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PHOTORECEPTORS IN THE CRAYFISH COMPOUND EYE: ELECTRICAL INTERACTIONS BETWEEN CELLS AS RELATED TO POLARIZED-LIGHT SENSITIVITY

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SUMMARY

1. The sensitivity to plane-polarized light and the electrical interactions of photoreceptors were examined with intracellular and extracellular micro-electrodes in excised compound eyes of the crayfish.

2. There are two types of photoreceptor: each photoreceptor cell responds best to polarized light when the electric-vector of the light is oriented in one of two orthogonal directions. Seven cells, representing each type, are grouped together to form ommatidia.

3. In each ommatidium, cells that are sensitive to the same orientation of the electric-vector of polarized light are coupled electrically. Cells having orthogonal polarized-light sensitivities are not coupled.

4. Nearly all cells studied were sensitive to orange light. A few cells of both types were found that were sensitive to blue light. Blue-sensitive cells were not coupled to orange-sensitive cells.

5. The photocurrents of both cell types produce negative extracellular potentials which can be greater than 10 mV when measured near the photoreceptive membranes within ommatidia. Evidence suggests that the extracellular potentials produced by one type of cell can effectively reduce the receptor potentials recorded in the other cell type. It is proposed that such a mutual non-synaptic interaction can make a cell more sensitive to the orientation of polarized-light than would be predicted from the cell's differential absorption of polarized light (i.e. its dichroic ratio).

INTRODUCTION

Many arthropods use the sun as a compass, even on cloudy days when only a small area of blue sky is visible. This ability to infer the position of the obscured sun is based on the polarization of natural skylight and on

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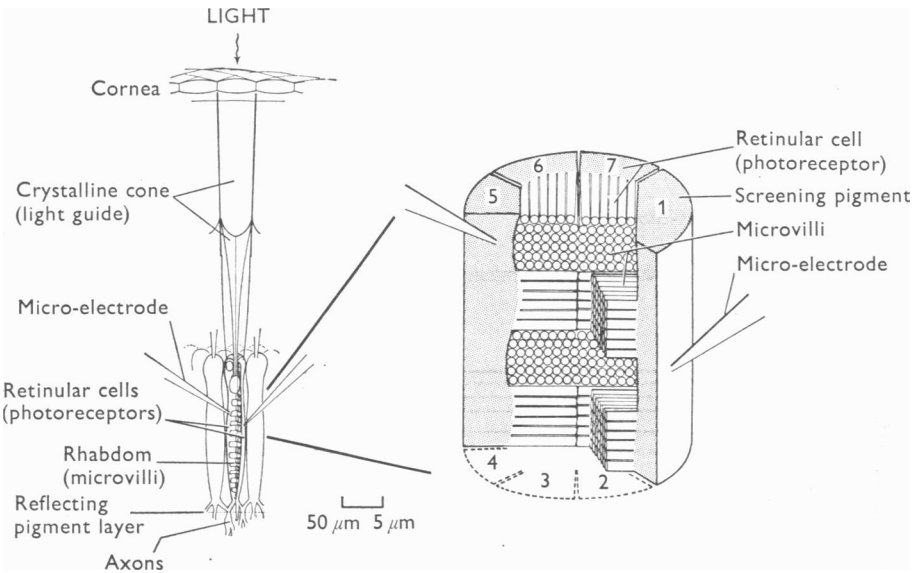
the detection of the plane of polarization of light by photoreceptors in the arthropod compound eye (von Frisch, 1950; Schöne, 1963). Natural skylight is polarized by Rayleigh scattering so that the electric-vector is at right angles to a line from the sun to the point of scatter. Sunlight scattered underwater is similarly polarized. Thus, by analysing the direction of polarization of light at different points in the sky, for example, the position of the sun can be determined by many arthropods. Each photoreceptor in these arthropods will respond maximally to light polarized at a given orientation and minimally to light polarized at right angles to that orientation (Kuwabara & Naka, 1959; Shaw, 1966, 1967, 1969*a*, *b*). The ommatidium, a cluster of photoreceptor and accessory cells, forms the structural unit of the arthropod compound eye (Text-fig. 1). Each eye contains hundreds or thousands of ommatidia, each looking at different but overlapping narrow regions of space. In the crayfish and many other arthropods with a closed or fused rhabdom, the photoreceptor cells in an ommatidium have the same angle of view. It is not known if the ommatidium is also the functional unit for polarized light sensitivity. That is, does a single ommatidium contain the information necessary to discriminate the angle of polarization of light?

The structural basis of polarized light discrimination is thought to be the array of parallel tubules of diameter less than 0·1 μm, the microvilli, which constitutes the photoreceptive membrane of each photoreceptor cell. The visual pigment (rhodopsin) in the microvilli of crayfish absorbs polarized light parallel to the long axis of the microvilli twice as well as light polarized perpendicularly giving a dichroic ratio of 2. This is consistent with the proposition that rhodopsin chromophores lie randomly in the plane of the microvillar membrane (Moody & Parriss, 1961; Waterman, Fernández & Goldsmith, 1969). In the crayfish compound eye the microvilli of the seven photoreceptors in each ommatidium are perpendicular to the path of the incoming light. Of the seven photoreceptors, three have their microvilli oriented horizontally, while the other four cells have their microvilli at right angles to those of the first three (Parker, 1897; Eguchi, 1965), suggesting that there are at least two classes of cells sensitive to polarized light in each ommatidium.

