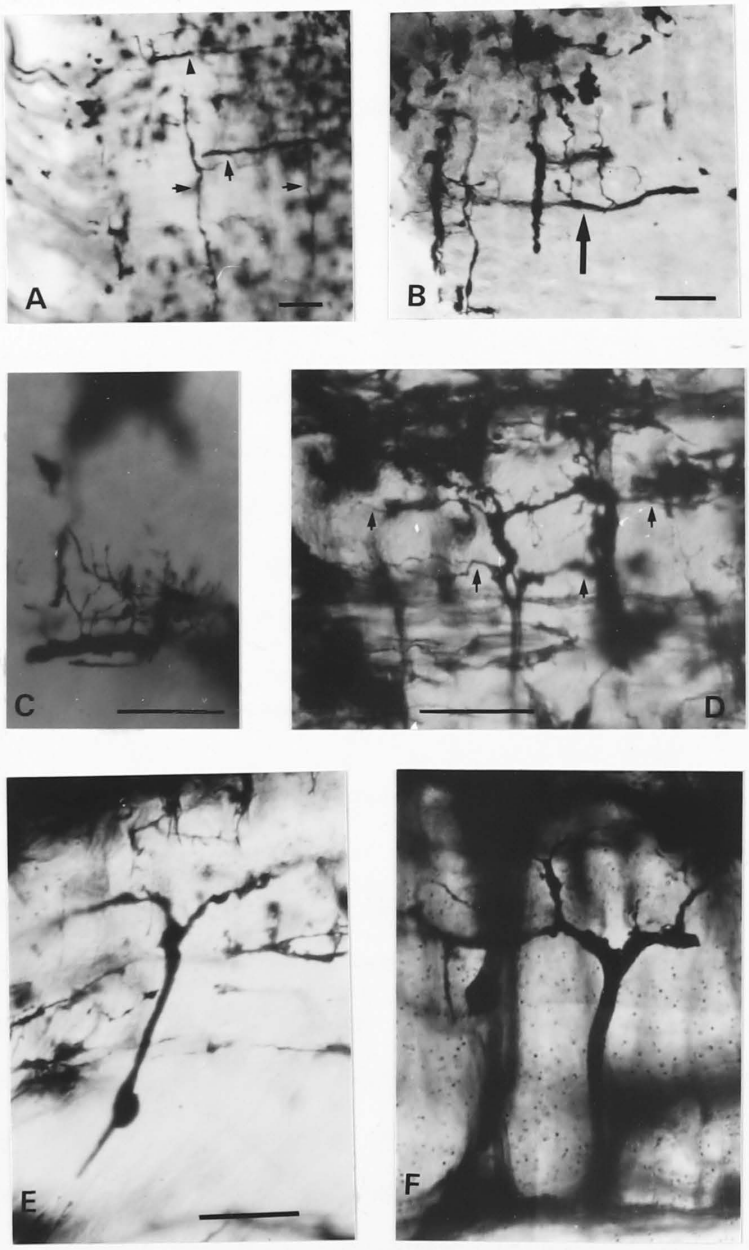


Fig.

Fig. 5.7 Tangential neurons in the lamina of Scylla. Golgi preparations.

A Tangential section through the lamina, showing two horizontal and two vertical tangential fibres (arrows). Scale 100µm.

B Part of a T3 neuron (arrow) showing secondary branches initially running at right angles to the main branch. Tangential section. Scale 50µm.

C Part of a large T3 neuron. Vertical section. Scale 50µm.

D Part of a T1 neuron, entering the lamina from below and sending branches (arrowheads) into both distal and proximal tangential layers. Vertical section. Scale 50µm.

E T2 neuron branching in the distal tangential layer (arrows). Vertical section. Scale 50µm.

F Bifurcation of a T2 neuron in the distal tangential layer. Scale 20µm.

T3 neurons (Fig. 5.7C) are in some respects similar to T2 neurons, but the primary branches are thicker (7-10μm) and run in the proximal tangential layer, mainly vertically. The primary branch of one of these neurons may be long enough to cover most of the vertical extent of the lamina.

There are several types of fine tangential neurons in the lamina some have cell bodies in or below the ganglion. The only one of this class which stained regularly and completely enough for positive identification was called T4. This has an axon which enters the lamina from the first chiasma, then branches to form a diffuse ramification distal to the large T3 neurons of the proximal layer. Its dendrites extend into the lower half of the plexiform layer, over an area with a diameter of about 160μm.

