AN ELECTROPHYSIOLOGICAL INVESTIGATION OF RETINULAR
CELLS OF THE CRAYFISH PROCAMBARUS CLARKII

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ABSTRACT

An Electrophysiological Investigation of Retinular Cells in the Compound Eye of the Crayfish, *Procambarus clarkii*

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Through each facet of the crayfish compound eye, a cluster (the ommatidium) of seven photoreceptor cells views a narrow angle of the world. Light causes the negative membrane voltage of the photoreceptor cell in the dark to change in a positive direction; this change (the receptor potential) is graded with intensity. The amplitude of the receptor potential is reversibly reduced when sodium is partially replaced by lithium or choline and when sucrose partially replaces sodium chloride. The reversal potential of the receptor potential is approximately +10 mv and is insensitive to changes of extracellular sodium. The amplitude of the receptor potential increases when extracellular calcium is removed. The resting membrane voltage is sensitive to the extracellular potassium concentration. Our experiments, using single electrodes, are largely consistent with the hypothesis that a light-induced increase in membrane conductance to sodium, and perhaps other ions, generates the receptor potential. However, it is paradoxical that the reversal potential of the receptor potential is independent of sodium ion concentration. We have also examined the effects of restricted extracellular space on the measurement of membrane voltage, and conclude that extracellular currents can effectively subtract from the transmembrane voltage in producing the intracellularly measured receptor potential.

The photoreceptor cell is sensitive to the orientation of the electric-vector of plane polarized light. Three of the cells in each ommatidium respond to plane-polarized light best when it is polarized horizontally, and the remainder when the light is polarized vertically. The anatomy of the ommatidium suggests that two orthogonal orientations of the photoreceptive membranes form the basis of the two classes of cells sensitive to polarized light. Using pairs of electrodes, we studied the electrical coupling between pairs of cells in the ommatidium. Cells of the same spectral sensitivity and sensitive to the same orientation of the electric-vector of polarized light are well coupled electrically, and cells having orthogonal polarized-light sensitivities are not coupled. We injected the dyes Procion Yellow and Procion Navy Blue into a pair of cells which were not coupled in the
same ommatidium, and confirmed the orthogonal orientations of the photoreceptive membranes as well as the location of the electrodes in the same ommatidium. Polarized light minimally stimulates a cell when the electric-vector is at right angles to that of light which maximally stimulates the cell. The ratio of intensities which produce identical responses with the minimal and maximal orientations of electric-vector is the polarization sensitivity ratio. We present evidence that the light-induced extracellular currents produced by cells sensitive to one orientation of polarized light can reduce the amplitude of the intracellularly recorded receptor potential of the other class. We propose that this mutual interaction can effectively increase the polarization sensitivity ratio which is measured intracellularly.

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The photoreceptor cells, or retinular cells, of the crayfish compound eye form a layer which begins about half a millimeter beneath the corneal surface of the eye. Each cell body is approximately 150 μ long along the path of the light and 15 μ in diameter. Retinular cells are clustered in groups of seven; each cluster is called an ommatidium (Fig. 1). If one penetrates a crayfish retinular cell with a microelectrode, one can measure an electrical response to light. Light causes the negative voltage (with respect to the outside) of the receptor cell in the dark to change in the positive direction. Increased intensities of light produce increased voltage changes, until a limiting voltage is reached. The response to light, called the receptor potential, is similar to the receptor potentials that have been recorded from other arthropod photoreceptors. Each crayfish retinular cell responds to plane-polarized light best when the electric-vector of the polarized light is oriented in one of two orthogonal directions. There is no evidence for electrical excitability of crayfish retinular cells.

The first of the two parts of this thesis concerns the sensitivity of retinular cells of the crayfish *Procambarus clarkii* to the plane of polarization of light. This part considers the significance of the correlations which can be made among (i) the sensitivity to polarized light, (ii) the anatomy of the retinular cells, and (iii) the electrical
Figure 1.

Schematic diagram of an ommatidium in the crayfish compound eye (not drawn to scale). (A) Longitudinal section, showing two retinular cells on opposite sides of the rhabdom. Light enters through the cornea and is focused onto the rhabdom. (B) Cross sections of two successive rhabdom bands. The seven retinular cells and their microvilli are numbered. There are about 25 such layers or bands of microvilli in the rhabdom.

(After Eguchi, 1965.)
Figure 1
interactions between retinular cells.

The second part concerns the nature of the ionic mechanisms which generate the receptor potential of crayfish retinular cells. Does light cause the ionic permeability of the receptor membrane to change? If so, what ions are involved? The experiments designed to answer these questions were conducted with single microelectrodes on excised eyes of *Procambarus clarkii*. Three series of elegant voltage clamp experiments have been reported for two other invertebrate photoreceptors: those of a related crustacean, the barnacle *Balanus* (Brown, Hagiwara, Koike, and Meech, 1970), and of the ventral eye of *Limulus*, another arthropod (Millecchia and Mauro, 1969a, b; Brown and Lisman, unpublished). It is believed that for both *Balanus* and *Limulus* an increased permeability of the membrane to sodium and perhaps other ions, such as calcium, generates the receptor potential. The *Balanus* and *Limulus* photoreceptors are larger than those of crayfish and may be isolated by dissection in a manner which facilitates study of the properties of the photoreceptor membrane. Crayfish retinular cells, on the contrary, are in close association with each other and with cells containing screening pigment. Some of the experiments described in this thesis demonstrate the effect of limited extracellular space on studying membrane mechanisms of such closely packed structures. By comparing the results from experiments on crayfish eye with those from barnacle and *Limulus* eyes, one may determine if the membrane mechanisms are similar.
PART I:

Introduction

Much natural skylight and reflected light is plane-polarized. Rayleigh scattering, for example, polarizes natural skylight so that its E-vector is at right angles to a line from the sun to the point of scatter. Light reflected from the surface of water is polarized in the plane of the water, whereas light transmitted from water is generally polarized at right angles to that reflected. Although the eyes of many animals cannot discriminate light polarized at different angles, the eyes of many cephalopods and arthropods, including the crayfish compound eye, do potentially permit polarized light discrimination.

A large number of arthropods and molluscs orient themselves with respect to the plane of polarization of light (see Waterman, 1966a). Von Frisch (1950) demonstrated that bees can detect the plane of polarization of skylight, and in fact use the sun as a compass both in locating flowers and in signaling to other bees, even on days when all but a small patch of blue sky is obscured by clouds. Schöne (1963) has shown that mangrove crabs (Goniopsis) leaving the water will walk inland perpendicularly to the shore. If the sun is obscured and the plane of polarization of the skylight is artificially altered, the crabs, like the bees studied by von Frisch, reorient accordingly. Both species also correct for changes in the sun's azimuth, presumably by means of their
Three basic mechanisms have been proposed to account for the behavioural data: (i) that animals analyse changes in the intensity of polarized light reflected from objects in their environment (Baylor and Smith, 1958); (ii) that the dioptric apparatus of the arthropod compound eye, through selective refraction (Stephens, Fingerman and Brown, 1953) and reflection (Baylor and Smith, 1953) is dichroic; and (iii) that the photoreceptors themselves are dichroic (Autrum and Stumpf, 1950).

Behavioral experiments such as those of Smith and Baylor (1960) with *Daphnia*, using a half-wave plate to change the polarization of incident but not reflected light, and of Moody and Parriss (1961) minimizing reflections with *Octopus* proved the first explanation to be insufficient.

There is less evidence against the second explanation, that polarized light is detected by selective transmission of the dioptric apparatus. Waterman found that the drug pilocarpine changed the phototaxis of *Daphnia* from positive to negative, but pilocarpine did not affect the animal's perpendicular orientation to the $E$-vector of a vertical beam of polarized light (Jander and Waterman, 1960; Hazen and Baylor, 1962). Waterman cited this as evidence against the second explanation (Waterman 1966b, 1967), but pilocarpine might well have an effect on phototaxis at neural "locations" beyond those required for orienting to polarized light.
I know of no behavioral experiments on polarized light sensitivity in crayfish. Work on the related decapod Carcinus showed the independence of detection of movement, color, and plane of polarization (Horridge, 1967). This study and that on Goniopsis, the mangrove crab, indicate that mechanisms found physiologically in crayfish might apply to polarized light discrimination in some anatomically similar photoreceptors of arthropods and molluscs.

The surface of a compound eye of the crayfish Procambarus is composed of an ordered array of several thousand facets. Each facet is square. Associated with each facet are a corneal lens and a light pipe called the crystalline cone. Without forming an image, the lens and crystalline cone (the dioptric apparatus) focus light from a narrow angle of view onto a cluster of seven light-sensitive retinular cells, which are cylindrically arranged somewhat like sections of an orange (Fig. 1). One cell (cell 1) is twice the size of the others (Parker, 1891), and is always posterior (Parker, 1897). The entire assembly of dioptric apparatus, retinular cells, and screening pigment behind a single facet constitutes an ommatidium (see Fig. 1). Each retinular cell sends a parallel array of tubular processes, termed microvilli, into the center of its cluster, perpendicular to the optic axis. The microvilli, which are at 45° to the sides of their corneal facet (Waterman and Fernandez, 1970), are stacked in alternate, orthogonally oriented bundles. Because the sides of the facets are at 45° to the horizontal (Parker, 1897), the two
orientations of microvilli are horizontal and perpendicular to the horizontal. The entire closely packed stack of microvilli, termed the rhabdom, is presumably the location of the visual pigment rhodopsin (Eguchi, 1965; Waterman, Fernandez, and Goldsmith, 1969). The two orthogonal orientations of microvilli found in the fused rhabdom of Procambarus are characteristic of the layered rhabdoms of other crustacea (Eguchi and Waterman, 1966; Rutherford and Horridge, 1965) as well as the non-layered fused rhabdom of the honeybee (Goldsmith, 1962). Dipteran retinular cells have parallel microvilli, but the microvilli of each cell are separated from those of other retinular cells, forming a so-called "open" rhabdom ommatidium (Boschek in Kirschfeld, 1969). Cephalopod eyes have a rhabdomeric-microvillar structure; the microvilli of a single cell are parallel (Moody and Parriss, 1961; Zonana, 1961).

Although in electron micrographs arthropod microvilli appear in cross section as a "honeycomb" structure, the microvilli are surrounded by extracellular space. Perrelet and Baumann (1969) demonstrated that there is extracellular space accessible to lanthanum but not ferritin throughout the rhabdom of the honeybee drone in the light. Stell and Ravitz (1970) showed that the space surrounding Limulus microvilli is accessible to microperoxidase, which is larger than lanthanum. At the base of the microvilli is an extracellular space which Perrelet and Baumann found is accessible to ferritin as well as to lanthanum. A similar space at the base
of the microvilli has also been seen in lobster (Rutherford and Horridge, 1965).

Eguchi and Waterman (1967) demonstrated that several hours of exposure to bright light increases the number of certain intracellular vesicles in glutaraldehyde-OsO₄ fixed retinular cells of the crab Libinia. Using light polarized at 0°, 45°, and 90° to the horizontal, they found that cells whose microvilli were parallel to the E-vector had more vesicles than those with microvilli at right angles (Eguchi and Waterman, 1968). Those cells with microvilli at 45° to the E-vector were all equally affected. This suggests that the microvilli are dichroic with a maximal absorption of plane-polarized light along their axes.

The proposal of Autrum and Stumpf (1950) that retinular cells themselves are dichroic can be tested by microspectrophotometric experiments on individual cells, examining the rhabdom for dichroism. The dichroic absorption might be expected to have a spectral dependence which could be attributed to rhodopsin. The chain of conjugated single and double bonds which is responsible for the light absorption of rhodopsin determines the molecule's dichroic axis; light is preferentially absorbed along the chain. One can therefore determine a preferential orientation of rhodopsin chromophores in a membrane by examining the membrane's dichroism. Liebman (1962) and Wald, Brown, and Gibbons (1963) investigated the dichroism of isolated frog rod outer segments by microspectrophotometry. They concluded that the chromophore lies
randomly in the plane of the disc membranes with dichroic ratios of from 4.5 (Wald et al.) to 6 (Liebman). Assuming that rhodopsin also lies randomly in the plane of the invertebrate photoreceptor membrane, the geometry of the microvilli suggest a basis for the dichroism postulated by the Autrum-Stumpf model. Because of electron micrographs which showed cylindrical microvilli in the octopus retina, Moody and Parriss (1961) calculated that ideally dichroic membranes rolled into cylindrical microvilli would have a dichroic ratio of 2, with maximal absorption along the microvillar axis and half maximal absorption in any direction perpendicular to the axis. Moody and Parriss' treatment was extended by Waterman, Fernandez, and Goldsmith (1969) by assuming that the membranes had components of absorption normal to their surfaces; thus, for small deviations from ideality a ratio close to 2 is expected.

The first experiments designed to detect dichroism of rhabdomeres were performed on single, dark-adapted receptors of diptera (de Vries, 1956). An eye was frozen and a section was cut containing the cornea and most of the length of the receptor to be examined. A photomultiplier tube was used to detect the intensity of blue, polarized light transmitted through the cornea and the rhabdomere. No dichroism was found. Later, Waterman, Fernandez, and Goldsmith (1969) illuminated isolated rhabdoms of the crayfish Orconectes transversely with a microbeam. Single bands (of which there are about 25 per rhabdom) containing microvilli of one orientation were
thereby examined. In agreement with Moody and Parriss' prediction, they found that the segments of rhabdom had the absorption spectra of rhodopsin and a dichroic ratio of almost exactly 2. Similar experiments with the crayfish *Procambarus* (Goldsmith, Fernandez, and Waterman, mentioned in Waterman and Fernandez, 1970) and the spider crab, *Libinia* (Hays and Goldsmith, 1969), also gave maximal dichroic ratios of about 2. Measurements on the single-cell rhabdomeres of the diptera *Calliphora* (Langer, 1965) and *Musca* (Kirschfeld, 1969) revealed a dichroic ratio of 1.3 for cells 1 through 6. These dichroic ratios were perhaps reduced from a ratio of 2 by depolarization of the light beam in passage through the long rhabdomeres (Shaw, 1969b).

Kirschfeld (1969) found, however, that the microvilli of cell number 7 in *Musca* absorb maximally perpendicular to their axes. As with cells 1 through 6 he passed light through the dioptric apparatus. Cell 7, is directly above cell 8, whose microvilli are at right angles to those of cell 7. Kirschfeld reported that he removed cell 8 before examining cell 7.

On the other hand, Moody (1964) reported that Hagins and Liebman found tangential:radial dichroic ratios as high as 6 in slices of living squid retina. This structure is supposedly like the octopus retina, on which Moody's model was based. If one assumes that the organization seen in electron micrographs is essentially that of the dark-adapted living preparation, it is then likely that in the squid the
dichroic axes of individual molecules are parallel to the squid microvillar axes.

Rhodopsin molecules randomly arranged in the plane of rod outer segment discs absorb 50% more light, polarized or unpolarized, than they would if the chromophores were randomly arranged in three dimensions (Commoner and Lipkin, 1949). Given a dichroic ratio of 4.5 (Wald, Brown, and Gibbons, 1963) for a membrane containing rhodopsin, the increased absorption over a random arrangement is 35% rather than 50%. Similarly, Moody-Parriss tubules absorb unpolarized light 12.5% better than a free solution of pigment (Waterman, Fernandez, and Goldsmith, 1969). An array of such microvilli stacked as in decapod rhabdoms will maintain this efficiency throughout the length of rhabdom (Shaw, 1969b).

While anatomical and microspectrophotometric results provide a basis for polarized light detection, it must be shown with intracellular recordings that in fact polarization sensitivity is retained at the retinular cell level.

The first electrophysiological measurements of arthropod responses to polarized light were electroretinograms (ERG's) Autrum and Stumpf (1950) found no change in the gross ERG of the bee Apis with rotation of a polarizing filter, and concluded that the individual retinular cells within each ommatidium are the basis of polarized light sensitivity. Because there are eight cells in the Apis ommatidium, they assumed four (rather than two) equally spaced orientations of the dichroic axes.

One prediction of a "two-channel" intra-ocular dichroism
model is that by selectively adapting units which respond to light polarized in one plane, and then illuminating with a rotating plane-polarized beam, one might be able to record a modulated ERG having maxima at 90° to the axes of the maximally adapted units. Baylor and Kennedy (1961) were unable to adapt selectively the bee's eye. It is not known if their negative result could, among other things, be explained by (i) equal angles of illumination of the adapting beam to both classes of photoreceptors (Shaw, 1969), (ii) a dark-adaptation of the light-adapted cells in the seven seconds before measurement (Bohn and Täuber, 1971), or (iii) adaptation of one cell producing adaptation of another. Selective adaptation of the ERG was eventually successful in the case of the crab *Cardisoma* (Waterman and Horch, 1966), the octopus and squid (Tasaki and Karita, 1966), and the water strider *Gerris* (Bohn and Täuber, 1971), but only after much evidence had been amassed using intracellular micro-electrodes. The unadapted ERG's of *Cardisoma* and *Gerris* have a 90° modulation of the ERG with 360° rotation of a polarizing filter.