The present paper seeks to determine if electrical interactions between photoreceptor cells affect the detection and analysis of polarized light by the crayfish compound eye. Therefore, electrical coupling was looked for between cells after ascertaining sensitivity to polarized light. Extracellular potentials were studied to determine not only if they affect the intracellular voltage of the photoreceptor cells which generate them, but also if extracellular potentials attenuate other cells' responses, thereby providing a non-synaptic, effectively inhibitory interaction between photoreceptors.

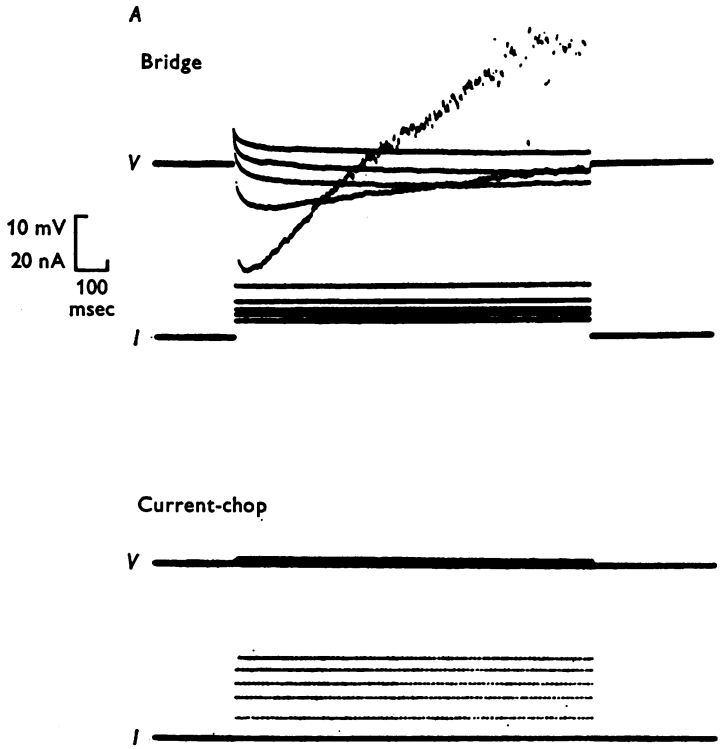
METHODS

Animals, chamber, and stimulus. Eyes and attached eyestalks were removed from the crayfish *Procambarus clarkii* 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° C). Bisected eyes could be maintained for over 12 hr in van Harreveld's solution (van Harreveld, 1936). The stimulus was light from a 150 W xenon arc lamp, which passed through an electromechanical shutter and a heat absorbing filter, and was focused on the



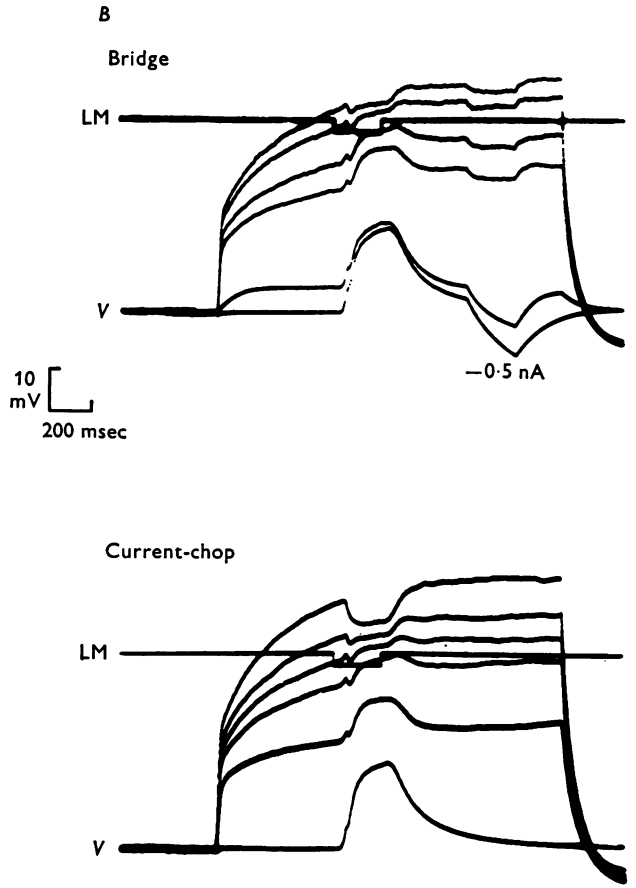
Text-fig. 1. Cutaway view of a crayfish ommatidium with two micro-electrodes penetrating separate photoreceptor cells. On the left, light enters the eye perpendicularly to the cornea and stimulates the photoreceptors as it passes through the rhabdom. This region, shown in a 10× enlargement at the right, is formed by the stacking of thousands of photoreceptor membrane tubules, the microvilli, of diameter 0·1 μm (not drawn to scale). The banded pattern of the rhabdom is due to the alternate stacking of arrays of microvilli. Screening pigment within the photoreceptor cells does not extend into the microvilli. In this diagram cells 2, 3, and 4 have been removed while half of the microvilli of cell 1 have been trimmed. Cell 1 is twice as large as the other six cells and is always located posteriorly in the ommatidium (adapted from Parker, 1897, and Eguchi, 1965).