The axons of the tangential fibres T1, T2 and T3 may be seen, in reduced silver sections, to cross the first chiasma together with the monopolar neurons and enter the external medulla. The cell bodies of the large tangential neurons were not found.

DISCUSSION

The architecture of the crab lamina, as shown by Scylla and Leptograpsus, is very similar to those of the prawn Pandalus (Nässel, 1975), the crayfish Procambarus (Hafner, 1973) and Pacifastacus, and the lobster Nephrops (Nässel 1977), both in its gross organisation and in the types of retinula, monopolar, and tangential cells present. The pronounced stratification of the lamina makes it comparable to the A-type lamina (Strausfeld 1976) among insects. Short retinula fibres end at two levels in the lamina, showing a functionally unproved but anatomically striking correlation with the branching patterns of M3 and M4 monopolars. Perhaps the most likely reason for this division is the separation of incoming information according to polarisation plane. In Pandalus, Nässel (1976) demonstrated that four photoreceptors terminate in the distal plexiform

layer, while three end in the proximal plexiform layer. The identity of the photoreceptors was not determined, but the numbers in each layer do correspond to the numbers of photoreceptors in an ommatidium with vertical and horizontal polarisation sensitivity. It was further recported by Nässel and Waterman (1977) that M3 is postsynaptic to the receptors in the distal plexiform layer, and M4 to the receptors in the proximal plexiform layer. M2 is postsynaptic to all seven photoreceptors, and may provide a polarisation-independent channel for R1-7. There are of course other possibilities, such as a division according to spectral class, although it is doubtful, as discussed in Chapter III, if R1-7 of decapods have different spectral sensitivities. In bees, polarisation sensitivity is now generally considered to be due mainly to the long visual fibres of retinula cell 9 (Menzel and Snyder, 1974; Ribi, 1975), but the long visual fibre of decapods does not necessarily fulfil this function. Polarisation sensitivity of cells 1-7 is high in the crab, in many cases up to 9:1 (Shaw, 1966). Shaw (1969) reported a small percentage of retinula cells which showed four low peaks per 360° of polaroid rotation. This corresponds with what might be expected from the anatomy of retinula cell 8, which in the crab has orthogonal microvilli (Eguchi and Waterman, 1973), and forms the long visual fibre. It is therefore likely that cell 8 is transmitting relatively polarisation-independent information in the crab. In Pacifastacus however, Nässel (1976) reports that the microvilli of the eighth cell are oriented only in the horizontal direction.

Monopolar neurons M1-4 of Scylla may well be homologous with M1-4 of Pandalus (Nässel 1975) Pacifastacus and Nephrops (Nässel, 1977). M1 of Scylla usually bears more processes than M1 of Pandalus, although this is variable. Occasionally neurons similar to M1 except for a finer, more diffuse branching pattern have been observed, but it is not certain whether these constitute a distinct type. M2 of Scylla is very similar to M2 of Pandalus, both probably being the central fibre of a cartridge. Both have a distal

cell body, thick axis fibre, and dendrites extending in both halves of the plexiform layer, although the width of the arborisation of M2 of Scylla is slightly greater than that of Pandalus. M3 and M4 in both species have distal cell bodies and dendrites confined to the distal and proximal parts of the plexiform layer respectively. Neuron B-5 of Procambarus (Hafner, 1973) strongly resembles M3, although no equivalent to M4 has been discovered in this species. M5 has not been described before in Crustacea, although it may well be functionally analogous to the wide-field monopolar neurons with a unilateral branching pattern found by Hafner (1973) in Procambarus.

Neuron C1 of Scylla, which may be centrifugal, bears some resemblance to a cell (no. 10) described by Hanström (1924) in Palinurus, although it is less diffuse. It is similar but not equivalent to the neuron C1 in Pacifastacus (Nässel, 1977), and to neuron C2 in the fly (Strausfeld, 1970), lacking the proximal dendrites.

Only one monopolar cell, M1, is unmistakably confined to a single cartridge. M2 may extend into immediately adjacent cartridges, as do dendrites of neurons M3 and M4. M5 has a restricted, unilateral branching pattern.

The crab lamina possesses the structural basis for treating information from the retina in a variety of ways before delivering it to the external medulla. The diversity of spatial integration alone which may take place in the lamina is evident if one considers that the external medulla may receive information from areas ranging from a single ommatidium (via the long visual fibres) to a large proportion of the visual field (via the large tangential fibres).

CONCLUSION

Leptograpsus is active both at night and in the day, sometimes in very bright sunlight. Its eye is well adapted to function over a wide range of illumination conditions, using many strategies for keeping a high acuity and a roughly constant spectral sensitivity.