The first intracellularly recorded differential sensitivity to polarized light was demonstrated for retinular cells of the dipterans *Lucilia* (Kuwabara and Naka, 1959) and *Calliphora* (Burkhardt and Wendler, 1960; Autrum and von Zwehl, 1962). Rotating a polaroid 360°, they obtained maximum responses separated by 180°. This was expected from the assumption that the parallel microvilli absorb preferentially along their axes. Burkhardt and Wendler reported a
polarization sensitivity ratio of 2. However, Autrum and von Zwehl found no polarization sensitivity for half their cells.

The highest polarization sensitivity has been found in decapod crustacean retinular cells. Shaw (1966, 1969b), using a 0.3° source which he positioned precisely along the optic axes of ommatidia of the crab Carcinus, measured polarization sensitivity ratios as high as 11 (mean = 8) for single retinular cells. He found that Carcinus retinular cells fall into two distinct classes having orthogonal polarization sensitivities. This finding agrees with both Waterman and Horch's (1966) adaptation experiments and the anatomical reports that over wide regions of the retina there are two orthogonal alignments of the microvilli (Rutherford and Horridge, 1965, and Waterman and Horch, 1966). Shaw (1969b and 1969c) found retinular cells of the crayfish Astacus have polarization sensitivities as high as 9 (mean = 6.2). Measured similarly, the polarized light sensitivity ratios of locust retinular cells are as high as 4 (mean = 2.3) (Shaw, 1966, 1967, and 1969a), and for the honeybee drone are as high as 2 (mean = 1.3) (Shaw, 1969a).

Shaw (1969a,b) proposed that microvilli have a basic dichroic absorption ratio of roughly 10:1. If the rhodopsin chromophores lie in the microvillar membrane, as in rod discs, but have their chromophore axes parallel to the microvillar axis, and if the chromophores themselves have a dichroic ratio of at least 10:1, then the microvillar dichroic ratios
could be 10:1. Shaw explained the low polarization sensitivity values of dipteran retinular cells by the depolarization of the light beam as it penetrates the rhabdomere. A fused rhabdom structure is not expected to depolarize light. Shaw argued that coupling between cells is responsible for polarization sensitivity values lower than 10:1 for arthropods with a fused rhabdom. Penetrating cells in the same ommatidium (determined by coincident angular sensitivity of the two cells) with single electrodes, he was able roughly to correlate coupling with polarization sensitivity. Locust ommatidia, which have three classes of cells with microvilli at 120° to one another, are weakly coupled, and honeybee drone cells, having lower polarization sensitivities, are more strongly coupled (Shaw, 1969).

Shaw's experiments on crayfish have been corroborated in part by Waterman and Fernandez (1970); using the crayfish Procambarus, they measured polarization sensitivities as high as 12, with averages of 3.1.

A small minority of crayfish retinular cells are maximally sensitive to blue light (Nosaki, 1969; Waterman and Fernandez, 1970, and Muller, unpublished), but Waterman and Fernandez found no noticeable differences in the polarization sensitivities of blue- and yellow-sensitive cells.

While Waterman and Fernandez admit that their average polarization sensitivities might be low because of less than optimal illumination through the dioptric apparatus, resulting in scattered light from other facets (Shaw, 1969c), they feel
that the basic dichroic ratio of the retinular cells is 2, and that something unknown, "beyond the primary photon absorption", is enhancing the polarization sensitivity. If Shaw is correct, the resolution of the problem lies in explaining the dichroic ratio of only 2 found for crayfish microvilli (Waterman, Fernandez and Goldsmith, 1969). If Waterman, Fernandez, and Goldsmith are correct, what then is the mechanism which enhances the polarization sensitivity of decapod retinular cells? In answering these questions it seems necessary first to examine more thoroughly the mutual electrical coupling between retinular cells having the same and orthogonal polarization sensitivities within an ommatidium, as well as other forms of interaction between retinular cells, if such exist.
Methods

Adult Procambarus clarkii were obtained from Waubun Laboratories, Shreve, La. Eyes, including eyestalks, were removed from the crayfish and bisected longitudinally with a razor blade. Half an eye was mounted in a perfusion chamber permitting direct access to retinular cells. The experiments were done at room temperature (18°-22°). Bisected eyes were bathed in van Harreveld's saline (van Harreveld, 1936). The stimulus was light from a 150-watt xenon arc lamp, which passed through an electromechanical shutter and a heat absorbing filter, and was focused on the preparation with a 0.25 N.A. objective. Neutral density and Wratten type 70 (red) or 94 (blue) filters were at times interposed between the light source and the objective. To provide plane-polarized light with different E-vector orientations, single Polaroid type HN38 or KN36 filters were interposed in the beam and were rotated 360°. To avoid selective polarization of the beam at the air-saline interface, the light entered the solution perpendicularly through a coverslip. The intensities of light polarized at different angles at the preparation were the same to within 5% (0.02 ND), measured with an Eppley thermopile and Keithly Model 150 B microvoltmeter. In making the measurements, the thermopile itself was also rotated. Normally, single ommatidia were illuminated by passing the beam outside but as nearly as possible parallel to the dioptric apparatus along the cut surface. In a few
cases, to be noted, illumination was through the cornea. The beam was aligned along the ommatidial optic axis in both cases before the microelectrodes were inserted.

Micropipettes were filled with 1M KCl, or 4% Procion Yellow M4RS or 4% Procion Navy Blue M3RS (Stretton and Kravitz, 1968). A pipette was introduced normal to the ommatidial optic axis and inserted into the cell. In most cases, current was injected through the same electrode used to monitor the membrane potential of retinular cells. A standard D.C.-bridge circuit (Frank and Becker, 1964) was used to subtract from the signal the voltage developed across the electrode resistance (see Fig. 2).

Another means of injecting current through the electrode used to monitor membrane potential dispenses with the bridge, permitting the electrode resistance to change. This method relies upon both the relatively rapid return (relaxation) of the electrode to its pre-injection state and the long time constants of the crayfish retinular cells. Current is injected for 1 msec and at the end of the next msec the voltage recorded by the same electrode is measured. This cycle is then repeated at 500 cycles per second. If the electrode "relaxes" within 1 msec, the voltage sampled at the end of the cycle is the membrane voltage. Since the time constant of the membrane is greater than 50 msec, the relaxation of the membrane voltage will be less than 2%. As successive current pulses are applied, the membrane charges

* This technique was suggested by John Lisman.
in the manner that it would if half the electrode current were applied continuously. By placing the electrode in the saline bath, one may readily detect as a non-zero voltage a change in the voltage measuring capabilities of an electrode that is passing current. Fig. 3 shows that because the electrode does not have a constant resistance, it is possible to pass larger currents through a microelectrode (integrated over the period during which current is being passed) using this so-called "current-chop" technique than with the bridge.

Figure 4 shows that the voltages measured with the current-chop technique and with the bridge agree. In Fig. 4 a reversal potential was first determined by injecting current pulses of various amplitudes and delivering light flashes when the membrane voltages approached a steady level. The voltage drop across the electrode resistance was initially balanced out with the bridge circuit; a small, negative current pulse during each long current pulse demonstrated that the bridge was balanced. Alternatively, an equally long series of 1 msec constant current pulses at 500 per second was given using the current-chop technique. Again light flashes were delivered as the membrane voltage approached a steady level. The voltage beyond which the receptor potential changes direction is reversal potential, and is the same for the two techniques.

The reversal potential is independent of when during the current pulse the light is flashed (Fig. 5), even though the membrane voltage might not have reached a steady level.
The slow change in membrane potential most likely represents a resistance increase of the membrane rather than a capacitative charging. The smaller current pulses show that capacitative charging occurs more rapidly.

To inject dye iontophoretically, we passed -4 to -10 namp current pulses for one half second each second. Injection lasted for about half an hour. The tissue was fixed overnight at 4° in a solution containing 4.3 ml acetate buffer at pH 4, 0.6 ml glutaraldehyde, and 0.1 ml acrolein (Stretton and Kravitz, 1968). After dehydrating with methanol and embedding in paraffin, 6 μ sections were cut and mounted in a non-fluorescent mounting medium (Lustrex). A Zeiss fluorescence microscope was fitted with a BG3 filter for excitation and barrier filters 44 and 50.

The screening pigment of all eyes studied was in the "light-adapted" position. Shaw (1969c) found, as did we, that the screening pigment of eyes which were originally dark-adapted migrated even in the dim lights used during the experiment.
Figure 2.

The bridge circuit used to inject current through the recording electrode. The voltage across the electrode has a shorter time constant than that of the cell membrane, and is subtracted from the observed signal by adjusting the 20K potentiometer.
Figure 2
Figure 3.

A comparison of the voltages (V) measured by an electrode in the saline bath during current injections (I) using the bridge and current-chop techniques. More current (averaged during the pulse) may be passed with the current-chop (see text) technique. $V_{cal}$ is a 10mv-100msec calibration pulse (see Figure 2).
Figure 3
Figure 4.

The agreement between reversal potentials measured using the bridge and current-chop techniques. The light monitor trace (LM) indicates the timing of the 200 msec flash and is positioned at zero voltage. $V$ is the membrane voltage and $V_{cal}$ is a 10mv-100msec calibration pulse. The current pulses of various amplitudes were delivered every 10 seconds. The continuity of the voltage trace during a $-0.5$ na current pulse demonstrated that the bridge was balanced.
Figure 4
Figure 5.

The reversal potential is independent of when during the current pulses the light is flashed. This is demonstrated for 2 cells. The light monitor trace (LM) indicates the timing of the 200msec flashes and is positioned at zero voltage. The -0.5 na current pulse demonstrated bridge balance.
Figure 5
Results

Impaled cells could be identified as retinular cells by their location just distal to the basilar membrane, their -30 to -70 mV resting potentials, and their large photoreceptor potentials (Fig. 28). We confirmed this identification by injecting such cells with Procion dyes (Fig. 6). To record from one cell with two electrodes or two cells in the same ommatidium with two electrodes simultaneously, the electrodes were advanced sequentially. With one cell impaled, the ommatidium containing that cell was precisely located by finding the position of a 20-40 μ spot which, moving in two dimensions on the retina, elicited a maximal response of the cell. If the second electrode impaled a cell in the same ommatidium, then the cells responded similarly to moving the small spot of light in two dimensions. The situation in which each electrode has penetrated a cell will be referred to as "a pair of cells". At times, of course, it is possible that "a pair of cells" is in fact two penetrations of the same cell. This condition will be considered in detail later.

We illuminated the retina directly rather than through the cornea because our optics usually did not illuminate precisely along the optic axis of the impaled ommatidium. By stimulating through the cornea we could not ascertain that a pair of cells was in the same ommatidium, because as the beam passed from one facet of the light-adapted eye to the next, the response in each cell passed through a series of maxima.
Figure 6.

Fluorescence micrographs showing injections of crayfish retinular cells with Procion yellow. (A) A nearly longitudinal section showing successive bands and the nucleus. (B) A cross section in which a single cell occupies one-fourth the area of the rhabdom in its layer. The 6 μ sections included parts of at least 2 layers. The calibration lines are 20 μ.
and minima. It seems unlikely that one could determine which facet is associated with which cell without stimulating with a remote point source, even for the light-adapted eye.

Penetrations of cells in any small region of the retina show, in confirmation of Shaw (1966), that retinular cells respond best to light polarized in one of two directions. Half the cells respond best when the $E$-vector is horizontal to the crayfish, the other half when it is rotated 90°. Therefore, two cells in the same ommatidium had either identical polarized light responses or responses with their maxima orthogonal to each other.

Pairs of cells having the same sensitivity to $E$-vector angle. After demonstrating that a pair of cells was in the same ommatidium, we determined the angle of maximum polarization response of each cell. One way this was done was to rotate the polarizing filter at a constant rate during steady illumination. Figure 7 shows coincident responses to polarized light for one pair of cells. Another way to determine which $E$-vector angle produces a maximum response was to rotate the polarizer in 15° steps between 200 msec flashes delivered every 3 or 4 seconds, as shown in Fig. 8 (for the same pair of cells as in Fig. 7). By comparing the responses to flashes of different intensities with the responses obtained by rotating the polarizer during constant intensity flashes, the polarization sensitivity ratio as measured by Shaw (1969a) was determined. In Fig. 8 the polarization sensitivity ratio is 2. An equivalent, more
Figure 7.
A pair of cells in an ommatidium responding identically to rotation of the polarizer during steady illumination. LM indicates the timing of the light. The resting voltages measured by the two electrodes were nearly identical.

Figure 8.
Same cells as Figure 7. 200 msec flashes were delivered every 2.5 sec while rotating the polarizer 15° between flashes. Then a series of flashes with the polarizer fixed, increasing in steps of 0.6 log units of intensity, were delivered. The difference in intensities which produce responses like the minimal and maximal responses to the polarizer rotation is about 0.3 log units, or 2 on a linear scale. This value is the polarization sensitivity.
rapid way to measure this ratio is as follows. First, a response is produced with the polarizing filter positioned to stimulate maximally. The polarizer is then rotated 90° for minimal stimulation; neutral density filters are removed until the response to a flash matches that before rotation of the polarizer. The density of the removed filters, converted to linear units of intensity, is the polarization sensitivity ratio, or polarization sensitivity.

For pairs of cells in the same ommatidium, we found fifteen cases in which both cells displayed the same angle of polarization sensitivity. Each pair was checked for coupling by passing current alternately through both electrodes. For one pair (see below) there was no coupling. For the other fourteen pairs, injecting current through at least one of the electrodes produced equal voltage changes in both cells (Figs. 9 and 10). We consider such cells to be "well coupled". One can model the D.C. electrical coupling between cells by the circuit of Fig. 11 (Bennett, 1966). By a straightforward calculation (see Appendix I), the record of Fig. 9 yields a coupling resistance $R_c = 12.5 \pm 2$ Megohms for a 5% error of measurement, and resistances $R_1 = 43$ Meg and $R_2 = 7.7$ Meg. Sometimes the degree of coupling improved slightly during the experiment, while the resistance measured by passing current through one of the electrodes (i.e. the "input resistance") increased.

One cannot be certain that a given pair of penetrations is of a single cell or a pair of cells. That is, even for
Figure 9.

Partially coupled cells. The first column was measured shortly before the second column. $L$ indicates the timing of a light flash. $I_1$ and $I_2$ indicate the timing of currents injected into cells 1 and 2 respectively. The short pulses during $I_1$ were $-0.2$ na and during $I_2$ were $-0.5$ na. The bridge was not balanced in the first column during $I_2$. 
Figure 10.

Perfectly coupled cells. Currents ($I_1$ and $I_2$) injected into the cells of Figures 7 and 8 produce identical voltage changes. $V_{\text{cal}}$ is a 10mv-100msec calibration pulse.
Figure 10
Figure 11.

A schematic representation of D.C. coupling between two cells in the same ommatidium (see Appendix I). $R_C$ is the coupling resistance.
Figure 11
cases of partial coupling the electrodes could have been in electrically remote parts of the same cell.

It was difficult to determine the effect of light upon electrical coupling between retinular cells because of the large increase in membrane conductance induced by the light. The increased conductance made it hard to maintain bridge balance during the larger currents required to produce voltage changes. However, in cases in which cells appeared well coupled in the dark, measured with currents passed through both electrodes, that relationship held in the light as far as could be determined. On the other hand, for the cells shown in Figure 9, light produced a decrease in the coupling resistance between the cells (Fig. 12) from $12.5 \pm 2$ to $7 \pm 1$ Meg. Resistances $R_1$ and $R_2$ became 13 and 5 Meg. respectively.

The single exception to the generalization that strong coupling exists between pairs of cells in the same ommatidium and having the same angle of maximum polarization response was a pair of cells of which one cell was more sensitive to orange than to blue light, and the other more sensitive to blue light than orange.

**Pairs of cells having orthogonal sensitivity to E-vector angle.**

In eleven of the cases in which a pair of cells was in one ommatidium, the angles of polarization which produced maximum responses in each of the two cells differed by $90^\circ$. In all eleven cases the pairs of cells were either not at all coupled, or extremely weakly coupled. In Fig. 13 is
Figure 12.

Coupling between the cells of Figure 9 in the light. L indicates the timing of the light; I₁ and I₂ indicate the timing of currents injected into cells 1 and 2 respectively. The short current pulse during I₂ is -0.5 na and during I₁ is -0.2 na. Vcal is a 10mv-0.1 sec calibration pulse.
Figure 12
Figure 13.

The responses of a pair of cells having orthogonal polarization sensitivities to rotating the polarizer 360° during steady illumination. L indicates the timing of the light.

Figure 14.