preparation with a 0·25 numerical aperture 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 variable electric-vector orientation of plane-polarized light, single Polaroid type HN 38 or KN 36 filters were interposed in the beam. To avoid selective polarization of the beam at the air-solution interface, light entered the solution perpendicularly. The intensities of light polarized at different angles



Text-fig. 2 *A*, a comparison of the voltages (*V*) measured by a micro-electrode in the bath during current injections (*I*) using the bridge and current-chop techniques. As injected current was increased stepwise, the bridge balance changed because of changes in the micro-electrode resistance. The voltages recorded with the current-chop technique were measured 1 msec after the cessation of 1 sec pulses of the indicated currents. The micro-electrode measured zero voltage to within 1 mV. More current (averaged during the cycle) can be passed through the recording micro-electrode with the current-chop than with the bridge technique.

B, reversal potentials measured using the bridge and current-chop techniques agree. The light monitor trace (LM) indicates the timing of the 200 msec flash and is positioned at zero voltage. *V* is the membrane voltage during successive oscilloscope sweeps. The current pulses of various amplitudes were delivered every 10 sec. A reversal potential, the voltage beyond which the receptor potential changes direction, 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 micro-electrode resistance was initially balanced out with the bridge circuit; the continuity of the voltage trace during a $-0{\cdot}5$ nA current pulse demonstrated that the bridge remained balanced. Alternatively, an equally long series of 1 msec pulses of constant current at 500 pulses/sec was delivered using the current-chop technique. Again light flashes were delivered as the membrane voltage approached a steady level. The reversal potential was the same for both techniques.



Text-fig. 2*B*. For legend see facing page.

were measured with an Eppley thermopile at the plane of the preparation and were the same to within 5 % (0·02 neutral density units).

Before the micro-electrodes were inserted into the illuminated ommatidium, the light beam was aligned approximately along the ommatidial optic axis but passed outside the dioptric apparatus. This procedure minimized scattering of light to adjacent ommatidia when the beam was narrowed, but assured equal illumination of both types of cell by polarized light.

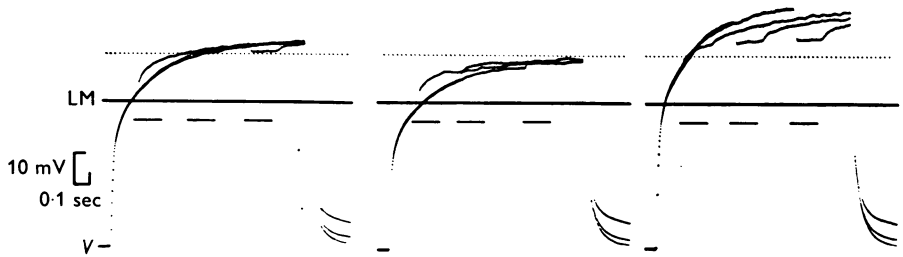
The screening pigment of all eyes studied was in the light-adapted position. The crayfish compound eye contains predominantly cells sensitive to orange light. Cells which respond best to blue light were sometimes penetrated but, except where mentioned, blue sensitive cells were not studied.

Micropipettes and current injection. Micropipettes were filled with 1 M-KCl, or 4 % Procion Yellow M4RS or 4 % Procion Navy Blue M3RS (Stretton & Kravitz, 1968). In most cases, current was injected through a micro-electrode which monitored the membrane potential using a standard d.c.-bridge circuit (Frank & Becker, 1964).

An alternative method of current injection through the recording micro-electrode

was used that permitted the membrane potential to be monitored even if the resistance of the recording micro-electrode changed. This method, which dispenses with the bridge circuit, is termed the current-chop technique. It relies upon both the relatively rapid return (relaxation) of the electrode potential to its original value after current has been passed and the long time constant (> 50 msec) of the membrane of a crayfish retinular cell. Current is injected for 1 msec; at the end of the next msec the voltage recorded by the same electrode is measured (Text-fig. 2*A*). This is repeated at 500 c/s. If the electrode relaxes within 1 msec, the voltage sampled at the end of the cycle is for all practical purposes (within 2 %) the membrane voltage. As successive current pulses are applied, the capacitance of the membrane charges as if half the current were applied continuously.

The maximum currents that can be passed using this current-chop technique and using the d.c.-bridge were compared as follows. The micro-electrode placed in the bath recorded zero voltage. Either technique should not have measured a voltage



Text-fig. 3. Reversal potential can be accurately measured when the membrane voltage has not reached a steady level. The membrane voltage (*V*) was shifted to near the reversal potential with pulses of constant current. The light monitor trace (*LM*) indicates the timing of 200 msec flashes, each delivered during a separate sweep. LM is positioned at zero voltage. For a given amplitude of current, the responses to three differently timed flashes were superimposed. Reversal potential is indicated by the dotted line. The reversal potential is the same whether the flash is delivered during the rising or the level portion of the voltage trace.

change while current was being injected. For small currents the bridge circuit remained balanced to produce no voltage change. For larger currents the electrode resistance increased, and non-zero voltages were recorded. Using the current-chop technique, the same average currents (e.g. half the current during each 1 msec pulse at 500 c/s) could be injected through a micro-electrode without recording a voltage change. Text-fig. 2*B* shows that the voltages measured within a cell with the current-chop technique and with the bridge technique agree over the range that the bridge remains balanced.