Two other crabs that are normally active at rather low light levels, Libinia, which lives in deep water, and Ocypode, (Nässel and Waterman 1979, in prep), have rhabdoms that are enlarged proximally, as if they were tending towards a crayfish-like superposition type of eye, although their optics have not been closely examined. In Ocypode, the proximal enlargement evidently only appears at night. Leptograpsus, in contrast, widens the distal end of the rhabdom at night. This means that it is maximising the amount of light captured at each facet, rather than increasing the overall aperture of the eye.

The screening pigment systems, apart from the dark and light distal pigments, are largely regulated in response to the level of ambient illumination. To see in more detail how pigment movements are affecting visual responses, in particular spectral sensitivity, it will be necessary to examine the theoretical effects of more combinations of pigments, and also to gain a more exact knowledge of the pigment positions under various circumstances.

Retinula cells R1-7 in Leptograpsus apparently contain only one type of photopigment, and despite the observed variation in spectral sensitivity of cells adapted to dim light, it is difficult to see how they

could provide the necessary input for colour vision. In crayfish, it has been suggested that R1-7 in each ommatidium consist of two different spectral classes (Nosaki, 1969, Waterman and Fernandez 1970, Eguchi et al. 1973). There is evidence against this in the crayfish, and it is not consistent with the study of Leptograpsus spectral sensitivity reported here. It is possible, although unlikely, that scattered ommatidia or single retinula cells have a different spectral sensitivity, as has been suggested for Scylla (Leggett 1979). But if crabs and other decapods do have colour vision, and it works on the same principle throughout the group, then there are a number of pieces of evidence which point to R8 being a short wavelength receptor:

a) There seems to be a gross structural difference between the long and short wavelength systems, since

   (i) The short wavelength component of the ERG is always small, and is masked by the long wavelength sensitive component except under special conditions of selective adaptation.

   (ii) There is a difference in the form of the long and short wavelength sensitive ERG (Wald 1968)

   (iii) The yellow/green and blue sensitive inputs to sustaining units have different latencies in the crayfish (Treviño and Larimer 1970)

b) The blue-violet receptor is either smaller or much scarcer than the longer wavelength receptor since

(i) A blue-sensitive photopigment has never been found in macruran decapod eye extracts, but a small quantity could easily be masked by photoproducts absorbing at short wavelengths.

(ii) A short wavelength absorbing pigment has never been found by msp of the rhabdom of R1-7, although some hundreds of rhabdoms have now been examined (discussed by Goldsmith 1978b). R8 has never been examined by msp.

(iii) Intracellular recordings of blue-violet receptors have only been reported in crayfish, and only by one group (Nosaki 1969, Waterman and Fernandez 1970). They have not been found by other workers in the field, although it must be admitted that reliable intracellularly determined spectral sensitivities of Crustacea are not common. Waterman and Fernandez reported that blue-violet receptors were held for shorter times than the yellow orange receptors. It is possible that they were recording from R8, but more probable, since peak sensitivites were apparently rather labile, that the cells were unstable and their absolute sensitivity was fluctuating.

c) The little that is known of R8 is consistent with it being a short wavelength receptor;

(i) It is generally much smaller than R1-7. There are exceptions, such as in the crab Grapsus, where R8 contributes 20% of the rhabdom (Eguchi and Waterman 1973), but these have not been investigated.

(ii) The distal position of R8 means that it is not affected by much of the UV-absorbing shielding pigment.

(iii) Not only the form of the rhabdom of R8, but also the connections made by its axon, are different from R1-7. The axon of R8 continues through the lamina (where it is not certain whether it synapses) and terminates in the external medulla. It may be relevant that the UV receptors in insects are often the long visual fibres (reviewed by Wehner 1976).

(iv) In the lobster Homarus it has not been possible to isolate a blue-sensitive ERG component. R8 in this species, although present, has been described as rudimentary (Rutherford and Horridge 1965).

Obviously, the problem cannot really be solved without recording from identified R8 cells.

Since many of the features of the retina are concerned with the maintenance of a high acuity, is this also a priority in the organisation of the optic lobe?

The direct projection of retinula cell axons from one ommatidium to one lamina cartridge means that there is no inevitable loss of resolution at this level. Indeed, if the M2, M3, and M4 monopolar neurons of the lamina have the same connection pattern as their homologues in the crayfish (Nässel and Waterman 1977) it is likely that the external medulla receives information from each ommatidium that is pooled according to the direction of polarisation sensitivity, and also pooled input from all R1-7, with no loss of spatial resolution.