The responses of the cells of Figure 13 to 200 msec flashes delivered every 2.5 sec. The polarizer was rotated 15° between flashes and then an intensity series in steps of 0.6 log units of intensity was presented. The difference in amplitudes with rotation of the polarizer was that between steps of intensity, therefore the polarization sensitivities were about 4.
Figure 13

Figure 14
shown the responses of such a pair to a steady light of constant intensity while the polarizer was rotated 360°. The maxima are seen shifted 90° from each other. Rotating the polarizer 15° between 200 msec flashes of light as in Fig. 8 also displayed the orthogonal relation between maxima (Fig. 14). The two cells of Fig. 14 each had polarization sensitivity ratios of 4. Because the recording electrodes were filled with dye solutions, it was difficult to pass current and maintain bridge balance. However, it is clear (Fig. 15) that the two cells are not electrically coupled. Table I summarizes the results of our coupling measurements. In no case did a pair of cells having orthogonal polarization sensitivities appear coupled. In one case a pair of cells showed no measurable polarization sensitivity; this pair was coupled, but the light response was weak.

When pairs of cells in neighboring ommatidia were impaled, no coupling was ever seen.

Although we attempted to inject dye into a number of pairs of cells in the same ommatidium, only one such pair of injections successfully stained two cells. The cells in Fig. 16 had their polarization sensitivity maxima at 90° to each other and were not coupled. The dyes were located at different layers in the rhabdom. Yellow dye apparently filled the large double cell (cell 1 in Fig. 1); that is, only one nucleus was stained yellow, but half of the microvilli of one orientation were dyed. Blue dye filled one of the smaller cells whose microvilli are at right angles to the
Table I

Recordings from the same ommatidium with two electrodes.

<table>
<thead>
<tr>
<th>Polarization Sensitivity</th>
<th>Number of Pairs of Cells Coupled</th>
<th>Number of Pairs of Cells Not Coupled</th>
</tr>
</thead>
<tbody>
<tr>
<td>// polarization sensitivity</td>
<td>14</td>
<td>1 (blue-orange pair)</td>
</tr>
<tr>
<td>_ polarization sensitivity</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>No polarization sensitivity</td>
<td>1 (poor response)</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 15.

The pair of cells of Figures 13 and 14 was not coupled. Currents $I_1$ into cell 1 produced no measurable voltage change in cell 2, nor did currents $I_2$ into cell 2 affect the voltage recorded in cell 1. $V_{cal}$ is a 10mv-100msec calibration pulses. Because the electrodes were filled with dye and had high resistances, it was difficult to maintain bridge balance.
Figure 15

[Diagram showing two sets of voltage and current indicators, labeled \( I_1 \), \( V_1 \), \( I_2 \), \( V_2 \), and \( V_{cal} \).]
Figure 16.

Micrographs of cross sections of a pair of cells in the same ommatidium injected with Procion Yellow M4RS and Procion Navy Blue M3RS dyes. The cells had orthogonal polarization sensitivities. Calibration marks are 20 μ.

Figure 16-A.

The large cell (cell 1) was injected with Procion Yellow and fluoresces; the other cell was injected with Procion Navy Blue, which quenches the fluorescence of the tissue.

Figure 16-B.

The section in (A) in yellow light. Only the blue dye is visible.

Figure 16-C.

A more distal, oblique section than in (A) which suggests the banding pattern of the rhabdom. In more distal sections this pattern indicated that the microvilli of the 2 cells occupied different layers.

Figure 16-D.

Section (C) viewed with yellow light.
large cell. In all the other cases in which we determined that both electrodes were in the same ommatidium by moving a spot of light in two dimensions on the retina and noting coincident responses, either no cells were stained or, in a few instances, only one was stained. Twice when the electrodes appeared to be in perfectly coupled cells (or in the same cell), only Procion Navy Blue dyed a cell. In these eyes it is possible that Procion Yellow was not injected. However, Procion Navy Blue can quench the fluorescence of ten-times more concentrated Procion Yellow, and hence the yellow dye might not be seen even if it had been passed out of the electrode.

**Polarization sensitivity at different intensities.**

Over the range of light intensities for which the amplitude of a response to a 200 msec flash of light is a logarithmic function of intensity, the polarization sensitivity remains approximately constant. This relation is illustrated in Fig. 17 for a cell which had a polarization sensitivity of 4. The voltages at which both the // and \( L \) responses saturate are identical (where by // we mean the polarizer is aligned for maximal stimulation of the penetrated cell and by \( L \) we mean the polarizer has been rotated 90°). At low intensities it is difficult to measure accurately the polarization sensitivity.

**Negative-going ERG's.**

Light-evoked voltage changes can be recorded extracellularly from the region of the rhabdom with a microelectrode
Figure 17.

The relation between polarization sensitivity and intensity.

(A) 200 msec flashes were delivered every 30 seconds with the polarizer first maximally stimulating (stars) and then minimally stimulating (circles) a retinular cell.

(B) The difference in intensities required to match responses at each level of attenuation are approximately constant over the region that the receptor potentials are logarithmic with intensity. The polarization sensitivity at attenuations greater than 3.3 log units was difficult to measure accurately.
Figure 17
(Naka and Kuwabara, 1959). Such extracellular responses, or local ERG's, can be observed as negative-going potentials greater than -5 mv. which last for the duration of the light (Fig. 18). The amplitude of the response is the same whether the E-vector of the light is aligned for maximal stimulation of one class of retinular cells or the other (Fig. 19). The response amplitude is dependent upon intensity (Fig. 18). The amplitude of the ERG is not changed by currents passed through the recording electrode, even for currents larger than those which would change the amplitude of an intracellularly recorded receptor potential (Fig. 20).

By rotating the polarizing filter 15° between successive flashes, as was done for measurements of intracellular polarization sensitivity, one can measure a four-peaked modulation of the response while rotating the polarizer 360° (Fig. 21). Although the polarization sensitivity is hard to determine, it is between 1.05 and 1.30 when calculated for responses separated by 45°. In Fig. 21 the response amplitude is roughly logarithmic with intensity. Two cells within a few micra of the site of the extracellular recording were impaled during the same penetration. The cells' polarization sensitivities were at least 5. The peak responses to polarized light by the impaled cells correspond with the minimum responses of the ERG.

There are several ways to abolish irreversibly the receptor potential in a retinular cell without markedly
Figure 18.

The extracellular receptor potential (ERG) at 3 levels of attenuation of the flash. LM indicates the timing of the flash. Vcal is a lmv-100 msec calibration pulse. All three voltage traces are at zero voltage.

Figure 19.

The extracellular receptor potential is the same with horizontal (0°) and vertical (90°) positions of the polarizer. LM indicates the timing of the flash. Vcal is a lmv-100msec calibration pulse.
Figure 18

Figure 19
Figure 20.

The extracellularly recorded receptor potential is not changed by current through the electrode. L indicates the timing of the light flash. Vcal is a 10mv-0.1sec calibration pulse. (A) shows a negative-going ERG and (B) shows a biphasic ERG. Both may be recorded in the retinular cell layer, and neither component is affected by current passed through the recording electrode.
Figure 20
Figure 21.

(a) The extracellular response to light can be modulated. A series of 200 msec flashes every 2.5 seconds is presented while the polarizer is rotated 15° between flashes. The intensity-response series which followed was logarithmic with intensity. A larger amplitude of the extracellular response at the microvillar membrane is expected.

(b) A neighboring cell was penetrated and was stimulated as in (a). It had a polarization sensitivity of at least 5.

(c) The next cell penetrated had an orthogonal polarization sensitivity. Stimulated as in (a), it also had a polarization sensitivity of at least 5. The minima of the ERG correspond with the maxima of the intracellularly recorded receptor potentials.
a. 1 mv 

\( V_{\text{ext}} \) 

0° 180° 360° 0.3 ND

b. 10 mv 

\( V_1 \) 

0° 180° 360° 0.3 ND

c. 10 mv 

\( V_2 \) 

0° 180° 360° 0.3 ND

Figure 21
altering its resting potential or resistance. Filling a microelectrode with 3 M KCl rather than 1M KCl can cause the receptor potential to diminish until a hyperpolarization followed by a slow, small depolarization is seen (Fig. 22). (This does not happen with 3 M KCl-filled microelectrodes of resistance greater than 30-50 Meg.) The deterioration of the receptor potential is somewhat accelerated by passing depolarizing currents through the electrode. The light-evoked hyperpolarization is stable and, like the ERG, is not changed in amplitude by passing current through the recording electrode, although the membrane potential does change (Fig. 24). Furthermore, the amplitude of the intracellularly recorded hyperpolarization is the same regardless of the angle of polarization of the light (Fig. 23).

**Reversal potentials and polarization sensitivity.**

One means of reversibly "abolishing" the receptor potential is to hold the membrane at the reversal voltage of the photoresponse. This can be done with a single electrode in many crayfish retinular cells using either the D.C.-bridge technique or the current-chop technique described earlier. For many retinular cells from which we recorded, most having a polarization sensitivity of about 2, polarization sensitivity was independent of membrane potential up to reversal potential. Several cells exhibited an increased polarization sensitivity, as determined with matching responses, when the cell was depolarized to a voltage near
Figure 22.

The intracellularly recorded response of a cell penetrated with an electrode filled with 3 M KCl. The light monitor trace (LM) is positioned at zero voltage and indicates the timing of the flash.

Figure 23.

The intracellular hyperpolarization of Figure 22 is the same for horizontal and vertical positions of the polarizer. LM indicates the timing of the flash. Only the small positivity following the negativity disappeared when the electrode was withdrawn from the cell (Extracell.).
Figure 24.

The intracellularly recorded hyperpolarization is not affected by changing membrane potential. Same cell as Figure 22. The light monitor (LM) is positioned at zero voltage and indicates the timing of the flash. $V_{cal}$ is a 10mv-100msec calibration pulse.
reversal potential (Fig. 25). In the most extreme cases, the response with light polarized for maximal stimulation reversed at a higher voltage than did the response to light polarized at right angles (Fig. 26). In other words, in the experiment of Fig. 26, the polarization sensitivity was shifted from 4 to infinity.
Figure 25.

Polarization sensitivity can be dependent upon intracellular voltage. The light monitor (LM) indicates the timing of the light and is positioned at zero voltage. Below each voltage trace (V) is the position of the polarizer and the attenuation of the light in log units. At rest the polarization sensitivity is 2 (a and d). During the current injections shown, it increased to over 4 (c).
Figure 25
Figure 26.

The dependence of reversal potential upon polarizer orientation. The legend is that of Figure 25. During constant current pulses the light is flashed; the intracellular voltage (c) lies between the reversal potentials of a and b.
Figure 26
Discussion

Coupling between cells.

When polarized light falls on a crayfish ommatidium, the light differentially stimulates two classes of cells. Information about the plane of polarization of the light is sensed because each cell is itself dichroic, and preserved because cells having orthogonal directions of maximal absorption are not electrically coupled. On the other hand, cells with the same spectral sensitivity and sensitivity to the same angle of light polarization are apparently well coupled electrically.

We also have evidence that color information is preserved at the retinular cell level. Cells which are maximally sensitive to blue light are split about equally into two classes on the basis of angle of polarized light sensitivity. Moreover, in the one case of a pair of cells in the same ommatidium with the same angle of polarization sensitivity and no electrical coupling, one cell was maximally sensitive to orange light, whereas the other was maximally sensitive to blue light.

When measuring the electrical coupling between pairs of cells, it is important that currents be passed through each electrode. In order to support this statement, let us consider two electrically coupled cells, each of which is internally isopotential; the two cells are modeled as in
Fig. 11.

The two cells are considered to be perfectly coupled electrically when a change in voltage in each cell appears unattenuated in the other. That is, passing current into cell 1 produces voltage changes $\Delta V_1 \equiv \Delta V_2$, and similarly, a current in cell 2 produces voltage changes $\Delta V'_2 \equiv \Delta V'_1$. According to the model of Fig. 11, we must then have $R_c << R_1$ and $R_c << R_2$ (where $R_c$ $\equiv$ coupling resistance).

A pair of cells is said to be poorly coupled electrically when changes in membrane potential produced by currents injected into each cell produce little or no voltage change in the other cell. That is, current injected into cell 1 produces a voltage $\Delta V_1 \gg \Delta V_2$ and a current injected into cell 2 produces a change in voltage $\Delta V'_2 \gg \Delta V'_1$. In the model of Fig. 11, both $R_1 << R_c$ and $R_2 << R_c$.

Partial coupling is said to exist between two cells when the change of voltage produced in one cell by current through an electrode appears somewhat attenuated in the other cell. That is $\Delta V_1 > \Delta V_2$ and/or $\Delta V'_2 > \Delta V'_1$. This coupling can be approximately reciprocal if the coupling resistor $R_c$ is about the same magnitude as $R_1$ and $R_2$. Partial coupling can apparently be produced by damaging one or both of two cells which are normally perfectly coupled. Producing a leakage in cell 1 would be analogous to decreasing $R_1$. If $R_1 \neq R_c$, then passing current into cell 1 will produce a $\Delta V_1 \equiv \Delta V_2$, but passing current into cell 2 will produce a $\Delta V'_2 > \Delta V'_1$. In this case damage has not actually
changed the coupling resistance $R_c$. Generally such damage-induced partial coupling will be asymmetrical.

Another way damage could produce partial coupling between cells is by increasing the coupling resistance, $R_c$. Damage to another crayfish neuron has been shown to increase its coupling resistance to another cell (Asada and Bennett, 1971). The increased coupling resistance persists even after the damaged membrane has restored its original resistance. The degree of partial coupling of cells is therefore likely to be highly dependent on the quality of the electrode impalement.

If the coupling resistance $R_c$ is not constant, but instead voltage dependent, one must pass both hyperpolarizing and depolarizing currents to measure the properties of the coupling membrane. The giant motor synapses of the crayfish are examples of such "rectifying" membranes (Furshpan and Potter, 1959), as are the junctions between retinular cells and the eccentric cell in an ommatidium of the Limulus lateral eye (Smith and Baumann, 1969). Similarly, to detect the effects of non-linear resistances $R_1$ and $R_2$, both hyperpolarizing and depolarizing currents must be passed through each electrode.

If partial coupling is measured between two electrodes, it is also possible that no membrane separates them. That is, the electrodes may have penetrated distant parts of the same cell, such that the tips are separated by a large access resistance.

Although we do not have direct proof by means of dye
injections that coupled cells were in fact two distinct cells, a number of lines of evidence indicate that this must be so, at least in some cases. We penetrated about the same number of pairs of cells in which the polarization sensitivity of one cell was at right angles to the other, as pairs of cells in which both cells had parallel polarization sensitivities. This is expected if the second electrode penetrated cells at random, rather than a particular cell, or cells of one class, preferentially. Direct proof that it is possible to impale two separate cells of the same polarization sensitivity in an ommatidium is the singular case of simultaneous impalement of a blue- and an orange-sensitive cell (see Table I). That this happened only once is not surprising because of the paucity of recordings from blue-sensitive cells.

This same data argues against the objection that cells were coupled artificially. Presumably for artificial coupling to happen, one electrode could have passed through one cell on its way to the other, the hole between the two cells providing the coupling. The second electrode would then have been introduced into the first cell. This might have happened, possibly for the pair of cells with no measureable polarization sensitivity which were coupled but had weak responses (see Table I)*, or when the light response of at least one cell was nearly abolished. However in no cases

*Although a twisting of the rhabdom in penetration might have produced the same result.
were cells of opposite polarization sensitivity coupled. In addition, if cells having the same polarization sensitivity were not normally coupled, then I should have occasionally impaled uncoupled pairs of cells of the same polarization sensitivity; no such cases were found.

Cells on opposite sides of the ommatidium having parallel microvilli are in contact with each other where their microvilli abut at the midline of the rhabdom. At the level of the rhabdom the cell bodies of such cells do not touch; interposed between them are the cell bodies of cells with orthogonally oriented microvilli (see Fig. 1). There is also the possibility that there are distant points of contact between the axons of cells on opposite sides of the rhabdom. However, their axons pass through the basement membrane not with those from cells on the opposite side of the same ommatidium, but instead with their nearest neighbors in the next ommatidium (Parker, 1897). Cells in adjacent ommatidia never were coupled. One concludes that unless there is coupling in the region distal to the rhabdom, where the retinular cell nuclei are situated, coupling between retinular cells located on opposite sides of the ommatidium is most likely through their microvilli.

Although no tight junctions or gap junctions have been described or looked for at the midline of the crayfish rhabdom, where microvilli of cells on opposite sides of the rhabdom meet, specialized contacts between microvilli have been seen in the Limulus lateral eye (Lasansky, 1967, and
The tight junctions are presumably the channels for the current which is known to flow between Limulus retinular cells and the eccentric cell.