By passing current through the recording micro-electrode it was often possible to shift the intracellular voltage sufficiently positive that the receptor potential became negative-going. The voltage beyond which the receptor potential changes direction is the reversal potential. The reversal potential can be measured accurately even when the membrane voltage has not reached a steady level. Text-fig. 3 shows that the reversal potential is the same whether measured on the rising portion or on the steady portion of the voltage trace. The slow change in membrane potential most likely represents a resistance increase of the membrane rather than a capaci-

tative charging. The smaller current pulses in Text-fig. 2*B* show that capacitative charging occurs more rapidly.

Dye injection. Dye was injected iontophoretically with 0·5 sec pulses of −4 to −10 nA delivered each second for about half an hour. The tissue was fixed overnight at 4° C in a solution containing 4·3 ml. acetate buffer at pH 4, 0·6 ml. 25% glutaraldehyde, and 0·1 ml. acrolein (Stretton & Kravitz, 1968). After dehydrating with methanol and embedding in paraffin, 6 μm sections were cut and mounted in Lustrex.

The localization of photoreceptor cells. The photoreceptor cells, or retinular cells of the crayfish compound eye form a layer which begins about 0·5 mm beneath the corneal surface of the eye. Each cell body is approximately 150 μm long along the path of the light and 15 μm in diameter. Retinular cells are cylindrically arranged in groups of seven, somewhat like sections of an orange, to form an ommatidium. One cell is twice the size of the other six (Parker, 1891) and is always posterior (Parker, 1897). The microvilli of the seven retinular cells in each ommatidium are stacked in alternate, orthogonally oriented bundles about 5 μm thick. Impaled cells can be identified as retinular cells by their location just distal to the layer of white reflecting pigment, by their −30 to −70 mV resting potentials, and by their large photoreceptor potentials. In response to bright stimuli the receptor potential can overshoot zero voltage. This identification was confirmed by injecting such cells with Procion dyes.

In many experiments an attempt was made to position two micro-electrodes in the same ommatidium. The dense screening pigment surrounding each ommatidium made precise visual localization of the micro-electrode tips unreliable. Therefore, the positions of the impaled cells were determined by using the cell's electrical responses to a minute spot of light. A single ommatidium could be illuminated at its distal end with a 20–40 μm spot of light by aligning the stimulus beam approximately along the axis of the rhabdom, but with the beam skirting the cornea and dioptric apparatus to reduce light scattering. Two intracellular micro-electrodes could be shown functionally to be in the same ommatidium when they recorded similar responses to the light spot as it was moved in two dimensions, from ommatidium to ommatidium, across the surface of the retina. When two micro-electrodes were in adjacent ommatidia the difference in recorded responses to moving the beam was clear; this was confirmed by injections of Procion dyes through electrodes. The situation in which each electrode penetrated a cell will be referred to as 'a pair of cells'. At times, of course, it is possible that 'a pair of cells' was in fact two simultaneous penetrations of the same cell.

RESULTS

The electrical responses to light. Light produces a positive-going change in the negative membrane voltage of a crayfish photoreceptor. This voltage change, the receptor potential, is graded with light intensity (Glantz, 1968) and resembles the receptor potentials of other arthropod photoreceptors. Unlike the retinular cells of *Limulus* (Benolken, 1959, 1965; Fuortes & Poggio, 1963), *Apis* drone (Naka & Eguchi, 1962) and *Homarus* (K. Muller, unpublished), crayfish retinular cells do not appear to be electrically excitable.

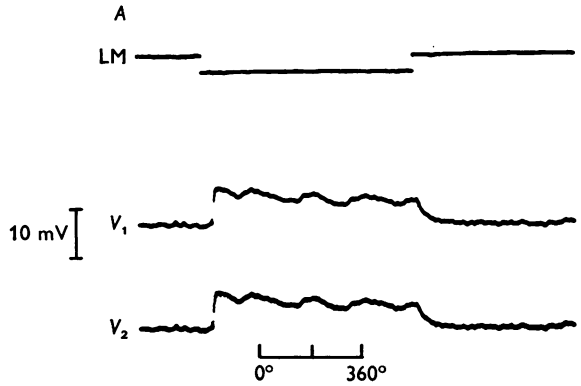
Micro-electrode penetrations of cells in any small region of the crayfish retina show that the retinular cells respond to plane-polarized light best

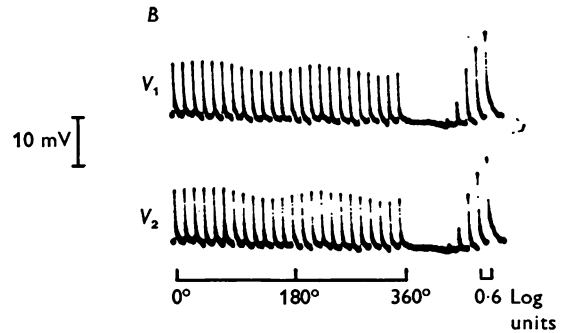
when the electric-vector is oriented in one of two particular orthogonal directions. About half of the cells respond best when the electric-vector is horizontal to the crayfish, the other half when it is rotated 90°. Most cells examined were more sensitive to orange than to blue light. Blue-sensitive cells, which were found inconsistently, were observed in both classes of polarization sensitivity. In a sample of thirteen blue-sensitive cells, seven were sensitive to a horizontal polarization and six to a vertical polarization of light.