The columnar organisation of the neuropil is maintained beyond the lamina into the external and internal medullas, and is not lost until the terminal medulla is reached. Since the anatomy shows many small-field units connecting the external and internal medullas, and the internal and terminal medullas (pers. obs.) it seems likely that the precise spatial localisation of visual input is retained through at least three stages of processing.

REFERENCES

Aréchiga, H. (1978). Modulation of Visual Input in the Crayfish. In "Identified Neurons and Behaviour of Arthropods" (ed. G. Hoyle) Plenum, New York, London.

Aréchiga, H., Wiersma, C.A.G. (1969) Circadian rhythm of responsiveness in crayfish visual units. J. Neurobiol. 1, 71-85.

Baker, J.R., Williams, Elizabeth G. (1965) The use of methyl green as a histochemical agent. Quart. J. micr. Sci. 106, 3-13.

Baylor, E.R. and Smith, F.E. (1957) Diurnal migration of planktonic crustaceans. In "Recent Advances in Invertebrate Physiology" (ed. B.T. Scheer) Univ. of Oregon Publ.

Bäuerlein, R. (1969) Morphophysiological Studies on the Visual System of the crab Potamon potamios rhodium - PARISI (Decapoda, Potamonidae). forma et functio, 1, 285-331.

Blest, A.D. (1976) A new method for the reduced silver impregnation of the arthropod central nervous system. Proc. Roy. Soc. Lond. B. 193 199-207.

Blest, A.D. (1978). The rapid synthesis and destruction of photoreceptor membrane by a Dinopid spider: a daily cycle. Proc. R. Soc. Lond. B. 200 463-483.

Blest, A.D. and Davie, P.S. (1977) A new Fixative Solution to Precede the Reduced Silver Impregnation of the Arthropod Central Nervous System Stain Tech. 52 273-276.

Boden, B.P., Kampa, E.M. and Abbott, B.C. (1961) Photoreception of a planktonic crustacean in relation to light penetration in the sea. Progr. in Photobiology, Proc. 3rd int. Congr. on Photobiology, 1960, 189-196.

Boschek, C.B. (1971) On the fine structure of the peripheral retina and lamina ganglionaris of the fly Musca domestica. Z. Zellforsch. microsk, Anat. 118 369-409.

Bowmaker, J.K. (1973) Spectral Sensitivity and Visual Pigment Absorbance. Vis. Res. 13 783-792.

Braitenberg, V. (1967) Patterns of projection in the visual system of the fly. I. Retina-lamina projections. Exp. Brain Res. 3, 271-298.

Briggs, M.H. (1961) Visual Pigment of Grapsoid Crabs Nature (Land) 190 784-786.

Bruno, M.S., Barnes, S.N. and Goldsmith, T.H. (1977) The Visual Pigment and Visual Cycle of the Lobster, Homarus J. comp. Physiol. 120 123-142.

Bruno, M.S. and Goldsmith, T.H. (1974) Rhodopsin of the blue crab Callineates: Evidence for absorption differences in vitra and in vivo. Vis. Res. 14 653-658.

Bruno, M.S., Mote, M.I. Goldsmith, T.H. (1973) Spectral Absorption and Sensitivity Measurements in Single Ommatidia of the Green Crab, Carcinus. J. comp. Physiol. 82 151-163.

Butler, R. (1971) Very rapid selective silver (Golgi) impregnation and embedding of invertebrate nervous tissue. Brain Res. 33, 540-544.

Butenandt, A., Biekert, E., Linzen, B. (1958) Über Ommochrome, XIII. Isolierung und Charakterisierung von Omminen. Hoppe-Seylers Z. physiol. Chem. 312 227-236

Collet, T.S., and Land, M.F.C. (1975). Visual Control of Flight Behaviour in the Hoverfly, Syritta pipiens J. Comp. Physiol. 99 1-66

Colonnier, M. (1964) The tangential organization of the visual cortex. J. Anat. (Lond.) 98, 327-344

Crane, J. (1975) Fiddler Crabs of the World (Ocypodidae: genus Uca) Princeton University Press.

Dartnall, H.J.A. (1953) The Interpretation of Spectral Sensitivity Curves. Brit. Med. Bull. 9 24-30.

de Bruin, G.H.P. and Crisp, D.J.(1957) The influence of pigment migration on vision of higher crustacea. J. exp. Biol. 34 447-463.