Is it theoretically feasible for a voltage appearing in one retinular cell to appear attenuated by less than 10% in a retinular cell on the opposite side of the ommatidium? The microvilli of Procambarus retinular cells were measured by Eguchi (1965) to be 7.5 μ long and 0.07 μ in diameter. If we assume that in the dark the membrane and cytoplasm have resistivities similar to non-myelinated crustacean axon, then the axial resistivity \( R_a = 50 \text{ ohm.cm} \) and the membrane resistivity \( R_m = 5 \times 10^3 \text{ ohm.cm}^2 \) (Katz, 1966, p.75). For a microvillar radius \( r = 0.035 \mu \), the length constant, \( l = \left( \frac{r}{2 R_a} \right)^{1/2} = 132 \mu \) (Appendix II). This is the value for a single microvillus in an infinite, conducting medium. At twice the microvillus length, the attenuation of a signal will be by 11% of its initial value. If one considers further that each microvillus is in a restricted space rather than each in an infinite bath, the attenuation is decreased. For example, were the extracellular space the same volume and resistivity as that inside the microvillus, and the path to ground was at the microvillar base, then the attenuation would be reduced (by a factor of 3%) to a value closer to 10% for whole crayfish rhabdom (see Appendix II). But the electron micrographs indicate that the extracellular volume might be considerably smaller than this, which would be expected to decrease the attenuation along the microvillus correspondingly.

Since the microvilli form a closely packed
parallel array along the length of the rhabdom and retinular cell, and the input resistance of the retinular cell is high compared to the total microvillar axial resistance, the magnitude of a voltage in the microvilli should be preserved in the body of the coupled retinular cell as well if the membrane resistance separating the microvilli is low.*

Light is believed to increase the membrane conductance of the photoreceptor (Fuortes, 1959; Kikuchi, Naito, and Tanaka, 1962; Muller and Brown, 1969; Brown, Hagiwara, Koike, and Meech, 1970), specifically that of the microvilli (Hagins, Zonana, and Adams, 1962). This would be expected to reduce the apparent coupling through the microvillus by reducing the membrane length constant. A 100-fold decrease in the membrane resistivity from $5 \times 10^3$ to 50 ohm.cm, for example, would cause the length constant to drop to 13.2 $\mu$m. A signal will be attenuated by 65% through two microvilli, but by only 40% if an equivolume, equiresistant extracellular space is considered. A light-induced decrease in apparent coupling between electrodes at opposite ends of a single cell could also be expected, for as the microvillar shunt increases, the cell's length constant will shorten. While such a coupling decrease was not routinely found, it apparently can occur (Fig. 12).

What is the value to the crayfish of coupling between cells which have the same polarization sensitivity, spectral

*Given the dimensions of the rhabdom, the total microvillar axial resistance for a 50 ohm.cm resistivity should be approximately $10^4$ ohms. The membrane resistance of the cell is at least 5 or 10 meg.
sensitivity, and angle of view? At bright levels of illumination the responses of the cells would be expected to be identical, hence one would expect to see no effect of coupling. At extremely dim levels, however, coupling might have the effect of a smoothing or averaging process on the output of a single retinular cell. This could be especially desirable in crayfish, for which the axons of different retinular cells apparently have different points of termination in the first ganglion in the eyestalk (Parker, 1897).

The basis of polarization sensitivity.

It is attractive to assume that rhodopsin molecules are randomly arranged in the plane of the microvillar membrane, for this is what has been found for vertebrate rod disc (Liebman, 1962; Wald, Brown, and Gibbons, 1963) and cone (Marks, 1965) membranes. Moreover, such a membrane when rolled into a cylindrical microvillus of the sort seen in electron micrographs would have a dichroic absorption ratio of close to 2 (Waterman, Fernandez, and Goldsmith, 1969). Now let us assume that pigment molecules in the receptor membrane are equally "coupled" to the generator of the primary electrical events, independent of the orientation or location of a molecule in the plane of the microvillar membrane. In this case the polarization sensitivity should also be 2.

Using microspectrophotometry, the dichroic absorption ratios of 2 measured by Waterman, Fernandez, and Goldsmith (1969) agree with the above predictions. However, the
polarization sensitivity ratios which are measured to be as high as 11 by Shaw (1969b) and Waterman and Fernandez (1970) and 6 by us do not agree with the above predictions. What accounts for the disparity between the size of the measured polarization sensitivity ratios and the measured dichroic absorption ratios?

A possible explanation of the apparent physiological and spectrophotometric disparity is that reflections in the dioptric apparatus might cause light polarized in one plane to strike photoreceptors in one section of the ommatidium more than if it were polarized at right angles. All indications are, however, that a fused rhabdom acts as a waveguide in which no such preferential stimulation is likely (Varela and Wiitanen, 1970). Since polarization sensitivity ratios higher than 2 seem to be symmetrical (see Fig. 14, for example, but contrast with Shaw, 1967), at least part of the high polarization sensitivity could not be due to selective absorption of the optics. It is conceivable that if in penetrating a cell illuminated through the optics the rhabdom is bent at an angle with respect to the crystalline cone, then the symmetry of the optics would be disrupted. In this case, higher than normal polarization sensitivities could occur for some of the cells. While it is possible that such an effect could produce the extreme ratios seen by Shaw (1966, 1969b, and 1969c) and Waterman and Fernandez (1970), both of whom illuminated through the optics, this explanation cannot account for the lack of low values reported by Shaw.
Another possible explanation for the difference between dichroic absorption ratios determined for isolated rhabdoms and the polarization sensitivity ratios which can be measured electrophysiologically is that the dichroic ratio of the intact rhabdom could be higher than 2, perhaps as high as 10, as Shaw implies. Shaw (1969a) proposed that the chromophore axes are aligned with the microvillar axes. The effect of the procedure used to isolate the rhabdoms would presumably be to rotate the chromophore axes out of the membrane approximately 45°, or to simply randomize their orientation within the membrane. There is some support for this view from the unpublished data of Hagins and Liebman (Moody, 1964), demonstrating an apparent dichroic ratio as high as 6 in fresh slices of squid retina. The microvilli of squid retina resemble those of crayfish in shape and orientation after fixation and histological preparation. It is possible, however, that living microvilli are not cylindrical, but instead flattened. Since the complete data and methods of Hagins and Liebman (c.f. Hagins and Liebman, 1963) are not published, it is difficult to draw any direct conclusions; however, a fixation procedure could cause the microvilli to swell, resulting in cylindrical tubules.* One of the implications of flat microvilli whose maximal absorption is tangential to the retina is that, like rod discs, they should display no dichroism when viewed from above. However, Moody and Parriss report that they have trained

* It would be interesting to know the dichroic absorption ratio for fixed squid microvilli.
octopuses to discriminate polarized light in the absence of environmental clues, and hence the eye itself must be dichroic (Moody and Parriss, 1961). If the optics of the eye are not "analysing" polarized light, then the receptors themselves must be dichroic. Although the amount of dichroism required by the octopus might be small, this would argue that either the visual pigment molecules are probably not randomly arranged in the plane of the microvillar membrane, or the microvilli are not flat in the plane of the retina, or both.

The consideration of microvilli whose cross section is elliptical, or in the extreme flat, rather than round, permits another explanation of the high polarization sensitivity ratios found in crayfish retinular cells. If the major axis of an elliptical microvillus were collinear with the ommatidial optic axis, and the orientation of rhodopsin in the microvillar membrane were similar to that in the rod disc membrane, then the dichroic absorption ratio of a living microvillus could be as high as 6, which is the dichroic ratio measured for rod discs (Liebman, 1962).

The polarization sensitivity of flat microvilli can be greater than that of cylindrical microvilli. This is not because the efficiency of capture of light polarized parallel to the microvillar axis would be increased; it would still be 50% more than the absorption by a random array of molecules. Instead it is because there would be little photon capture for light polarized at right angles to the microvillar
axis. As a result, the absorption of unpolarized light by such flat microvilli would be 25% less than that of randomly oriented molecules, rather than 12.5% more as for cylindrical microvilli.

Elliptical microvilli have in fact been seen in *Procambarus* (Eguchi, 1965), although the ellipses' major axes in the micrograph do not seem to be preferentially oriented. This, however, implies that the ellipticity is not an artifact caused by cutting cylindrical microvilli obliquely.

The outline of an isolated rhabdom can be extremely scalloped rather than straight, the bulging portions corresponding to microvilli cut in cross section (Waterman, Fernandez, and Goldsmith, 1969). If the rhabdom functions as a conventional waveguide, it would most likely have straight sides. It is possible that a swelling of microvilli could produce part of the scalloping. Disruption of the cell, as in the procedure used to isolate rhabdoms, fixation procedures, or even light (White, 1967, and Gribakin, 1969) could cause the microvilli to swell, thereby decreasing the dichroic ratio to 2 as predicted for round microvilli.

The hypothesis that osmotic swelling produces round microvilli from normally flat ones suggests a number of experiments based on artificially induced osmotic swelling and shrinking. For example, a flat microvillus should become somewhat cylindrical in hypotonic saline if its membrane is chiefly permeable to one ionic species. If this happens while
one is recording from the cell with a microelectrode, the recorded polarization sensitivity ratio might be expected to decrease. However, if the microvillar membrane is permeable to both anions and cations, then osmotic swelling induced by bathing the eye in hypotonic saline will be only transient. A similar transient effect would appear when the cell was returned to normal saline after prolonged bathing in hyperosmotic saline. For rod outer segments in hypertonic NaCl, transient volume changes, recovering on the order of 2% per second, are observed (Korenbrot and Cone, 1971). The large tissue volume and the structure of the ommatidia of the crayfish eye are likely to prevent rapid exchanges of bathing solutions. Therefore, transient changes might be obscured by slow exchange times. Preliminary experiments, perfusing with saline made hyperosmotic with sucrose and then returning to normal saline showed no repeatable changes of polarization sensitivity. That is, in only one case in five was a transient reduction of polarization sensitivity seen in the shift from hyperosmotic to normal saline. On the other hand, concentrated KCl leaking from the electrode into the cell could have caused microvilli to swell, causing a fixed, low, polarization sensitivity. The use of different concentrations of electrolyte in the recording electrode (from 0.1 M KCl to 3 M KCl) showed no marked difference in polarization sensitivities. Until there is definite evidence to the contrary, then, one is inclined to assume that crayfish microvilli are indeed cylindrical in the living state.
Assuming that the dichroic absorption ratio of a single microvillus is 2, then a cell in isolation would have a polarization sensitivity ratio of 2 if light absorption by one rhodopsin molecule is physiologically equivalent to absorption by another. Since higher polarization sensitivity ratios than 2 have been measured (Shaw, 1969b; Waterman and Fernandez, 1970; and Fig. 14, 17, and 21), we propose that interactions between cells can explain the enhancement of the "basic" polarization sensitivity.

One such interaction could be a synaptic lateral inhibition acting between cells having orthogonal orientations of microvilli. A cell responding to light polarized parallel to the cell's microvilli would be stimulated more than cells with microvilli at right angles to the E-vector. If mutually inhibitory synapses existed, then the more strongly stimulated cell would also more strongly inhibit its neighbors than be inhibited by them. Depending upon the intensity-vs.-response characteristics of the cell and the characteristics of the inhibitory synapse, nearly any polarization sensitivity ratio would seem plausible. This idea can be tested by stimulating one cell with current and looking for inhibition in the other cell. We found that injecting depolarizing current into cells having one orientation of microvilli produced no discernable change in the membrane voltage or resistance of cells in the same ommatidium having microvilli at right angles to those stimulated. Unless only light and not changes in voltage activate the synapse, inhibitory
synapses appear not to be important in enhancing the crayfish retinular cells' polarization sensitivity.

All studies thus far on the polarization sensitivity of decapod retinular cells have been conducted on isolated eyestalks. Waterman and Wiersma (1963) found that cutting the blood supply to the eyestalk in several decapods abolished, within four minutes, all visual signals in the optic nerve. It is possible, therefore, that normally a lateral inhibition enhances the polarization sensitivity of crayfish retinular cells further than that recorded in isolated eyestalks.

There is another type of mutual interaction that is not synaptically mediated which might provide an enhancement of the polarization sensitivity. The currents through the microvillar membranes share a common extracellular path to ground. The flow of current through the resistance of this pathway produces a voltage which can be recorded as the local ERG (Fig. 18). With unpolarized light the contributions of both classes of cells to the ERG should be nearly equal. If polarized light is used, the cells which are maximally stimulated will also contribute maximally to the ERG. This is, in fact, the basis of the selective adaptation experiments of Waterman and Horch (1966), Tasaki and Karita (1966), and Bohn and Täuber (1971).

The intracellularly recorded receptor potential is measured between the electrode tip inside the photoreceptor and a reference ground in the saline bath. The local ERG is recorded between the reference in the bath and a point
near the outside of the photoreceptor membrane. If the inside of the cell is relatively isopotential, the true transmembrane potential at any region of the cell will be the difference between the voltage recorded inside the membrane and the voltage outside the membrane, both referred to the same ground. A negative ERG component in series with the photoreceptive membrane will effectively subtract from the true transmembrane potential, yielding an attenuated intracellularly recorded receptor potential. Such an interaction of the transmembrane receptor potential with the ERG has in fact been inferred for the fly *Calliphora* (Burkhardt, 1962).

How, then, would extracellular currents enhance a basic polarization sensitivity? We will refer to cells whose microvilli are parallel to those of the impaled cell as "// -cells" and cells whose microvilli are at right angles to those of the impaled cell as "\(-cells". We shall assume, for example, a basic polarization sensitivity for an isolated cell of 2. Consider the following two stimulus conditions. In the first case, if the cell penetrated by the electrode is stimulated with light of intensity \(I\) polarized parallel to its microvilli, then cells with orthogonally aligned microvilli, the \(-cells, will be responding as they would to light of intensity \(\frac{1}{2}I\) polarized parallel to their microvilli. Now in the second case the polarizer is rotated 90° and the intensity of the light is doubled to 2I. Were the intracellular receptor potential of \(-cells independent of the responses of the \(-cells, the \(-cells would now respond
as in the first case, before the polarizer was rotated and the intensity doubled. However, \( L \)-cells are now responding to light which is effectively 4-fold brighter than in the first case, and their contribution to the extracellular current is accordingly increased. The result is a depression of the intracellularly recorded responses in the \( H \)-cells. Hence, additional light is required to match the response seen in \( H \)-cells after rotation of the polarizer with the response produced before rotation, the amount of additional light will depend upon the balance between the intrinsic intensity-vs.-response characteristics of the \( H \)-cell membrane and the extracellular potential generated by currents from both the \( H \)-cells and the \( L \)-cells. Appendix III shows that with some assumptions, but independent of the dichroism of the microvilli and the incident light intensity, the increased extracellular potential upon rotating the polarizer 90° and doubling the intensity will be constant.

The local ERG of the crayfish has the characteristics which this model requires. The extracellular potential recorded close to or in the rhabdom is the same size with both horizontal and vertical orientations of the polarizer. This indicates that the contributions to the ERG from both classes of cells are symmetrical. Furthermore, there is evidence that this extracellular potential contributes to the intracellularly recorded voltages. The "ERG" can be several millivolts negative inside a cell whose light response mechanism has been damaged (Figs. 22, 23, and 24). However,
during the light response, when the photoreceptive membrane's conductance is high, the extracellular voltage is expected to appear largest inside the cell.

If extracellular currents can increase the basic polarization sensitivity of crayfish retinular cells, then one would predict that by reducing the size of the response of a cell with depolarizing currents passed through the electrode, the polarization sensitivity of the cell should increase. The reasoning which leads to this prediction is as follows. We have proposed that a balance between (1) the intensity-vs.-response characteristics of the transmembrane potential and (2) the intensity-vs.-response characteristics of the extracellular potential determines the polarization sensitivity. First, as the membrane of a retinular cell is depolarized with current, the size of the receptor potential decreases. Since the difference between the intracellularly recorded responses at two intensities also decreases, the slope of the intensity-vs.-response curve decreases. Second, the extracellular current from $I$-cells, which are not coupled to the impaled cell, is not affected by changing the membrane potential of $II$-cells.* Moreover, it is the extracellular

* Although I never saw uncoupling of cells when one cell was depolarized with current, uncoupling would not be expected to alter qualitatively the predicted change of polarization sensitivity. The possibility of such uncoupling cannot be eliminated in view of the uncoupling, seen at voltages near reversal potential, between retinular cells of Limulus lateral eye (Smith and Baumann, 1969).
current from \(L\)-cells, both normally and when the \(II\)-cells are depolarized with current, which is predicted to have the more significant role in increasing the polarization sensitivity. Since the relative contribution of the extracellular currents to the intracellularly recorded receptor potential of a cell is expected to increase when the cell is depolarized with current from the microelectrode, the polarized-light sensitivity ratio should also increase. Figure 27 shows that this can happen for crayfish retinular cells. In such a case, the closer the membrane potential is to the reversal potential, the higher is the polarization sensitivity. Another possible explanation is that depolarizing the cell with current might change the cell in such a way that the ERG becomes significant when before it was not. Or, the intrinsic polarization sensitivity of a single cell might, through some unknown mechanism, be voltage dependent.