The polarization sensitivity ratio as measured by Shaw (1969*a*) was determined by first finding the angle of the polarizer at which the cell responded least well. Then the responses to flashes of a series of intensities at this angle were compared with the responses elicited by flashes of constant intensity while rotating the polarizer. The polarization sensitivity is defined as the ratio of intensities which produce identical responses to the minimal and maximal orientations of the polarizer. In Text-fig. 4*B*, for example, the polarization sensitivity ratio is 2 for both records. As one would expect, over the intensity range that the receptor potential amplitude was approximately logarithmic with intensity, the polarization sensitivity ratio of cells was constant.

Pairs of cells having the same sensitivity to electric-vector. Cells in the same crayfish ommatidium which have similar responses to the polarization and wave-length of light ought to be functionally indistinguishable, and might therefore be electrically coupled. To demonstrate coupling, two cells were simultaneously impaled. After ascertaining that a pair of cells was in the same ommatidium, the polarization angle eliciting maximum response from each cell was determined. In fifteen out of twenty-seven experiments both members of the pair had the same angle of maximum response. This was shown by rotating the polarizing filter at a constant rate during steady illumination as in Text-fig. 4*A*. Alternatively, the polarizer was rotated in 15° steps between 200 msec flashes delivered every 3 or 4 sec, as in Text-fig. 4*B* (for the same pair of cells). A 200 msec flash at any given intensity will elicit the maximum amplitude of the receptor potential for that intensity.

Each of the fifteen pairs of cells with the same angle of polarization sensitivity was tested for electrical coupling by passing current alternately through one and then the other electrode. For fourteen pairs, injecting positive and negative currents through at least one of the electrodes produced equal voltage changes in both cells (Text-fig. 5). Such cells were considered to be well coupled. The remaining pair of cells was not coupled. This pair was moreover exceptional in that one cell was more sensitive to orange than to blue light, and the other more sensitive to blue than to orange light.



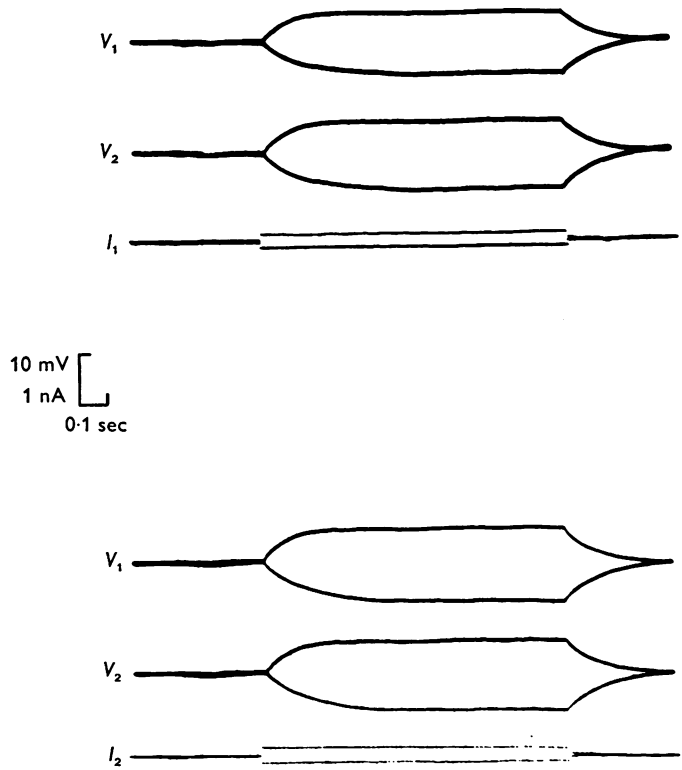


Text-fig. 4. A pair of cells in an ommatidium which respond identically to rotation of the electric-vector of polarized light.

A, the polarizer was rotated during steady illumination. LM indicates the timing of the light. The resting voltages measured by the two electrodes were nearly identical.

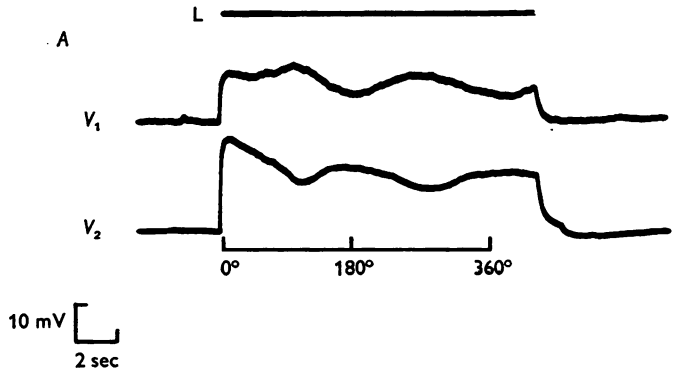
B, flashes of 200 msec duration were delivered every 2·5 sec while rotating the polarizer 15° between flashes. Then with the polarizer position fixed, a series of flashes increasing in steps of 0·6 log units of intensity were delivered. The difference in intensities which produced responses like the minimal and maximal responses during the polarizer rotation was about 0·3 log units, or 2 on a linear scale. This value is the polarization sensitivity ratio.