Drury, R.A.B., Wallington, E.A.(1967) Carleton's histological technique 4th ed. Oxford Univ. Press.

Edwards, A.S. (1969) The fine structure of the eye of Ligia oceania. Tissue and Cell 1, 217-228.

Eguchi, E.: (1965) Rhabdome structure and receptor potentials in single crayfish retinula cells. J. cell. comp. Physiol. 66, 411-430.

Eguchi, E. (1971) Fine structure and spectral sensitivities of retinula cells in the dorsal sector of compound eyes in the dragonfly Aeschna. Z. vergl. Physiol. 71 201-

Eguchi, E., Waterman, T.H. (1966) Fine structure patterns in crustacean rhabdomes. In: The functional organisation of the compound eye (C.G. Barnard, ed), p. 105-124. Oxford: Pergamon Press.

Eguchi, E., and Waterman, T.H., (1967) Changes in retinal fine structure induced in the crab Libinia by light and dark adaptation. Z. Zellforsch, 79 209-229.

Eguchi, E., Waterman, T.H. (1973) Orthogonal microvillus pattern in the eighth rhabdomere of the rock crab Grapsus. Z. Zellforsch. 137 145-157.

Eguchi, E., Waterman, T.H., Akiyama, J. (1973) Localisation of the violet and yellow receptor cells in the crayfish retinula. J. Gen. Physiol. 62, 355-374.

Elofsson, R. (1969) The development of the compound eyes of Panaeus duorarum (Crustacea: Decapoda) with remarks on the nervous system. Z. Zellforsch. 97, 323-350.

Elofsson, R., and Kauri, T. (1971) The ultrastructure of the chromatophores of Crangon and Pandalus (Crustacea) J. Ultrastr. Res. 36 263-270.

Elofsson, R. and Hallberg, E. (1973) Correlation of ultrastructure and chemical composition of crustacean chromatophore pigment. J. Ultrastruct. Res. 44 421-429.

Erber, J. and Sandeman, D.C. (1976) The Detection of Real and Apparent Motion by the Crab Leptograpsus variegatus II Electrophysiology. J. comp. Physiol. 112 189-197.

Fernandez, H.R. (1965) A survey of the visual pigments of decapod Crustacea of South Florida. Thesis, Univ. of Miami, Coral Gables, Florida. (not sighted).

Fernandez, H.R. (1973) Spectral Sensitivity and Visual Pigment of the Compound Eye of the Galatheid Crab Pleuroncodes planipes Mar. Biol. 20 148-153.

Fuortes, M.G.F., and O'Bryan, P.M. (1972) Generator potentials in invertebrate photoreceptor. In: "Handbook of Sensory Physiology" Vol. VII/2. (ed. M.G.F. Fuortes) Springer, Berlin-Heidelberg-New York.

Glantz, R.M. (1973) Five classes of visual interneurons in the optic nerve of the hermit crab. J. Neurobiol. 4, 301-319.

Glantz, R.M. (1974) Habituation of the motion detectors of the crayfish optic nerve: Their relationship to the visually evoked defense reflex. J. Neurobiol. 5, 489-510.

Goldsmith, T.H. (1978a). The effects of screening pigments on the spectral sensitivity of some crustacea with scotopic (superposition) eyes. Vis. Res. 18 475-482.

Goldsmith, T.H. (1978b). The Spectral Absorption of Crayfish Rhabdoms: Pigment, Photoproduct and pH Sensitivity Vis. Res. 18 463-473.

Goldsmith, T.H. and Bernard, G.D. (1974) The Visual System of Insects. In: 'The Physiology of Insecta' Vol. II, 2nd ed. (ed. M. Rockstein) pp. 165-272. Acad. Press.

Goldsmith, T.H. and Bruno, M.S.C. (1973) Behaviour of rhodopsin and metarhodopsin in isolated rhabdoms of crabs and lobsters. In: Biochemistry and Physiology of visual pigments. (ed. H. Langer) 147-153. Springer, New York.

Goldsmith, T.H., Fernandez, H.R. (1968) Comparative studies of crustacean spectral sensitivity. Z. vergl. Physiol. 60, 156-175.

Goldstein, E.G. and Williams, T.P. (1966) Calculated effects of "screening pigments" Vis. Res. 6, 39-50.

Green, J.P. (1972) Pigmentation of the Eyes of Nebalia bipes. crustaceana, 22 206-207.

Hafner, G.S. (1973) The neural organisation of the lamina ganglionaris in the crayfish: a Golgi and EM study. J. comp. Neurol. 152 255-280.