Related to the prediction that making the membrane potential more positive should increase the polarization sensitivity is the prediction that the reversal potential of the receptor potential of the impaled cell will change when the angle of the polarizer is changed. Let us assume that in the absence of extracellular currents the reversal potential is independent of the position of the polarizer. A negative extracellular potential will shift the intracellularly recorded reversal potential to a less positive value. From a polarizer position which maximally stimulates the impaled cell, rotating the polarizer 90° and increasing the light intensity should, therefore, shift the reversal potential
to a more negative value. Figure 26 shows that this does happen. In Fig. 26, the membrane potential actually lies between the two reversal potentials.*

Another prediction is that over the range that both the receptor potential and extracellular potential are logarithmic with intensity, polarization sensitivity will be independent of intensity. The reasoning is as follows. Assuming that the extracellular voltage is logarithmically proportional to the light intensity, we have shown that the difference in extracellular potential when the polarizer is rotated 90° and the intensity doubled is independent of initial intensity (Appendix III). By definition, over the range that the intracellular receptor potential is logarithmic with intensity, increasing or decreasing the intensity by a constant multiplicative factor should change the receptor potential amplitude by a constant amount. If the transmembrane potential is the difference between the receptor potential and the extracellular potential, then the transmembrane potential will also be logarithmic with intensity. Now, if the light is polarized so that it is maximally stimulating a cell and the polarizer is rotated and the intensity doubled, the intensity of light absorbed by the cell will change by a constant factor, and the voltage in the absence of an extracellular current (the transmembrane voltage) would change by a constant amount. The extracellular

* Were an intracellular contribution from \( J \)-cells possible, the reversal potential would shift to a more positive value when \( J \)-cells were preferentially stimulated.
voltage change is also constant. Therefore, the difference in the intracellularly recorded voltage before and after rotating the polarizer and doubling the intensity should be constant. The logarithm of the intensity difference required to match the response amplitude after rotation to that before should therefore be constant. This implies that the polarization sensitivity is constant over the range of intensities for which our assumptions hold. We find, in fact, that the polarization sensitivity is roughly constant over the range for which the receptor potential is logarithmic with intensity (Fig. 17).

The polarization sensitivities which we recorded were markedly less than Shaw's average of 6.2 for Astacus (Shaw, 1969b), and were instead very similar to the results of Waterman and Fernandez (1970), who measured an average of 3.1. Waterman and Fernandez found that over 40% of their cells had polarization sensitivities less than 5. Consideration both of the apparatus for illuminating and of the preparation suggests a basis for the differences. Shaw illuminated with a point source which was perfectly aligned on the optic axis of the penetrated ommatidium. This arrangement keeps scattered light within the eye to a minimum, and light scattering is likely to reduce considerably the amount of polarization of light falling on retinular cells. With a microscope objective, Waterman and Fernandez focused light on the cornea as I did, although I also illuminated the retina directly. While using an objective lens enables one to deliver much brighter stimuli,
the light scattered is likely to be sizeable since illumination is not along the optic axis and several ommatidia are illuminated. (See, for example, Shaw, 1969c, in which he estimates that over half the light striking a light-adapted ommatidium can come through facets other than its own.)

In addition, precise alignment of the beam along the optic axis was not possible, introducing further opportunities for scattering. Furthermore, illuminating the retina directly might well produce more scattering than illuminating through the optics of the eye (although no difference in polarization sensitivities between retinal and corneal illumination was noticed). Finally, light passing through the microscope objective will itself become depolarized, although for a low N.A. objective this effect should be small, and was measured to be less than 5%.

Shaw's preparation was evidently an entire eyestalk, the penetrating electrode passing through a small hole in the cornea. Both my preparation and that of Waterman and Fernandez was a bisected eyestalk; the ommatidia were penetrated by way of the sliced surface. The tissue could have been more easily distorted with the sliced preparation. Were microvilli not normally circular in cross section, they could have become so with a change in both the structural and, possibly, the ionic environment. But perhaps most important, the extracellular resistances have been vastly altered. While the bisected preparation is better suited for studying transmembrane potentials, a number of physiological effects are
likely to be missing which rely on structured extracellular resistive pathways. One example is the possible contribution to the retinular cell's receptor potential of extracellular currents from adjacent ommatidia (Burkhardt and Autrum, 1960; Burkhardt, 1962). Another is that the D.C. bias voltages which might be recorded in the intact eye (see, for example, Burtt, Catton, and Cosens, 1966, or Mote, 1970) are also abolished or altered. It is difficult to evaluate the importance of having an intact eye to a mechanism which enhances polarization sensitivity by means of extracellular potentials. It is possible that such effects could contribute to the high polarization sensitivities measured by Shaw (1969b).

There is a test of the extent to which dichroism, whether of the optics or the retinular cell, produces the large polarization sensitivities seen by Shaw. The test is based on the assumptions: (1) that the ERG is of the form $V_{\text{ERG}} = V_0 \log I$; (2) that both classes of cells contribute equally to the ERG; (3) that cells of one class do not affect the membrane current of the other; and (4) that the absorption of polarized light is proportional to $\cos^2 \theta$ (the Law of Malus). By combining these assumptions (see Appendix IV, based on a similar treatment of Bohn and Täuber, 1971) one concludes that the amplitude of the ERG produced by a rotating polarizer placed in a constant intensity light beam should be modulated. There will be four equal maxima at $45^\circ$ to the microvillar axes and minimal responses when the $E$-vector is in line with
the axes. To match the amplitude of the response to light polarized at 45°, light polarized parallel to the microvilli must be made brighter. The factor by which the intensity must be increased is used to calculate the dichroic ratio N. For example, if N = 2, then 7% more light is required; if N = 5, 34% more light is needed; and if N = 10, one must deliver 73% more light at 0° or 90° to match the response at 45°. A 4-peaked modulation of the amplitude of the ERG with rotation of the polarizer has been seen before (Waterman and Horch, 1966; Bohn and Täuber, 1971). However, the sensitivity ratio has never been determined; it is only from the polarization sensitivity ratio that the dichroic ratio can be calculated. To make such a calculation, the polarization sensitivities of cells near the electrode must be maximal and of similar size. I am unable adequately to apply this test of dichroic ratio, because the polarization sensitivities I see are usually between 2 and 4, and never above 6. The effective dichroic ratio would be less than the polarization sensitivity, hence little modulation would be expected. However, the experiment of Fig. 21 shows the predicted 4-peaked modulation in crayfish. Accurate measurement of the polarization sensitivity of the ERG is difficult, but a "dichroic ratio" of 3± 1 is obtained. Although the polarization sensitivities of the neighboring cells are both at least 5, the difference is not significant enough to prove the hypothesis that extracellular currents increase a basic polarization sensitivity. From Shaw's experiments, we should
be able to measure dichroic ratios on the order of 6 or 7. However, an apparatus such as that used by Shaw would be required. The difference between the dichroic ratio measured from the ERG and the polarization sensitivity of the intracellular response should indicate the contribution of "enhancement" effects to the overall polarization sensitivity of the retinular cell's receptor potential.

The lack of electrical coupling which we found between cells whose maximum polarized-light sensitivities were at right angles is consistent with maintaining a maximal sensitivity to polarized light. However, in this respect it is surprising that the retinular cells in the ommatidium of honeybee drones, whose relatives the workers are the best examples of the functional importance of polarized light detection, are reported by Shaw (1969a) to be weakly coupled and to have a polarization sensitivity less than 2. The drone and locust microvilli are not stacked in bands as in decapod rhabdoms. This indicates that extracellular current paths of retinular cells may be more separate for honeybee drones and locusts than for decapods. One wonders if this could account for part of the low polarization sensitivity.

It seems likely that the neural analysis of the angle of polarization, consisting of a comparison of responses not only of cells with different microvillar orientations, but perhaps also of different spectral sensitivities,
takes place at one of the four ganglia in the eyestalk.* An understanding of this region is next required.

Large extracellular potentials have been demonstrated in the arthropod compound eye. Although the intact preparation is not well suited to understanding membrane phenomena, the final resolution of the physiological functioning of the organism will require experiments on the intact eye.

I have hypothesized that the dichroic absorption ratio of crayfish retinular cells is lower than the polarization sensitivity, and that the process which increases the polarization sensitivity is mediated, at least in part, by extracellular currents. Although the case for such currents increasing the polarization sensitivity is strong, the magnitude of the contributions from extracellular currents has not been shown. Yet to be answered are such questions as what is the dichroic ratio of living retinular cells, and is the alignment or position of a rhodopsin molecule in the membrane important in its coupling to the generator mechanism? If extracellular currents increase the polarization sensitivity measured in the cell body, one wonders if the same polarization sensitivity is preserved in the axon terminals.

* To make this analysis it seems likely that the crayfish must move its eyes or its body, for light polarized at $45^\circ$ to the horizontal would otherwise be indistinguishable from unpolarized light. Another ambiguity could arise from the identity between the degree of stimulation by light polarized at angles of $\theta$ and $\pi-\theta$. A locust, which has three orientations of microvilli, should have no such points of ambiguity.
Finally, our experiments have not only been important in understanding polarized-light sensitivity in arthropods, but they have suggested interactions which must be considered in other investigations. The membrane phenomena associated with the receptor potential cannot be studied in isolation in crayfish, but any elucidation of receptor potential mechanisms will require taking into account the effects of coupling, extracellular potentials, and a high access resistance to the rhabdom.
PART II:

Introduction

The first electrical responses to light that were recorded with microelectrodes in the compound eye of the crayfish *Procambarus clarkii* were local electroretinograms (Naka and Kuwabara, 1959). Naka and Kuwabara placed a microelectrode in the retinular cell layer and observed that white light produced a biphasic ERG. At the onset of the light, they recorded a brief (~40 msec) transient, positive voltage change as large as +10 mv., followed by a negative potential as large as -10 mv. which lasted for the duration of the light, and which returned to ground voltage within a second. Naka and Kuwabara recorded from eyes which had been bisected longitudinally and from eyes which were penetrated through a small hole in the cornea. The ERG's measured distal to the basement membrane were similar in the two preparations. They reported that during long-term recording the positive component disappeared before the negative component did, but was less affected by light-adaptation.

Eguchi (1965) impaled single crayfish retinular cells with microelectrodes and was able to measure receptor potentials which were stable for several hours. He was interested in correlating changes in the fine structure of the rhabdom with changes in the receptor potential produced by months of continuous darkness or by long-term perfusions of the excised
eye with various solutions. The changes in fine structure have been questioned as a fixation artefact (Kabuta, Tominaga, and Kuwabara, 1968). Relevant to our study, however, are Eguchi's findings that replacing sodium chloride with sucrose in the perfusate reversibly reduced the initial large transient and the smaller plateau components of the receptor potential.

Glantz (1968) examined light-adaptation in the excised Procambarus eye by recording intracellularly from single photoreceptors. He studied light-adaptation in the intact eye by measuring a local ERG with an intraretinal micro-electrode. Glantz did not see the receptor potential overshoot zero voltage. He consequently suggested that the receptor potential is generated by a non-specific increase in membrane conductance similar to that described for the motor endplate (del Castillo and Katz, 1954).

There have been two studies on wavelength sensitivity of crayfish retinular cells. Noguchi (1969) found that in the summer, 10% of the cells from which he recorded in Procambarus clarkii were most sensitive to 460 nm light, while the remainder were sensitive to light of wavelength greater than 600 nm. In the winter, cells maximally sensitive to blue light were not found, and the predominant wavelength of maximal sensitivity was 560 nm. Using Nosaki's apparatus in the autumn, Waterman and Fernandez (1970) found that 20% of the cells from which they recorded in the anterior-dorsal quadrant of the eye responded maximally to 440 nm light, while the remainder responded maximally to light of from 538 to 634 nm.
It is likely that much of the variation in long-wavelength sensitivity is due to filtering by the screening pigment. Recording from the optic nerve of Procambarus, Woodcock and Goldsmith carefully studied the effects of screening pigment migration and pigment filtering on spectral response curves. Ninety percent of dark-adapted units had a maximal sensitivity to 560 to 570 nm light. Filtering by migration of the screening pigment to the light-adapted position produced a shift in the maximum sensitivity of such units to longer wavelengths by as much as 80 nm. Pigment migration had little effect on short wavelength units. The excised eye, when stimulated with dim lights, exhibits a pigment migration to the light-adapted state even when initially dark-adapted (Shaw, 1969). Using excised eyes, Nosaki illuminated retinular cells from the side, often passing light through several layers of ommatidia, and therefore through much screening pigment. Also using excised eyes, Waterman and Fernandez did not illuminate along the ommatidial optic axis, and therefore screening pigment filtered the incident light in their preparations. One can conclude that there are two spectral classes of cells in the crayfish eye; no more than 10 to 20% of retinular cells are maximally sensitive to blue light (450 nm), while the remainder are most sensitive to yellow light (560 nm).

The ionic mechanisms which generate the intracellularly recorded receptor potential in crayfish have not been previously investigated. However, the receptor potential of
another crustacean, the barnacle *Balanus* (Brown, Hagiwara, Koike, and Meech, 1970; Koike, Brown, and Hagiwara, 1971), and that of the horseshoe crab *Limulus* (Millecchia and Mauro, 1969a, b; Brown and Lisman, unpublished; Brown and Mote, in press) have been recently studied using the voltage-clamp technique. In the dark, the membrane voltages of both *Balanus* and *Limulus* were found to be dependent upon the external potassium concentration. The barnacle and *Limulus* membranes were insensitive to replacing chloride with isethionate; the membrane is hence considered to be less permeable to chloride than to potassium. An increase of the membrane potential in barnacle and *Limulus* was pronounced following stimulation with bright lights. This hyperpolarization has been characterized as probably produced by an electrogenic pump which preferentially removes sodium from the cell (Koike, Brown, and Hagiwara, 1971; Brown and Lisman, unpublished). The hyperpolarization was reduced or abolished with cardiac glycosides, such as ouabain and strophanthidin, or by reducing the temperature of the preparation. Removing external potassium or replacing external sodium with lithium also reduced the hyperpolarization. Intracellular injections of sodium, even in sodium-free medium, mimicked the effect of illumination of a cell in normal saline by producing a hyperpolarization after the injections.

The conclusions drawn from the voltage-clamp experiments on *Balanus* and *Limulus* were that the receptor potential is caused by an increase of membrane conductance, principally
to sodium ions. The reversal potentials for rapid changes in light-induced current averaged +27 mv for Balanus and about +15 mv for Limulus. Small replacements of external sodium with TrisH or choline produced a change of the reversal potential of from 10 to 15 mv per 10-fold change of external sodium concentration in Balanus. Millecchia and Mauro (1969b) reported a similar small dependence of reversal potential on sodium concentration for total replacement of sodium by Tris, lithium, and choline. However, Brown and Mote (in press) found the reversal potential for the transient component of the receptor potential highly dependent on changes in extracellular sodium. The reversal potential changes by 55 mv per 10-fold replacement of sodium by TrisH or sodium chloride by sucrose, and by 20 to 30 mv. per 10-fold replacement by choline. There is little or no change during replacement with lithium, yet the amplitude of the receptor potential is reduced. That is, from Brown and Mote's observations it appears that the light-induced conductance change functions as a sodium conductance increase would. Since a light-induced membrane current is measured in preparations bathed in sodium-free saline, all three groups postulated that other ions, possible calcium or magnesium, might carry part of that current. In sodium-free saline, for example, the reversal voltage for the barnacle receptor potential was found to vary by as much as 20 mv per 10-fold change of calcium. A calcium-selective (Nernst) electrode would vary by 29 mv per 10-fold change of calcium.
Furthermore, when the barnacle eye was perfused with calcium-free saline, the reversal potential changed by no more than -20 mv per decade reduction in external sodium.

Calcium appears to be capable of controlling the light-induced sodium flux through the membrane. In both Balanus and Limulus, reducing the concentration of external calcium in the presence of 430 mM Na⁺ increased the membrane current without appreciably altering the reversal potential. Increasing the concentration of external calcium produced the opposite effect.

Another light-induced change in membrane current has been identified in Limulus by Lisman and Brown (in press). The time course of this component of the light response is much slower than the component studied by Millecchia and Mauro (1969b). The slowly changing current is small at voltages more negative than resting potential; it appears as a negative (inward) current for voltages from resting potential up to +30 or +40 mv., which is well above the reversal potential for the more rapid light-induced current. Beyond +30 or 40 mv. the slow current again becomes small and may reverse in sign. As a consequence of the slower light-induced current, which lasts for many minutes, the current-voltage relation (I-V curve) in the dark following illumination is "steeper" (i.e. has a higher slope resistance) from resting voltage up to ground voltage than the I-V curve before illumination.