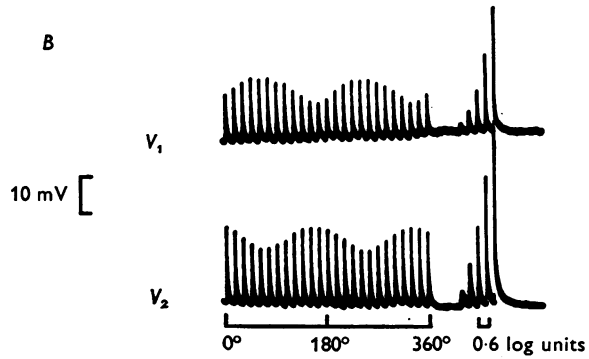
When the coupling between pairs of cells was measured, both positive and negative currents were passed through each electrode. This procedure permitted the detection of non-linear junctional resistances and gross differences in the membrane resistances of the impaled cells. It is the *relative* values of resistance between cells and input resistances of those cells which determine the extent of signal transmission between them.



Text-fig. 5. When cells in the same ommatidium had the same spectral sensitivity and responded best to the same electric-vector orientation, they were well coupled. Current (I_1 and I_2) injected, using the d.c.-bridge, into each cell of Text-fig. 4 produced equal voltage changes in the other cell.

For example, if the recording electrode damages a cell's membrane, that cell can appear to be less well coupled to neighbouring cells. But the transmission to a neighbouring cell of a signal generated in the damaged cell will not be appreciably affected by the damage. Between crayfish retinular cells the coupling resistance is apparently linear, but intercellular transmission is affected by the quality of the electrode impalement.





Text-fig. 6. The receptor potentials of a pair of cells in an ommatidium which have orthogonal polarization sensitivities to rotating the polarizer 360°.

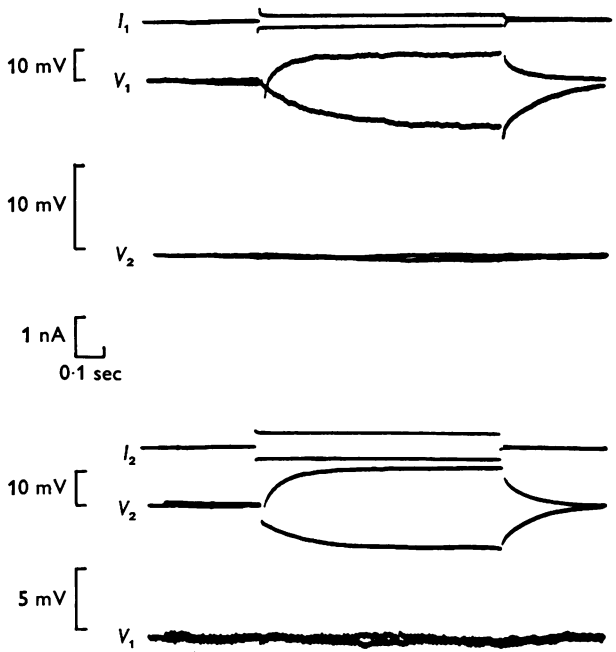
A, the polarizer was rotated during steady illumination. *L* indicates the timing of the light. The responses (V_1 and V_2) of the two cells are shifted in phase by approximately 90° with respect to each other.

B, every 2·5 sec, a 200 msec flash of constant intensity was delivered; the polarizer was rotated 15° between flashes. Then an intensity series in steps of 0·6 log units of intensity was presented. The difference in maximal and minimal amplitudes with rotation of the polarizer was equal to the difference in amplitudes between steps of intensity, therefore the polarization sensitivity ratios were about 4.

When less than perfect coupling was measured, coupling was asymmetrical and thus can be attributed to damage of one cell of the pair.

In no cases were cells which had the same sensitivity to electric-vector coupled if they were not in the same ommatidium.

Pairs of cells having orthogonal sensitivity to electric-vector angle. In eleven of the twenty-seven experiments in which a pair of cells was in one ommatidium, the angles of electric-vector which produced maximal responses



Text-fig. 7. Cells having orthogonal polarization sensitivities to rotating the polarizer 360° were not coupled electrically. Currents I_1 into cell 1 of Fig. 6 produced no measurable voltage change in cell 2, nor did currents I_2 into cell 2 affect the voltage recorded in cell 1. Because the electrodes were filled with dye and had high resistances, it was difficult to maintain bridge balance.

in each of the two cells differed by 90° (Text-fig. 6). In all eleven cases the pair of cells was not electrically coupled (Text-fig. 7). The two cells of Text-fig. 6 each had polarization sensitivity ratios of 4, which is larger than the presumed dichroic ratio of 2.

Two cells in the same ommatidium which were successfully stained by injection are shown in Pl. 1. Their polarization sensitivity maxima were at 90° to each other and the cells were not coupled. The dyes were located at

different layers in the rhabdom; the cells therefore had orthogonally oriented microvilli.