Hafner, G.S. (1974) The ultrastructure of retinula cell endings in the compound eye of the crayfish. J. Neurocyt. 3, 295-311 (1974).

Hallberg, E. (1977) The Fine Structure of the Compound Eyes of Mysids (Crustacea: Mysidacea). Cell. Tiss. Res. 184, 45-65.

Hamdorf, K., Paulsen, R. and Schwemer, J. (1973). Photoregeneration and Sensitivity Control of Photoreceptors of Invertebrates. In: "Biochemistry and Physiology of Visual Pigments" (ed. H. Langer) 155-166 Springer, New York-Heidelberg-Berlin.

Hámori, J., Horridge, G.A. (1966a) The lobster optic lamina. I. General organisation. J. Cell. Sci. 1, 249-256.

Hámori, J. Horridge, G.A. (1966b) The lobster optic lamina. II. Types of synapse, J. Cell Sci. 1, 257-270.

Hámori, J., Horridge, G.A. (1966c) The lobster optic ganglion. III. Degeneration of retinula cell endings. J. Cell Sci. 1, 271-274.

Hámori, J. Horridge, G.A. (1966d) The lobster optic lamina. IV. Glial cells. J. Cell Sci. 1, 275-280.

Hanström, B. (1924) Untersuchungen über das Gehirn insbesondere die Sehganglien der Crustaceen. Ark. Zool. 161-119.

Hardie, R.C. (1979) Electrophysiological Analysis of Fly Retina. I: Comparative Properties of R1-6 and R7 and 8 J. comp. Physiol. 129 19-33.

Hays, D., and Goldsmith, T.H. (1969) Microspectrophotometry of the Visual Pigment of the Spider Crab Libinia emarginata Z. vergl. Physiol. 65 218-232.

Herrnkind, W.F. (1972). Orientation in shore-living arthropods, especially the sand fiddler crab. In "Behaviour of Marine Animals", Vol. I. (ed. H.E. Winn, and B.L. Olla) Plenum Press, New York.

Horridge, G.A., Meinertzhagen, I.A. (1970) The accuracy of the patterns of connections of the first- and second-order neurons of the visual system of Calliphora. Proc. Roy. Soc. Lond. B. 175, 69-82.

Hyatt, C.W. (1975). Physiological and Behavioural Evidence for Colour Discrimination by Fiddler Crabs (Brachyura, Ocypodidae, genus Uca). In: "Physiological Ecology of Estuarine Organisms" (ed. F.J. Vernberg). U. of S. Carolina Press, Columbia.

Kampa, E.M., Abbot, B.C. and Boden, B.P. (1963) Some Aspects of vision in the Lobster, Homarus vulgaris, in relation to the structure of its eye. J. Mar.biol. Ass. U.K. 39 227-238.

Kennedy, D., and Bruno, M.C. (1961) The spectral sensitivity of crayfish and lobster vision. J. gen. Physiol. 44 1089-1102.

Kirschfeld, K. (1967) Die Projection der optischen Umwelt auf das Raster der Rhabdomere im Komplexauge von Musca. Exp. Brain Res. 3, 248-270.

Kleinholz, L.H. (1936) Crustacean eyestalk hormone and retinal pigment migration. Biol. Bull. 70 159-184.

Kleinholz, L.H. (1976) Crustacean neurosecretary hormones and physiological specificity. Amer. Zool. 16 151-166.

Kolb, G., and Autrum, H. (1972) Die Feinstruktur im Auge der Biene bei Hell- und Dunkel adaptation. J. comp. Physiol. 77 113-125.

Kong, K.L., and Goldsmith, T.H. (1977) Photosensitivity of Retinula Cells in White-Eyed Crayfish (procambarus clarkii) J. comp. Physiol. 122 273-288.

Krebs, W.D. (1972) The fine structure of the retinula of the compound eye of Astacus fluviatilis. Z. Zellforsch. 133, 399-414.

Kunze, P. (1967) Histologische Untersuchungen zum Bau des Auges von Ocypode cursor (Brachyura). Z. Zellforsch. 82, 466-478.

Kunze, P. (1968) Die Orientierung der Retinulazellen im Auge von Ocypode. Z. Zellforsch. 90, 454-462.

Land, M.R. (1976) Superposition images are formed by reflection in the eyes of some oceanic decapod crustacea. Nature (Lond) 263 764-765.

Laughlin, S.B. (1974) Receptors, Interneurons and Integration in an Insect Visual System. Ph.D. Thesis. A.N.U.