The nature of the receptor potential in retinular cells
of the honeybee drone has been studied by Fulpius and Baumann (1969) using single electrodes. For this study they did not attempt to change membrane potential with current, but they did alter the sodium, potassium, and calcium ion concentrations in the bathing medium. They found that the membrane resting voltage was sensitive to potassium; the resting potential increased by more than 25 mv. when the external potassium was increased 10-fold.* Also, replacing the external sodium by lithium caused the membrane to depolarize. As was found for Balanus and Limulus, replacing sodium with TrisH or choline diminished both the initial transient and smaller plateau phases of the receptor potential. The absolute magnitude of the receptor potential declined in preparations for which lithium replaced sodium, but the voltages reached by both the transient and plateau components increased. The effect of replacing sodium chloride by sucrose was similar to sodium replacement by choline. Finally, Fulpius and Baumann observed that low calcium increased and high calcium decreased the amplitude of the plateau component of the receptor potential. The main effect on the transient phase was a prolongation in low calcium and depression in high calcium. In an earlier study, Fulpius and Baumann (1966) reported that when chloride was replaced by propionate, the amplitudes of both the transient and plateau components were increased (although the transient did not increase

* Another effect of high external potassium was to increase the voltage reached by the receptor potential in response to less than saturating intensities of light.
Fulpius and Baumann concluded that the receptor potential of the honeybee drone is generated principally by a conductance increase to sodium ions, and possible also to potassium and chloride ions. They argued that calcium acts not as a charge carrier, but as a regulator of sodium- and perhaps other currents.

Although the ionic dependence of the intracellularly recorded receptor potential has not been studied in crayfish, Stieve and Wirth (1971) examined the ion dependence of the gross ERG of the retinular cell layer of the crayfish Astacus leptodactylus. They found that when sodium was replaced almost entirely by choline or entirely by potassium, the ERG reversibly diminished. Removing calcium increased the amplitude of the ERG. These results are consistent with those mentioned for honeybee drone, barnacle, and Limulus, but the ERG is not an interpretable measure of membrane current or of intracellular voltage. It can only be assumed that it is produced by retinular cells. The ERG is likely to be exquisitely sensitive to the structure of the extracellular space, the current paths in that space, and the mobility of charge carriers. Hence, in order to study membrane mechanisms it is important to measure intracellular receptor potentials.

One cannot expect an alteration of the ionic content of the saline which bathes the crayfish eye to produce as rapid effects on the receptor potential as do ion changes.
with _Limulus_ ventral eye and barnacle preparations. Crayfish retinular cells cannot readily be freed from the surrounding tissue. Furthermore, each rhabdom is surrounded by seven cells whose membranes are closely apposed and create a diffusion barrier.

The intracellularly measured voltages might be affected by extracellular voltages which are generated by membrane currents in the rhabdom. Moreover, the coupling which exists between cells, at least in the dark, might affect voltage measurements made during the injection of currents through the recording electrode.

The small size of crayfish retinular cells, suspended in the opaque, soft tissue of the eye, makes penetration of cells with more than one electrode extremely difficult. The amount of current which can be injected while recording voltages with the same electrode is limited and a voltage-clamp cannot be used.

We must take into account the possible effects described above which might be attendant on recording from a confined system when we interpret our experiments on the ionic mechanisms which generate the receptor potentials of crayfish retinular cells. As a result, the experiments which we will report do not prove that the mechanisms found for _Balanus_ and _Limulus_ do or do not generate the receptor potential in crayfish. Instead, the results indicate that there are differences which might be attributed to the structure of the crayfish compound eye, or possibly to differences in mechanisms which regulate the distribution of ions across the cell membranes.
Methods

Bisected eyestalks of the crayfish *Procambarus clarkii* were prepared as previously described (Methods, Part I) and perfused with solutions which were nearly isosmotic with van Harreveld's saline (van Harreveld, 1936) and, where possible, of the same total ionic strength (see Table II). Because light entered the perfusion chamber through a thin glass window, changes in the level of the solution during perfusion did not affect the light path. The volume of the perfusion dish was about 0.5 ml, and could be exchanged within a fraction of a minute. The light stimulus and the apparatus used to inject current through the recording electrode were described earlier (Methods, Part I). From the voltages measured during pulses of constant current, a current-voltage relation (I-V curve) can be plotted. We also displayed "steady-state" I-V curves directly by monitoring current on the horizontal axis of the oscilloscope while slowly and continuously varying the current injected into the cell (dV/dt was less than 5 mv/sec). The I-V curves determined by injecting current pulses of known amplitude and measuring the "steady-state" membrane changes were equivalent to those I-V curves displayed directly on the oscilloscope (Fig. 27).

Except where otherwise noted, the cells which we studied were maximally sensitive to orange rather than blue light;
Table II. Compositions of crayfish Ringer solutions.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Choline</th>
<th>Li⁺</th>
<th>Sucrose</th>
<th>Cl⁻</th>
<th>HCO₃⁻</th>
<th>Ac⁻</th>
<th>(in mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Ringer</td>
<td>207.7</td>
<td>5.4</td>
<td>13.6</td>
<td>2.6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>243.0</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Choline for Na⁺</td>
<td>--</td>
<td>5.4</td>
<td>13.6</td>
<td>2.6</td>
<td>207.7</td>
<td>--</td>
<td>--</td>
<td>243.0</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Lithium for Na⁺</td>
<td>--</td>
<td>5.4</td>
<td>13.6</td>
<td>2.6</td>
<td>--</td>
<td>207.7</td>
<td>--</td>
<td>243.0</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Sucrose for NaCl</td>
<td>--</td>
<td>5.4</td>
<td>13.6</td>
<td>2.6</td>
<td>--</td>
<td>--</td>
<td>415.4</td>
<td>35.3</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Na⁺ for Ca²⁺</td>
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<td>5.4</td>
<td>--</td>
<td>2.6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>229.4</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Mg²⁺ for Ca²⁺</td>
<td>207.7</td>
<td>5.4</td>
<td>--</td>
<td>16.2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>243.0</td>
<td>2.4</td>
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<td></td>
</tr>
<tr>
<td>Choline for Ca²⁺</td>
<td>207.7</td>
<td>5.4</td>
<td>--</td>
<td>2.6</td>
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<td>--</td>
<td>--</td>
<td>229.4</td>
<td>2.4</td>
<td>--</td>
<td></td>
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<tr>
<td>Li⁺ for Ca²⁺</td>
<td>207.7</td>
<td>5.4</td>
<td>--</td>
<td>2.6</td>
<td>13.55</td>
<td>--</td>
<td>--</td>
<td>229.4</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Li⁺ for Na⁺(Ca²⁺-free)</td>
<td>5.4</td>
<td>5.4</td>
<td>--</td>
<td>2.6</td>
<td>--</td>
<td>221.3</td>
<td>--</td>
<td>229.4</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>1 x K⁺</td>
<td>154.0</td>
<td>5.4</td>
<td>13.6</td>
<td>2.6</td>
<td>53.7</td>
<td>--</td>
<td>--</td>
<td>243.0</td>
<td>2.4</td>
<td>--</td>
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</tr>
<tr>
<td>0.1 x K⁺</td>
<td>154.0</td>
<td>0.54</td>
<td>13.6</td>
<td>2.6</td>
<td>58.5</td>
<td>--</td>
<td>--</td>
<td>243.0</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>5 x K⁺</td>
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<td>2.6</td>
<td>26.9</td>
<td>--</td>
<td>--</td>
<td>243.0</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>10 x K⁺</td>
<td>154.0</td>
<td>53.7</td>
<td>13.6</td>
<td>2.6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>243.0</td>
<td>2.4</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Acetate for Cl⁻</td>
<td>207.7</td>
<td>5.4</td>
<td>13.6</td>
<td>2.6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>2.4</td>
<td>243.0</td>
<td></td>
</tr>
</tbody>
</table>

pH of all solutions adjusted to 7.0-7.8.
Figure 27.

The current-voltage (I-V) curve (open circles) obtained by plotting the amplitudes of injected current pulses vs. the steady-state voltages produced by those pulses is identical with the I-V curve displayed directly on the oscilloscope screen (solid lines). Calibration is 25 mv, 0.4 na per division, and inset cal is 10 mv, 0.16 na, 100 msec.
the cells were normally stimulated with white light. Although qualitative differences appeared in the waveforms of receptor potentials recorded from blue- and orange-sensitive cells, other differences were not found. The amplitude of the steady or plateau phase of the receptor potential of blue-sensitive cells was usually disproportionately smaller than the amplitude of the transient phase.

To change the temperature of the preparation, a chamber containing the bisected eye was placed upon a Peltier module. A thermistor probe placed in the saline bath measured the temperature.
Results

Characteristics of the retinular cell in normal Ringer.

As Eguchi (1965) and Glantz (1968) observed, a sufficiently bright pulse of light produces a large, transient, positive change in the intracellular voltage of crayfish retinular cells; this transient component is followed by a smaller depolarization, the "plateau", which is coterminal with the light (Fig. 28). At low light levels the initial depolarization does not exceed the plateau. With bright stimuli, the transient component of the receptor potentials of sensitive cells is seen to overshoot zero voltage by up to 10 mv. In Fig. 29 are plotted the intensity-vs.-response curves for the transient and plateau components using, in one case, filters of decreasing and then increasing density, and in the other, neutral density filters selected at random. In cases in which intensities greater than that which causes the transient to saturate were used, the series of increasing and then decreasing intensities showed "hysteresis". A flash of light 200 msec long is sufficient for the transient to attain its maximum height for that intensity.

The healthy crayfish photoreceptor does not generate either a "spike-like" component on the rising phase of the light response or an electrically evoked "spike" such as have been described in Limulus (Benolken, 1959, 1965; Fuortes and Poggio, 1963; Millecchia and Mauro 1969a).
Figure 28.

Responses to increasing intensities of 1 second illumination. Numbers to the left of voltage traces are the attenuation of the incident light in log units. LM indicates the timing of the flashes and is positioned at ground voltage for the brightest flash. The membrane resting voltage was constant during the series.
Figure 28
Figure 29.

Intensity vs. response curves for the cell of Figure 28. The slope of the least squares fit for the transients of the continuous series (solid line on the left and dashed line on the right) is the same as that for the random series (solid line). One sec flashes were delivered every 30 sec.
Figure 29
A typical I-V curve is not linear in the dark (Fig. 27); its slope is less for more positive voltages. In a dark-adapted cell, the resting voltage is at or below the "break-point" of the I-V curve. During illumination, the slope resistance of the I-V curve is lower at any particular voltage than is the slope resistance at that voltage following illumination (Fig. 30). The slope resistance in the light decreases more the brighter the stimulus. The voltage at which the I-V curves measured during the light and following illumination intersect is the voltage at which the "off" of the receptor potential reverses (Fig. 30). The reversal potential is approximately constant for a given cell, and is usually between +5 and +15 mv (range 0 to +20mv).

The effect of changing the temperature.

Over the range of temperatures measured, from 2 or 3° C to above 25° C, the resting voltage of the retinular cell was an approximately linear function of temperature (Fig. 31). For 8 of 9 cells, the change in voltage was from 2 to 3 times greater than would be expected of a membrane whose voltage was dependent upon the Nernst equilibrium potential of a single ion \( E_j = \frac{RT}{zF} \ln \frac{[\text{out}]}{[\text{in}]} \). (The factor for the ninth cell was 1.5.) The amplitude above resting voltage of the response to a 20 msec flash delivered every 10 seconds diminished as the temperature was reduced below 15° C (Fig. 31).

The effect of changing the concentration of external potassium.

The resting potential of the crayfish retinular cell was
The reversal potential is the voltage at which the I-V curves measured in the light (L) and dark (D) intersect. The reversal in the figure at left is indicated on the right by a dashed line. The light monitor trace (LM) is positioned at zero voltage and indicates the timing of the light flash. \textit{Vcal} is a 10mv-100msec calibration pulse. The -0.2 na current pulse tests bridge balance.
Figure 30
Figure 31.

The temperature dependence of the resting potential (circles) and responses to 20msec light flashes (triangles). The temperature was increased (open symbols) and then decreased (filled symbols). The slope of the solid line is 2.5 times that of a membrane voltage dependent upon a Nernst potential.
Figure 31
sensitive to the external concentration of potassium (Fig. 32). For intermediate light intensities, the voltage reached by the receptor potential was constant up to 5 times the normal concentration of potassium. Above this concentration, the voltage at the peak of the receptor potential increased. Reversible changes larger than in Fig. 32 were not seen.

**Alteration of the concentration of external sodium.**

When sodium ions were partially replaced by lithium ions, the retinular cell depolarized (Fig. 33). The voltage at saturation of the response to a 200 msec flash was nearly unchanged with partial replacement of sodium by lithium (Fig. 33); however, the amplitude of a response to less than saturating light was reduced. The voltage reached by the receptor potential did not always change, but the resting voltage became markedly less negative. When 90% of the external sodium was replaced with lithium, the reversal potential measured during the perfusion remained nearly constant (Fig. 34).

Replacing sodium with choline ions slightly depolarized the cell in the dark. The voltages reached by both the transient and plateau phases of the receptor potential were more negative, but the reversal potential was not measureably changed (Fig. 35). The voltage at which the transient saturated also did not change (Fig. 36a). The intensity-vs.-response curves for this cell can be thought of as having shifted to the right along the intensity scale with decreasing
Figure 32-A.

The time course of voltage changes during potassium perfusion. Fixed intensity flashes of 200msec duration were delivered every 15 seconds. $V$ is the intracellularly recorded voltage and $V_{\text{ref}}$ is the voltage recorded by an electrode placed in the tissue near the impaled cell.

Figure 32-B.

The dependence on the concentration of external potassium of the resting potential ($V_{\text{rest}}$) and response to a fixed intensity flash ($V_{\text{resp}}$) of the cell in (A). The open circles are the voltages measured by the electrode in the tissue.
Figure 32-A
Figure 32-B

A

0

0

0

0

5

mM

K+

0

10

20

V

V_{\text{resp.}}

-20

-30

-40

-50

V

V_{\text{rest}}

-60

0.5

2

50

mM K^+

Figure 32-B
Figure 33.

Sodium replacement by lithium. The triangles are the voltages reached by receptor potentials to 200 msec flashes of saturating intensity. The circles are the responses to light flashes of fixed intensity (V 4.8); and the resting voltages are indicated by squares (Vrest).
Figure 33
Figure 34.

The effects of sodium replacement by lithium on the reversal potential, $V_{\text{rev}}$ (triangles), the voltage reached by a response to a fixed intensity flash 50 msec long ($V_{\text{resp}}$, circles) and the resting membrane potential, $V_{\text{rest}}$ (squares).
Figure 34

Graph showing the changes in $V_{\text{rev}}$, $V_{\text{resp}}$, and $V_{\text{rest}}$ over time in minutes. The graph includes data points with error bars for $V_{\text{rev}}$ and $V_{\text{resp}}$. Notations for time points include '0', '5', '10', '15', and '20' minutes._labels: #1

- $V_{\text{rev}}$ (voltage, mv)
- $V_{\text{resp}}$ (voltage, mv)
- $V_{\text{rest}}$ (voltage, mv)

Time, min.

Figure 34
Figure 35.

The effects of sodium replacement by choline on the reversal potential, $V_{\text{rev}}$ (open triangles); the voltages reached by the transient, $V_T$ (solid triangles), and plateau, $V_p$ (circles), components of the receptor potential upon flashing a bright, fixed intensity light, and the resting membrane potential, $V_{\text{rest}}$, (squares).
Figure 35
Figure 36.

A. A comparison of the reversal potentials (V\text{rev}, triangles) of responses to 200 msec flashes with the voltages reached by responses to saturating intensities of light (V\text{sat}, circles) during sodium replacement by choline. V\text{rest} (squares) is resting voltage.

B. Intensity-vs.-response curves for the series in A. Filled symbols are peak responses and open symbols are resting membrane voltages.
Figure 36
concentrations of sodium (Fig. 36b).

When the external sodium chloride was partially exchanged for sucrose, the transient and plateau components were again diminished while the reversal potential was unchanged (Fig. 37). With 200 msec flashes, the voltage at which the response saturated was not constant (Fig. 38). In Fig. 39 we compare the saturating responses and reversal potential measurements for choline replacing sodium and for sucrose replacing sodium chloride. When sucrose replaced sodium chloride, a negative component, which became more pronounced as reversal potential was approached, appeared on the initial phase of the receptor potential. The latency of the negative-going component was approximately the same as that of the peak of the response to saturating light intensities. Such a negative component was not seen when choline replaced sodium.

The effect of removing external calcium.

When either sodium or magnesium replaced calcium ions in the bathing solution, the amplitude of the receptor potential increased (Fig. 40). The voltages reached at late times during the flash of light increased more than did the initial voltages, resulting in a "squaring" of the waveform. When lithium was used to replace calcium, the reversal potential became slightly less positive (Fig. 41). When, in the absence of calcium, lithium also partially replaced sodium, the reversal potential continued to decrease by a few millivolts (Fig. 41). Similar experiments in which choline
Figure 37.