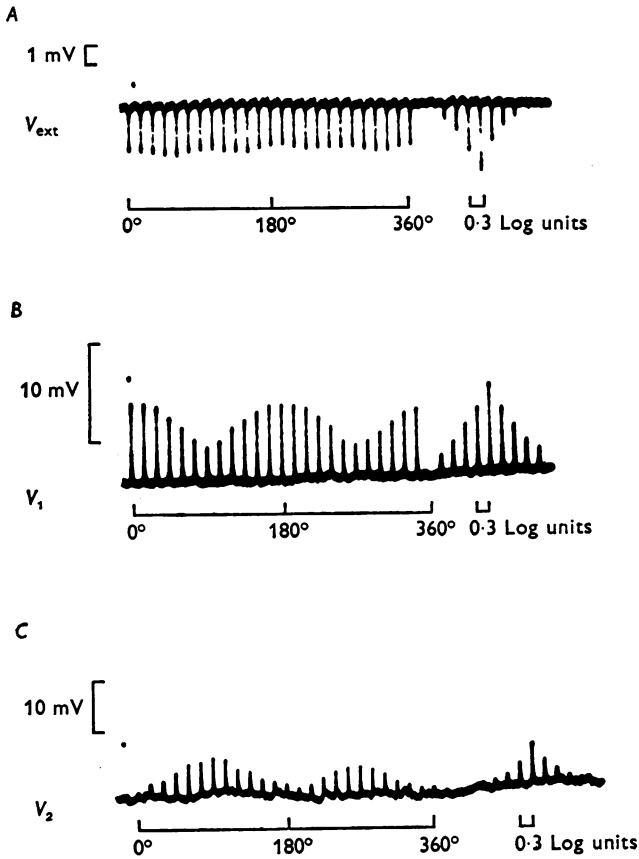
Negative-going extracellular and intracellular potentials evoked by light. There is no evidence that a voltage change in one class of cells synaptically affects the membrane voltage or light response of cells in the orthogonally sensitive class. However, the possibility was examined that there are non-synaptic interactions via extracellular resistive pathways shared by both classes of photoreceptor.

Light-evoked voltage changes can be recorded extracellularly from the region of the microvilli (the rhabdom) with a micro-electrode (Text-figs. 8 and 9*B*), as previously reported by Naka & Kuwabara (1959). Such locally recorded electroretinograms (local e.r.g.s) in response to general illumination can be observed as negative-going potentials as large as 10 mV which last for the duration of the light. The amplitude of the local e.r.g. is not changed by currents passed through the extracellular recording electrode, even for currents larger than those which would markedly change the amplitude of an intracellularly recorded receptor potential.

Both classes of cells sensitive to polarized light contribute to the local e.r.g. 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 local e.r.g. while rotating the polarizer 360° (Text-fig. 8). At later times during this experiment two cells near the site of the extracellular recording were impaled successively. The polarization sensitivity ratios of both cells were at least 5 and their response maxima were 90° apart. The peak responses to polarized light of the impaled cells corresponded with the minimum responses of the local e.r.g., as would occur if the extracellular responses of the cells of both classes sum algebraically to produce the local e.r.g. (Bohn & Täuber, 1971).

If extracellular potentials produced by other cells affect the intracellularly recorded potential, then by selectively reducing or abolishing the receptor potential of the impaled cell a response similar to the local e.r.g. ought to be unmasked. The response of the impaled cell may be selectively abolished as follows. If a retinular cell is impaled with a micro-electrode filled with 3 M-KCl rather than 1 M-KCl, the receptor potentials recorded by that electrode diminish, presumably because of leakage of electrolyte, until a light-induced hyperpolarization followed by a slow, small depolarization is seen (Text-fig. 9) (this does not happen with 3 M-KCl-filled micro-electrodes of resistance greater than 30–50 MΩ). Although the process is irreversible, the resting potential and input resistance of the retinular cell are not markedly altered. Like the local e.r.g. recorded extracellularly, the light-evoked hyperpolarization is stable and is not changed in amplitude by passing current through the recording electrode,

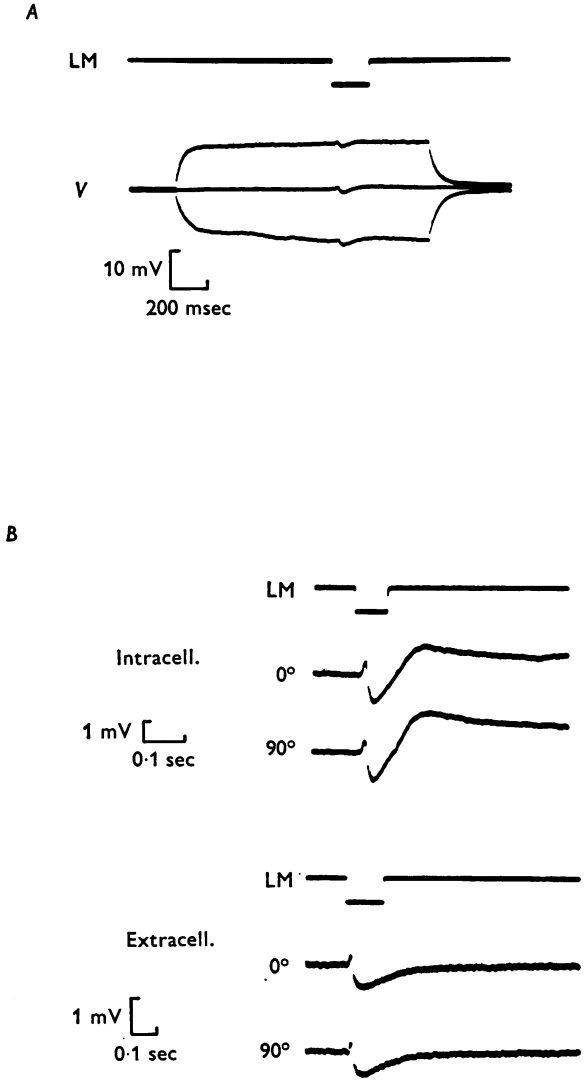
although the membrane potential does change (Text-fig. 9*A*). Furthermore, the amplitude of the intracellularly recorded negative-going potential is not as sensitive to the angle of polarization of the light as is the depolarizing receptor potential (Text-fig. 9*B*). It is not known if cells remain coupled under such conditions.