Laughlin, S.B. (1975) Receptor function in the Apposition Eye. An Electrophysiological Approach. In "Photoreceptor Optics" (ed. A.W. Snyder and R. Menzel) Springer, Berlin.

Laughlin, S.B. and Horridge, G.A. (1971) Angular sensitivity of the retinula cells of the dark-adapted worker bee Z. vergl. Physiol. 74 329-335.

Leggett, L.M. (1976) Polarised light sensitive interneurons in a swimming crab. Nature 262, 709-711.

Leggett, L.M.W. (1978) Some Specialisations of a Crustacean Eye Ph.D. Thesis, Australian National University.

Ludolph, D., Pagnanelli, D. and Mote, M.I. (1973). Neural control of migration of proximal screening pigment by retinula cells of the swimming crab Callinectes sapidus. Biol. Bull. 145 159-170.

Meinertzhagen, I.A. (1976) The organisation of perpendicular fibre pathways in the insect optic lobe. Phil. Trans. Roy. Soc. Lond. B. 274, 555-596.

Menzel, R. (in press 1979) Spectral Sensitivity and Colour Vision in Invertebrates. In "Handbook of Sensory Physiology. Vol.

Menzel, R., Snyder, A.W. (1974) Polarised light detection in the bee Apis mellifera. J. Comp. Physiol. 88, 247-270.

Meyer-Rochow, V.B. (1975) Larval and adult eye of the western rock lobster (Panulirus longipipes). Cell Tiss. Res. 162 439-457.

Nässel, D.R. (1975) The organisation of the lamina ganglionaris of the prawn, Pandalus borealis (Kroyer). Cell Tiss. Res. 163, 445-464.

Nässel, D.R. (1976) The retina and retinal projection on the lamina ganglionaris of the crayfish Pacifastacus leniusculus. (Dana). J. comp. Neurol. 167, 341-360.

Nässel, D.R. (1977) Types and arrangements of neurons in the crayfish optic lamina Cell Tiss. Res. 179, 45-75.

Nässel, D.R., Waterman, T.H. (1977) Golgi EM evidence for visual information channelling in the crayfish lamina ganglionaris. Brain Res. 130, 556-563.

Nassel, D.R. and Waterman, T.H. (1979 in prep) Massive diurnally modulated photoreceptor membrane turnover in crab light and dark adaptation.

Nosaki, H. (1969) Electrophysiological study of color encoding in the compound eye of the crayfish Procambarus darkii. Z. vergl. Physiol. 64 318-323.

Ohly, K.P. (1975) The neurons of the first synaptic region of the optic neuropile of the firefly, Phausis splendidula L. (Coleoptera). Cell Tiss. Res. 158, 89-109.

Olivo, R.F., and Larsen, M.E., (1978) Brief Exposure to Light Initiates Screening Pigment Migration in Retinula Cells of the Crayfish, Procambarus. J. comp. Physiol. 125 91-96.

Pantin, C.F.A. (1962) Notes on microscopical Technique for Zoologists (2nd ed.). C.U.P.

Parker, G.H. (1897) The retina and optic ganglia in decapods, especially in Astacus. Mitt. Zool. Stat. Neapel. 12, 1-73.

Schönenberger, N. (1977) The fine structure of the compound eye of Squilla mantis (Crustacea, Stomatopoda). Cell Tiss. Res. 176, 205-233.

Scott, S., Mote, M.I. (1974) Spectral sensitivity in some marine crustacea. Vision Res. 14, 659-663.

Shaw, S.R. (1966) Polarised light responses from crab retinula cells. Nature, Lond. 211, 92-93.

Shaw, S.R. (1969) Sense-cell structure and interspecies comparisons of polarised-light absorption in arthropod compound eyes. Vision Res. 9, 1031-1040.

Shivers, R.R. (1967) Fine structure of crayfish optic ganglia. Univ. of Kansas Sci. Bull. 47, 677-733.

Snyder, A.W. (1973) Polarization Sensitivity of Individual Retinula Cells. J. comp. Physiol. 83 331-360.

Snyder, A.W. and Pask, C. (1972) A theory for changes in Spectral Sensitivity Induced by off Axis Light. J. comp. Physiol. 79, 423-427.

Snyder, A.W. and Pask, C. (1973) Waveguide Modes and Light Absorption in Visual Photoreceptors. Vis. Res. 13 2605-2608.

Snyder, A.W. and Miller, W.H. (1972) Fly colour vision. Vis. Res. 12 1389-1396.