The effects of sodium chloride replacement by sucrose on the reversal potential, $V_{\text{rev}}$ (inverted triangles); the voltages reached by the transient, $V_t$ (triangles), and the plateau, $V_p$ (circles), components of the receptor potential upon flashing a bright, fixed intensity light; and the resting membrane potential, $V_{\text{rest}}$, (squares).
Figure 37
Figure 38.

The effects of sodium replacement by sucrose on the intensity-vs.-response curves measured with flashes 200 msec. long. Filled symbols are the peak voltages and open symbols are the resting membrane voltages.
Figure 38
Figure 39.

A comparison of receptor potentials at saturating intensities of light and reversal potentials, measured when sodium is partially replaced by choline and sodium chloride is partially replaced by sucrose. The light monitor trace (LM) indicates the timing of the flash and is positioned at ground voltage. The -0.5 na current pulses test bridge balance.
Figure 40.

The equivalence of calcium replacement by sodium or by magnesium. The traces in calcium-free solutions were measured about 5 minutes after perfusion was begun; the return to normal followed a similar time course. The light monitor traces (LM) indicate the timing of the constant intensity flashes and are positioned at zero voltage.
Figure 40
Figure 41.

The effects of calcium replacement by lithium and by choline on the reversal potential, $V_{\text{rev}}$ (inverted triangles), the response to a flash of fixed intensity, $V_{\text{resp}}$ (circles), and the resting membrane potential, $V_{\text{rest}}$ (squares). Open symbols are measurements in normal Ringer. When calcium was replaced by lithium, lithium was then used to replace sodium. The lithium and choline replacements were done with different cells.
replaced sodium were not successful (bathing in solutions low in calcium and with sodium partly replaced by choline irreversibly reduced the responses of cells). However, when choline replaced external calcium ions (Fig. 41), the reversal potential dropped by 10 mv to below zero voltage. The amplitude of the response increased when calcium was removed.

The effects of replacing external chloride ions by acetate.

When the chloride ions in the saline bath were replaced by acetate, the amplitude of the receptor potential diminished (Fig. 42). The reversal potential became negative by an even greater amount than the reduction in the response amplitude (Fig. 43).* One can compare the intracellular receptor potential with the local ERG, measured in the region of the rhabdom. At similar times following ion changes, the amplitude of the electroretinogram increased as the amplitude of the intracellularly recorded response decreased (Fig. 43).

Miscellaneous observations:

(i) The effects of ouabain.

After the response to a bright light, the membrane frequently became more negative than before illumination (Fig. 44a). This hyperpolarization lasted less than 2

* Only a portion of the voltage changes might be attributed to a change in the junction potential at the indifferent electrode. An indication of the size of such an effect is given by the -5 mv. rapid change in voltage measured by an electrode placed in the tissue near the site of intracellular recording when the eye was perfused with chloride-free solution (Fig. 43). Never were larger changes than -5 mv. seen.
Figure 42.

The time course of the effects of replacing chloride with acetate on the resting membrane potential and responses to 200 msec flashes of fixed intensity light delivered every 15 seconds (V), and on the voltage measured by an electrode placed in the tissue, \( V_{ref} \) (vertical lines are 10 mv, 100 msec calibration pulses).
Figure 42
Figure 43.

The effects of replacing chloride with acetate on the response to a fixed intensity light (first column), the reversal potential (second column) and locally recorded extracellular voltage, Vext (third column). The response amplitudes in normal Ringer are indicated with dashed lines on the figures in chloride-free solutions. The extracellular responses in chloride-free solution occurred 1.5 and 5 minutes after the perfusion was begun. The light monitor trace (LM) is positioned at zero voltage in the first and second columns, and in all cases indicates the timing of the 200 msec flash.
Normal Ringer

10 mv
0.1 sec

Chloride-free, Acetate

Normal Ringer

Figure 43
Figure 44.

The effects of 1 mM ouabain on the receptor potential, the hyperpolarization following the light, and the I-V curves.
Figure 44
minutes. Within 4 minutes after 1 mM ouabain was added, the membrane in the dark depolarized. The hyperpolarization which followed illumination was absent following the same intensity flash as that which hyperpolarized the cell before ouabain was added (Fig. 44d). The voltages measured by the electrode during the light were nearly unchanged after adding ouabain. Forty-five minutes later the amplitude of the response to the same light had diminished to the level shown in Fig. 44g. The I-V curves measured before and after the light (Fig. 44b and c) may be nearly superimposed by a translation along the current axis of the I-V curve measured after illumination. The I-V curves measured before and after light following addition of ouabain (Fig. 44e and f) can be almost directly superimposed (but differ slightly in slope). The break points of the curves occur at the same value of current. The I-V curve measured 4 minutes after the addition of ouabain (Fig. 44e) may be superimposed upon that measured before ouabain (Fig. 44b) by a negative translation along the current axis. The shapes of the I-V curves slowly changed while the response amplitude diminished (Fig. 44g, h, and i). The effects of ouabain were not reversible.

(ii) Other characteristics of the ERG.

As described previously (Naka and Kuwabara, 1959), the electroretinogram measured with a microelectrode in the region of the retinular cells can be biphasic. The waveform of the ERG can depend critically upon the position of the
electrode, the condition of the preparation, and the intensity of the stimulus. The relative amplitudes of the positive and negative components depend upon the wavelength of the stimulus. Fig. 45 shows the extracellular response to red light and the response to blue light. After a red adapting light was shone on the eye, the response to a blue light revealed that only the negative component was attenuated (Fig. 45). The response to red light was merely diminished by red adaptation. Although at times a positive component appeared on the response to red light, never was the amplitude of the positive component relative to that of the negative component larger in response to red light than to blue light. Blue adaptation was not convincingly seen to selectively adapt one component in response to either a red or blue flash. When current was injected through the recording electrode, neither the amplitude of the ERG changed nor was a charging curve present. When the electrode was removed from the recording sites, D.C. potential changes were never greater than 1 or 2 mv.
Figure 45.

The selective adaptation of the negative component of the extracellularly recorded response to blue light with a red adapting light. No positive component is seen here on the response to red light.
Figure 45
Discussion

Similarities with other invertebrate photoreceptors.

Crayfish retinular cells generate a receptor potential which resembles the light response of many other invertebrate photoreceptors, such as those of the barnacle Balanus, the horseshoe crab Limulus, and the honeybee drone. Because of the similarities between the mechanisms which apparently generate the receptor potentials of Balanus and of Limulus ventral eye photoreceptors, one would not be surprised to find that Procambarus retinular cells use a mechanism like that of Balanus or of Limulus photoreceptors to convert light to electrical signals which are meaningful to the animal's nervous system.

As was found for Balanus, Limulus, and honeybee drone, the resting potential of the crayfish retinular cell is dependent upon the concentration of external potassium. That the resting voltage does not vary as a Nernst potential with changing extracellular potassium indicates either (i) that the internal concentration of potassium changes during perfusion, (ii) that the concentration of potassium in extracellular spaces does not change as much as in the bath, or (iii) that mechanisms in addition to potassium permeability determine the resting potential. Although it is possible that the internal concentration of potassium could change during perfusion, the large intracellular
volume compared to the volume of extracellular spaces makes it more likely that the extracellular potassium concentration is regulated or that diffusion barriers limit changes in extracellular potassium. The close association of retinular cells with surrounding glia and the known permeability of the glial membranes to potassium make it possible that glial cells could "buffer" the external potassium concentration; this was found not to happen in the extracellular spaces between leech neurons and glia (Nicholls and Kuffler, 1964). In both the leech (Baylor and Nicholls, 1969) and the amphibian (Orkand, Nicholls, and Kuffler, 1966) central nervous systems, potassium efflux during nerve activity can cause potassium to accumulate in the spaces between cells. Light causes an efflux of potassium from Limulus photoreceptors (Holt and Brown, 1971). A similar efflux from crayfish retinular cells during stimulation could cause the level of potassium outside the cell membrane to differ from that in the saline bath.

It is likely that mechanisms other than potassium permeability also determine the resting potential. Because the resting potential did not change when chloride was replaced with acetate, but the potential measured with an extracellular electrode deep in the tissue did become negative, it is possible that a portion of the membrane resting potential is determined by chloride ions. There is evidence for a metabolic, hyperpolarizing component of the membrane potential in crayfish retinular cells (c.f. studies on mollusk neurons:
Carpenter and Alving, 1968; Gorman and Marmor, 1970), which we shall discuss in detail later. Furthermore, reduced temperatures depolarized the retinular cell in the dark 2.5 times more than would be expected of a cell whose membrane potential was dependent solely upon a potassium Nernst potential.

The amplitude of the crayfish receptor potential diminished in response to a flash of light of constant intensity when the external sodium was replaced by lithium or choline ions or when sodium chloride was replaced by sucrose. This behavior was similar to that of Limulus, Balanus, and Apis drone photoreceptors during such perfusions. Choline is presumably relatively impermeant to the membrane, and sucrose is uncharged. Lithium is likely, but not necessarily, less permeant than sodium because lithium has a larger hydrated radius. As a result, one would expect the membrane current to be reduced and the response amplitude to decline when sodium is partially replaced by an impermeant ion.

The depolarization which often accompanied sodium replacement, particularly replacement by lithium, might be related to the concurrent diminution of the hyperpolarization after illumination. Sodium replacement by lithium is known to reduce effects which have been attributed to an electrogenic sodium pump. If such a pump were active across the crayfish retinular cell membrane at rest, the depolarization which occurs during the perfusion of lithium-rich solutions could be due to the
inactivation of such a pump. Alternatively, a resting membrane more permeable to lithium than to sodium could produce a similar effect.

The increase in amplitude of the crayfish receptor potential when calcium is removed is similar to the effect of calcium removal seen in photoreceptors in other species. If calcium's role were solely that of a charge carrier, one would expect the receptor potential amplitude to diminish when calcium is removed. While it is likely that removing calcium increases the sodium current during the light as suggested for Balanus (Brown, Hagiwara, Koike, and Meech, 1970) and Limulus (Millecchia and Mauro, 1969b; Lisman and Brown, unpublished), other effects of calcium removal must be considered before the role of calcium as a "regulator" of sodium current is accepted.

One effect of low-calcium solutions is a partially reversible increase of coupling resistance between neurons of the septate axon of Procambarus (Asada and Bennett, 1971). Because all coupled retinular cells presumably respond identically, an uncoupling would not be expected to change their response to light. Uncoupling of partially coupled cells could, however, change the measured reversal potential, causing it to become less positive. This could account for part of the reduction in reversal potential with low calcium in both barnacle and crayfish. Because the receptor potential can nearly reach the reversal potential in crayfish, the measured reversal potential is likely the true reversal potential. Therefore,
the explanation of uncoupling of partially coupled cells seems insufficient.

Because removing calcium increases the receptor potential amplitude, it seems probable that one principal, immediate effect of removing calcium is to increase the relative permeability of the membrane to sodium in the light.* In calcium-free solutions, therefore, the reversal potential should become more dependent upon the external sodium concentration. Such an increased dependence, though weak (about 20 mv. per decade concentration change), was observed for Balanus. Because of the long times required for equilibration during perfusions of the crayfish eye, long-term, irreversible effects probably account for the difficulty in measuring reversal potentials in crayfish eye during sodium replacements in calcium-free solutions. Similarly, prolonged bathing in calcium-free solutions has an irreversible effect on Limulus photoreceptors (Millecchia and Mauro, 1969a).

The crayfish photoreceptor hyperpolarizes after stimulation with a bright light in the manner that honeybee drone, barnacle, and Limulus ventral eye photoreceptors hyperpolarize. Although we have not extensively studied the nature of this hyperpolarization, the results we have are consistent with its being generated by an electrogenic sodium pump. The cardiac glycoside ouabain is a known inhibitor of sodium-potassium dependent ATPases. When 1 mM ouabain was added to

* If in addition light normally increases the calcium permeability, one could expect the observed negative shift in reversal potential when calcium is removed.
the perfusing bath, it first eliminated the hyperpolarization which followed the light and it depolarized the cell. An inspection of the I-V curves showed that the depolarization could be interpreted as the removal of a hyperpolarizing current source across the voltage-dependent resistance of the membrane. Ouabain reduced the amplitude of the receptor potential with a very much slower time course.

Differences from other photoreceptors which generate depolarizing receptor potentials.

Despite the apparent similarity between the mechanisms which generate the receptor potentials of crayfish retinular cells, those of the honeybee drone, Balanus photoreceptors, and those in the ventral eye of Limulus, some results of our experiments differ from those of these other systems. We shall see that this does not imply that the membrane mechanisms generating the light response do themselves differ.

One of the most puzzling results is that the reversal potential of the light response is insensitive to replacing sodium by molecules to which the membrane is presumable impermeable. If the light-induced change in the membrane were an increase in conductance to sodium ions alone, then the reversal potential would be expected to decrease by about 60 mv for a 10-fold decrease in external sodium concentration. This assumes that the internal concentration of sodium is constant and that the external concentration of sodium is identical with that in the bath. Because in normal solutions the voltage at which the response to a 200 msec flash
saturates is nearly the reversal potential, we felt that a measure of the voltage for saturation at different sodium concentrations could support our reversal potential results. When only sodium was replaced by another ion, we found that in fact both the reversal voltage and the voltage for saturation of the light response were relatively unaffected by changes of external sodium.

Because the amplitude of the receptor potential elicited by a flash of fixed intensity decreased during sodium replacement by choline, it seems reasonable to assume that choline diffused to the photoreceptive membrane and that choline is less permeant than sodium. The effect of sodium replacement by choline was what would be expected from a reduction of light-induced current without a change in its driving potential. If this is in fact what is happening in crayfish photoreceptors, there are a number of ways of effecting it. One way would be for the cell to regulate the internal sodium concentration so that the ratio of internal to external sodium concentrations remained constant. Such a regulation might be a consequence of a pump whose rate of sodium extrusion was directly proportional to the internal concentration of sodium, and an influx of sodium which was directly proportional to the external sodium concentration. One would then expect that pump inhibitors might cause the reversal potential to become dependent upon the external concentration of sodium. Preliminary experiments using ouabain indicated that this does not happen. Alternatively, we have
evidence that negative-going extracellular voltages induced by light can make the measured reversal potential less positive. Sodium replacement could reduce extracellular voltages (Stieve and Wirth, 1971) by reducing membrane currents, thereby offsetting a negative shift of true reversal potential. This explanation seems unlikely because the reversal potential did not appreciably change in low-sodium solutions even when the light intensity was increased, which would be expected to increase membrane currents.

Considering mechanisms which generate the receptor potential by conductance changes to more than one ion, the measured small dependence of the reversal potential on sodium ion concentration can result, even if sodium is the major charge carrier. For example, imagine a mechanism which involves a light-induced increase of conductance to sodium ions and a decrease of conductance to potassium ions. If we write

\[ V_{\text{rev}} = \frac{\Delta g_{Na} E_{Na} + \Delta g_{K} E_{K}}{\Delta g_{Na} + \Delta g_{K}} \]  

(Takeuchi and Takeuchi, 1960; Brown, Muller and Murray, in press) where here \( \Delta g_{K} \) is negative, then a decrease of sodium concentration which produces a decrease of \( \Delta g_{Na} \) as well as \( E_{Na} \) could result in any value for \( V_{\text{rev}} \), depending upon the values of \( \Delta g_{K} \) and \( E_{K} \). We have not been able to determine the reason for the reversal potential's independence of the external sodium concentration. On this point the results for barnacle are also somewhat paradoxical, for an examination of the data shows that it is only near the normal concentration of sodium that the reversal potential for
barnacle changes by more than a few millivolts. Hence such considerations as the rhabdom as a structural diffusion barrier might account for the slight differences between crayfish and barnacle. If part of the rhabdom were inaccessible to changes in external sodium, a smaller change in reversal potential might be expected for crayfish.

Perhaps the major difference between the experiments with crayfish retinular cells and with photoreceptors of *Limulus*, *Balanus*, and *Apis* drone is the effect of replacing chloride with larger anions, such as acetate. No large, long term effect has been reported during chloride removal in *Balanus* and *Limulus*, except for a deterioration of the *Limulus* ventral photoreceptor when propionate replaced chloride (Millecchia and Mauro, 1969a). Fulpius and Baumann (1966) report that replacing chloride with propionate increases the amplitude of the honeybee drone receptor potential for less than saturating lights. For the crayfish, replacing chloride with acetate can decrease the amplitude of the receptor potential. At least part of this decrease was shown to be real, and not due to a change in junction potential at the indifferent electrode. Because of the restricted extracellular space in the rhabdom and the large negative-going ERG which may be recorded locally in the crayfish ommatidium, we postulate that the reduction in amplitude of the intracellularly recorded receptor potential is due partly to an increased extracellular negativity during the light. Such a negative voltage would effectively subtract from the
transmembrane voltage in producing the receptor potential. If the membrane current did not change during the light response, which would occur if the response were generated as for Balanus or Limulus, but the mobility of extracellular charge carriers decreased thus increasing the extracellular resistivity, such as would be expected if chloride were replaced by acetate, then the negativity outside the photoreceptive membrane would increase during the light. The negative-going electroretinogram would therefore be expected to increase; this happens. The magnitude of the measured electroretinogram is likely to be smaller than the negativity just outside the receptor membrane by an unknown amount.