Snyder, A.W., Stavenga, D.G. and Laughlin, S.B. (1977) Spatial Information Capacity of Compound Eyes. J. comp. Physiol. 116 183-207.

Sommer, E.W., Wehner, R. (1975) The retina-lamina projection in the visual system of the bee, Apis mellifera. Cell Tiss. Res. 165, 45-61.

Stieve, H.C. (1960) Die spektrale Empfindlich Keitskurve des Auges von Eupagurus bernhardus. Z. vergl. Physiol. 43, 518-525.

Stowe, S.J. (1977) The Retina-Lamina Projection in the crab Leptograpsus variegatus Cell Tiss. Res. 185 515-525.

Stowe, S., Ribi, W.A., Sandeman, D.C. (1977) The organisation of the lamina ganglionaris of the crabs Scylla serrata and Leptograpsus variegatus. Cell Tiss. Res. 178, 517-532.

Strausfeld, N. J. (1976) Atlas of an insect brain. Springer, Berlin-Heidelberg-New York.

Strausfeld, N.J. (1970) Golgi studies on insects. Part II. The optic lobes of Diptera. Phil. Trans. B. 258, 135-223.

Strausfeld, N.J., Blest, A.D. (1970) Golgi studies on insects, Part I. The optic lobes of Lepidoptera. Phil. trans. B. 258, 81-134.

Struwe, G., Hallberg, E., and Elofsson, R., (1975) The Physical and Morphilogical Properties of the Pigment Screen in the Compound Eye of a Shrimp (Crustacea). J. comp. Physiol. 97 257-270.

Treviño, D.L., and Larimer, J.L. (1970). The Responses of One Class of Neurons in the Optic Tract of Crayfish (Procambarus) to Monochromatic Light. Z. vergl. Physiol. 69 139-149.

Trujillo-Cenóz, O., Melamed, J. (1966) Electron microscope observations on the peripheral and intermediate retinas of dipterans. In: The functional organisation of the compound eye (C.G. Bernard, ed.) London: Pergamon Press.

Viallanes, H. (1891) Sur la structure de la lame ganglionnaire des crustacés décapodes. Bul. Soc. Zool. de France XVI.

von Frisch, K. and Kupelwieser, H. (1913) Über den Einfluss der Lichtfarbe auf die phototaktischen Reaktionen niederer Krebse. Biol. Zentr. 33 517-552.

Walcott, B. (1974) Unit studies on Light-adaptation in the Retina of the crayfish, cherax destructor.

Wald, G. (1967) Visual Pigments of crayfish. Nature (Lond) 215, 1131-1133.

Wald, G. (1968) Single and multiple visual systems in arthropods. J. gen. Physiol. 51 125-156.

Waterman, T.H. (1978) The Bridge Between Visual Input and Central Programming in Crustaceans. In "Identified Neurons and Behaviour of Arthropods" (ed. G. Hoyle) Plenum, New York, London.

Waterman, T.H., and Fernandez, H.R. (1970) E-vector and wavelength discrimination by retinular cells of the crayfish Procambarus. Z. vergl. Physiol. 68 134-174.

Wehner, R. (1976) Structure and Function of the Peripheral Pathway in Hymenopterans. In: "Neural Principles in Vision" (ed. F. Zettler and R. Weiler) 280-333. Springer-Berlin-Heidelberg-New York.

Welsh, J.H. (1935) Further evidence of a diurnal rhythm in the movement of pigment cells in the eyes of crustaceans. Biol. Bull. 68 247-255.

Wiersma, C.A.G., Bush, B.M.H., and Waterman, T.H. (1964) Efferent Visual Responses of Contralateral Origin in the Optic Nerve of the Crab Podophthalmus. J. Cell. and Comp. Physiol. 64, 309-326.

Wiersma, C.A.G., Yamaguchi, T. (1966) The neuronal components of the optic nerve of the crayfish as studied by single unit analysis. J. Comp. Neur. 128, 333-358

Wiersma, C.A.G., York, B. (1972) Properties of the seeing fibres in the rock lobster: field structure, habituation, attention and distraction. Vision Res. 12, 627-640.

Woodcock, A.E.R., and Goldsmith, T.H. (1970) Spectral responses of sustaining fibers in the optic tracts of crayfish (Procambarus) Z. vergl. Physiol. 69 117-133.

Zyznar, E.S. and Nicol, J.A.C. (1971). Ocular reflecting pigments of some malacostraca. J. exp. mar. Biol. Ecol. 6 235-248.