We have shown that the local ERG is produced by current from cells having both orientations of microvilli (Figs. 19 and 21). When a cell is polarized to reversal potential with current, light-induced currents are still being generated in the rhabdom by the responses from other cells, and these currents might shift the apparent reversal potential to a more negative value than the "true" reversal potential. The reversal potential became more negative during the replacement of chloride with acetate, considerably more negative than the shift of junction potential. That the reversal potential changed by even more than the receptor potential amplitude might be explained by the following argument. If extracellular currents shift the measured reversal potential to more negative values than the true reversal potential, then there is still a net light-induced
membrane current at the measured reversal potential. If the extracellular resistance is increased by removing chloride, thus making the measured reversal potential more negative, the transmembrane potential at the measured reversal potential will be even further from reversal potential. This will cause an increase in light-induced membrane current at the measured reversal potential, which itself can increase the amplitude of the negative extracellular potential. It appears likely that the crayfish photoreceptive membrane is no more permeable to chloride than is that of the barnacle photoreceptor, but that the structure of the crayfish eye, with the resulting large extracellular potentials, accounts for the differences between Procambarus and Balanus.*

When sucrose replaces sodium chloride the number of charge carriers in the extracellular spaces drops. It is then possible that a subtraction by extracellular voltages from the transmembrane potential attenuates the intracellularly measured response and reduces the voltage at which the transient saturates. The largest membrane currents are probably generated soon after the light has flashed, and it is at this time that a negative component of the receptor potential was seen in solutions for which sucrose had partially replaced sodium chloride (Fig. 39).

Spectral adaptation of the electroretinogram.

The dependence of the ERG upon chromatic adaptation and electrode location was not investigated thoroughly. Our

*In fact, one would expect a positive shift in reversal potential with acetate replacement of chloride if the chloride conductance changed during the light.
results therefore merely suggest further investigations. It has been argued that the initial positive-going component of the *Procambarus* ERG is generated at a more proximal location (possibly at the retinular cell axons) than the negative component, which is believed to be generated in the region of the rhabdom (Naka and Kuwabara, 1959). In *Drosophila* it has been proposed by Alawi and Pak (1971) that cells in the lamina produce the initial, transient, positive component. For both these studies the retina was effectively considered a one-dimensional structure with regard to current flow. Neither group of investigators reported studies of the spectral dependence of the local ERG. Wald (1968) noticed a slight, positive inflection of the negative ERG of *Procambarus* in response to blue light; the inflection did not appear in response to red light. Our recordings effectively amplify the component which Wald noticed as an inflection. Blue-sensitive cells depolarize in response to blue light, hence the mechanism which produces a positive-going response to blue light in the ERG is unclear. (Possibly, for example, the electrode was near a current source. The involvement of capacitance must also be considered.) It is of course possible that the generator of the positive component of the ERG is a cell which receives synaptic inputs from blue-sensitive cells, but from the short latency of the positive component it seems likely that the positive component is at least partly produced by the blue-sensitive cells themselves. A source-sink analysis would be desirable to
determine the sites of generation of the components of the ERG.

The results of our experiments on the ionic mechanisms which generate the receptor potential of retinular cells in the compound eye of the crayfish largely agree or can be reconciled with the results of experiments which have explained the mechanisms which function in photoreceptors of the barnacle _Balanus_ and the horseshoe crab _Limulus_. Our results are also similar with those from _Apis_ drone. It is probable that sodium is a major charge carrier in generating the light response, and that one of the actions of calcium is to modify and regulate the response generated by sodium. The insensitivity of the reversal potential to changes in sodium concentration is nonetheless paradoxical. Other unspecified ions might contribute to the light induced current. The resting membrane potential seems to be determined by the potassium gradient, and possibly by the chloride gradient and by an electrogenic pump.

We have shown that the structure of the crayfish eye, with its restricted extracellular space, can influence the intracellularly recorded receptor potential. Because the coupling between retinular cells appears to be complete when it exists, coupling was unlikely to have influenced our measurements. Since crayfish microvilli are relatively long, one considers if they are equipotential. The close packing of the microvilli prevents a calculation of a length constant. However, results with reversal potentials were consistent,
and the amplitude of the receptor potential at saturation did approach the reversal potential in normal Ringer, which implies that the photoreceptors we studied were nearly isopotential.

The influence of extracellular voltages on the intracellularly recorded response has not been thoroughly studied in crayfish, but its effect appears to be significant. What is the extent to which one cell, or one ommatidium, influences another by means of extracellular currents? Perhaps most important of all, our studies have been with excised eyes. What are the mechanisms of vision in the intact eye? Electoretinograms recorded from living animals and from excised eyes are not different (Glantz, 1968), but possible synaptic interactions which could be absent in the excised eye might require intracellular recording for detection. The crayfish eye is not an ideal system for studying membrane mechanisms in isolation. But because we can largely assess the influence of the anatomy of the eye and the resistance of extracellular pathways upon the measurements of intracellular voltages, the crayfish compound eye is well suited for understanding the general effects of structure and intercellular interactions on the measurement of membrane mechanisms.
Appendix I

The Calculation of Coupling Resistance:

The recording configuration is represented in Fig. 11. Current $I_1$ is injected through electrode 1, producing voltage changes $V_1^*$ in cell 1 and $V_2$ in cell 2. Current $I_2$ is injected through electrode 2, producing voltage changes $V_2^*$ in cell 2 and $V_1$ in cell 1. In other words, voltages recorded with the electrode actively passing current will be marked with an asterisk. From elementary circuit theory we can write:

(1) $I_1 = \frac{V_1^*}{R_1} \left( \frac{1}{R_1} + \frac{1}{(R_c+R_2)} \right) = \frac{V_1^*}{R_1} \left( \frac{R_c+R_1+R_2}{R_1(R_c+R_2)} \right)$,

(2) $I_2 = \frac{V_2^*}{R_2} \left( \frac{R_c+R_1+R_2}{R_2(R_c+R_1)} \right)$,

(3) $\frac{V_2^*}{V_1} = \frac{R_2}{R_2(R_c+R_1)} \quad \text{or} \quad R_2 = R_c \left( \frac{V_2^*}{V_1} \right)$, and

(4) $\frac{V_1^*}{V_2} = \frac{R_1}{R_c+R_1} \quad \text{or} \quad R_1 = R_c \left( \frac{V_1^*}{V_2} \right)$.

On substituting (3) and (4) into (1) we obtain

(5) $R_c = \frac{V_1^* V_2^* - V_1 V_2}{V_1 I_1}$.

Similarly from (3), (4), and (2) we obtain

(6) $R_c = \frac{V_1^* V_2^* - V_1 V_2}{V_2 I_2}$.
From (4) and (5) we have

\[ (7) \quad R_1 = \frac{V_1^* V_2^* - V_1 V_2}{(V_2^* - V_1) I_1} \]

and from (3) and (6) we have

\[ (8) \quad R_2 = \frac{V_1^* V_2^* - V_1 V_2}{(V_1^* - V_2) I_2} \]

In order to calculate the standard error in \( R_c \), we assume independence of error in the variables, and that the standard error in each variable \( x_i \) is some constant \( \sigma \). We then have the expression for the standard error of \( R_c \),

\[ s(R_c) = \sqrt{\sum \left( \frac{\partial R_c}{\partial x_i} \right)^2 s(x_i)^2} \]

Therefore

\[ s(R_c) = \sigma \left( \frac{2 V_1^* V_2^*}{V_1 I_1} \right)^2 + 2 \left( \frac{V_2}{I_1} \right)^2 \] \( \frac{1}{2} \), and

\[ s(R_c) = \sigma \left( \frac{2 V_1^* V_2^*}{V_2 I_2} \right)^2 + 2 \left( \frac{V_1}{I_2} \right)^2 \] \( \frac{1}{2} \).

We give an example of the calculation of coupling resistance from the experiment of Figs. 9 and 12. If in the dark \( V_1^* = 3.0, V_1 = 2.5, V_2^* = 3.3, V_2 = 1.2, I_1 = 0.2 \) and \( I_2 = 0.5 \), then \( R_c = 13.8, 11.5 \) and \( \overline{R_c} = 12.7 \), where \( s(R_c) = 2 \) for \( \sigma = 0.05 \). Furthermore \( R_1 = 43.1 \) and \( R_2 = 7.7 \). Values for voltage are in mv., for current in namps, and for resistance in Megohms. The effect of light was to alter the measured voltage changes to the new values \( V_1^* = 1.2, V_1 = 1.5, V_2^* = 2.2, V_2 = 0.5, I_1 = 0.2, \) and \( I_2 = 0.5 \) giving \( R_c = 6.3, 7.6 \) and \( \overline{R_c} = 6.9 \) where \( s(R_c) = 1 \).
for $\sigma = 0.05$. Also, $R_1 = 13.5$ and $R_2 = 5.4$. These decreases in $R_1$ and $R_2$ are equivalent to a light induced increase in the conductance of cell 1 by $5 \times 10^{-8}$ mho, and the same conductance increase of cell 2's membrane. Such equal conductance increases are consistent with the supposition that the difference between $R_1$ and $R_2$ is the quality of electrode penetrations.
Appendix II

Modified cable equation for tubules packed in equivolume, equiresistant space:

The standard cable equation is

\[
\frac{a^2}{2Ra} \frac{\partial^2 V_i(x,t)}{\partial x^2} = (V_i - V_e) \sum_{i} g_i - 1 \frac{E_i}{R_m} + C \frac{\partial (V_i - V_e)}{\partial t},
\]

where \(x\) is the distance along the cable, \(a\) is the cable (tubule) radius, \(R_a\) is the axial resistivity, \(\sum_{i} g_i = 1/R_m\) is the membrane conductivity, \(V_i(x,t)\) is the internal potential, \(V_e(x,t)\) is the external potential, \(C\) is the membrane capacitance, and \(R_m \sum_{i} g_i E_i = E\) is the membrane resting voltage, where \(V_e(x,t) = 0\) for an infinite, conducting bath.

If the extracellular volume equals the intracellular volume, and if the extracellular resistivity is identical to \(R_a\), then

\[
\frac{a^2}{2R_a} \frac{\partial^2 V_e(x,t)}{\partial x^2} = (V_i - V_e) \sum_{i} g_i - 1 \frac{E}{R_m} + C \frac{\partial (V_i - V_e)}{\partial t}.
\]

Considering only the steady-state case, and adding (1) and (2), we have

\[
\frac{1}{2} \left( \frac{a}{R_a} \right) \frac{\partial^2 (V_i - V_e)}{\partial x^2} = (V_i - V_e) - E.
\]

The solution to equation (3) is of the form
\[ \delta V = V_i - V_e = A e^{-\sqrt{2}x/l} + B e^{\sqrt{2} x/l} + E, \] where \( l = \frac{2a R_m}{R_a} \).

At \( x = \) infinity, \( \delta V = E \), therefore \( A = 0 \). At \( x = 0 \), \( \delta V = V_o + E \), hence \( B = V_o \). We know from symmetry that \( V_e = 0 = V_o - V_i \), therefore \( V_e = V_o - V_i \) and \( \delta V = 2V_i - V_o = V_o e^{-2.5x/l} + E \).

Since \( E \) is effectively a reference voltage, setting \( E = 0 \) we have

\[ V_i(\text{closed}) = \frac{1}{2} V_o (e^{-\sqrt{2} x/l} + 1). \]

For the case of a microvillus in an infinite, conducting bath

\[ V_i(\text{open}) = V_o e^{-x/l}. \]

Therefore \( \frac{V_i(\text{closed})}{V_i(\text{open})} = \frac{e^{-0.414 x/l} + e^{x/l}}{2} \)

If \( x/l = 0.1 \), then \( V_i(\text{closed})/V_i(\text{open}) = 1.03 \).

If \( x/l = 1.0 \), then \( V_i(\text{closed})/V_i(\text{open}) = 1.69 \).
Appendix III

The increase of extracellular voltage when the polarizer is rotated 90° and the intensity doubled:

We shall assume that $i = k \log I$, where $i$ is the extracellular current, $I$ is the effective intensity, and $k$ is a constant. We assume further that $V_{\text{extracell.}} = (i R)$, where $R$ is a constant resistance.

For a two-channel system as for the crayfish, where the dichroic ratio is $N$, we have initially for maximal stimulation of $// -$-cells

$$i_{//} = k_{//} \log (N I)$$
$$i_{\perp} = k_{\perp} \log I.$$

If now the polarizer is rotated 90° and the intensity doubled, we have

$$i'_{//} = k_{//} \log 2I$$
$$i'_{\perp} = k_{\perp} \log 2NI.$$

The extracellular potential difference is

$$V'_{\text{ext.}} - V_{\text{ext.}} = R (i' - i)$$
$$= R (k_{//} \log 2I + k_{\perp} \log 2NI - k_{//} \log NI - k_{\perp} \log I)$$
$$= R (k_{//} \log (2/N) + k_{\perp} \log 2N).$$

If $N = 2$, then $\Delta V = R k_{\perp} \log 4 = 0.6R k_{\perp}$. Otherwise, if $k_{//} = k_{\perp}$, then $\Delta V = R k_{\perp} \log 4 = 0.6R k_{\perp}$.

For a three-channel system of the locust-type, if we maximally stimulate one class of cells, then we are less
stimulating two equivalent classes of cells whose microvilli are at 120° to those of the first class. Maximal stimulation of // -cells gives

\[ i_{//} = k_{//} \log ((N-1)\cos^2 0° + 1)I = k_{//} \log(N I) \]

\[ i_{120} = 2k_{120} \log((N-1)\cos^2 60° + 1)I = 2k_{120} \log\left(\frac{N+3}{4}\right)I. \]

If we now rotate the polarizer 90° and double the intensity, we have

\[ i'_{//} = k_{//} \log((N-1)\cos^2 90° + 1)2I = k_{//} \log 2I \]

\[ i'_{120} = 2k_{120} \log((N-1)\cos^2 30° + 1)2I = 2k_{120} \log\left(\frac{3N+1}{2}\right)I. \]

The extracellular potential difference is

\[ V'_{\text{ext.}} - V_{\text{ext.}} = R (i' - i) \]

\[ = R(2k_{120} \log\left(\frac{3N+1}{2}\right)I + k_{//} \log 2I - 2k_{120} \log\left(\frac{N+3}{4}\right)I - k_{//} \log NI) \]

\[ = R(2k_{120} \log\left(\frac{6N+2}{N+3}\right) + k_{//} \log\left(\frac{2}{N}\right)). \]

If \( N = 2, \Delta V = R k_{120} \log 7.9 = 0.9 R k_{120}. \)
Appendix IV

The relation of the polarization sensitivity of the ERG to the dichroic ratio $N$:

By the "Law of Malus", the effective intensity absorbed by a retinular cell is $I_{\text{effective}} = AI(N\cos^2\theta + \sin^2\theta)$, where $N$ is the dichroic ratio of the retinular cell, $I$ is the incident intensity, and $A$ is a proportionality constant. We wish further to assume a logarithmic dependence of the extracellular response upon $I_{\text{eff}}$, that is,

$$V_{\parallel} = k \log AI(N\cos^2\theta + \sin^2\theta),$$

and for cells of the other class,

$$V_{\perp} = k \log AI(N\sin^2\theta + \cos^2\theta).$$

Therefore if the extracellular voltages are proportionally additive by the factor $a$,

$$V = a V_{\parallel} + a V_{\perp}$$

$$= ak \log A^2 + ak \log I^2(N\cos^2\theta + \sin^2\theta)(N\sin^2\theta + \cos^2\theta)$$

$$= A^* + ak \log I^2(N + \frac{1}{4}(N - 1)^2 \sin 2\theta).$$

If $N = 2$, $\theta = 45^\circ$, then $V = A^* + ak \log (2.25 I^2)$,

and for $\theta = 0^\circ$, $90^\circ$, $V = A^* + ak \log (2I^2)$.

To produce a response at $0^\circ$ or $90^\circ$ which matches that at $45^\circ$, we must use $(2.25/2)^{1/2} = 1.07$ times more light.

For $N = 3$, $I_{0^\circ,90^\circ}/I_{45^\circ} = (4/3)^{1/2} = 1.15$.

For $N = 4$, $I_{0^\circ,90^\circ}/I_{45^\circ} = (6.25/4)^{1/2} = 1.25$.

For $N = 5$, $I_{0^\circ,90^\circ}/I_{45^\circ} = (9/5)^{1/2} = 1.34$.

For $N = 10$, $I_{0^\circ,90^\circ}/I_{45^\circ} = (30/10)^{1/2} = 1.73$. 
Bibliography


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