Text-fig. 8*A*, the extracellular response to light can be modulated. A series of 200 msec flashes every 2·5 sec was presented while the polarizer was rotated 15° between flashes. The intensity response series which followed shows that the response was logarithmic with intensity. At the microvillar membrane a larger amplitude of the extracellular response is expected.

B, a neighbouring cell was penetrated and was stimulated as in *A*, its response maxima corresponding with minima in *A*. It has 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 e.r.g. correspond with the maxima of the intracellularly recorded receptor potentials.

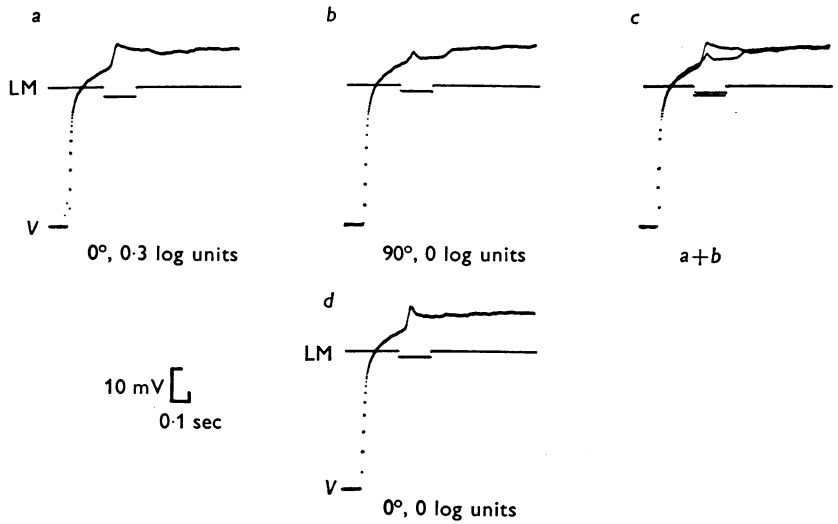


Text-fig. 9. The intracellularly recorded response of a cell penetrated with a micro-electrode filled with 3 M-KCl. The depolarizing receptor potential is nearly abolished.

A, the intracellularly recorded hyperpolarization is not affected by changing membrane potential. The light monitor (LM) is positioned at zero voltage and indicates the timing of the three successive flashes.

B, the intracellular hyperpolarization, like the local e.r.g., is the same for horizontal and vertical positions of the polarizer. LM indicates the timing of the flash. Only the small positivity following the negative potential disappeared when the electrode was withdrawn from the cell (Extracell). The extracellular potential is presumably larger at the rhabdom.

Reversal potentials and polarization sensitivity. By irreversibly abolishing the receptor potential in the impaled cell as shown in the previous section, a stable signal resembling the local e.r.g. was recorded intracellularly. Without irreversibly changing the impaled cell, can such a signal be observed? The receptor potential may be *reversibly* abolished by holding the *membrane* at the reversal voltage of the photoresponse. This can be done with many crayfish retinular cells by using a single micro-electrode with either the d.c.-bridge technique or the current-chop technique described earlier.

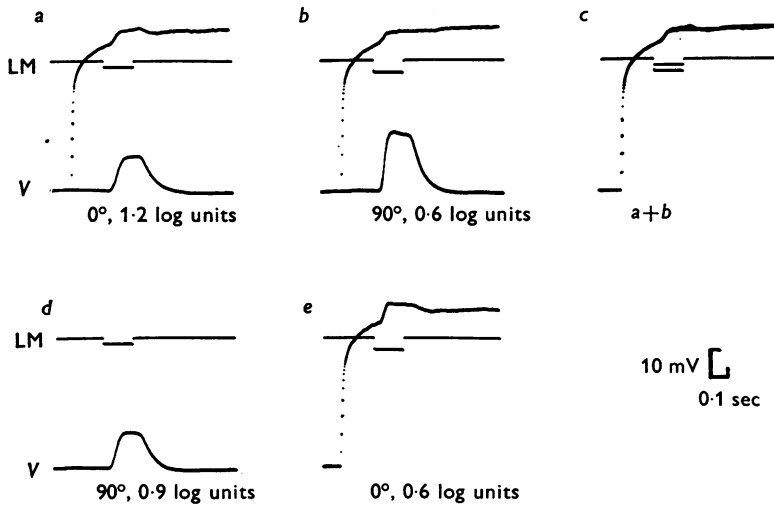


Text-fig. 10. The value of the reversal potential can depend upon polarizer orientation. Each voltage trace (*V*) represents the intracellular response to constant current pulses of the same amplitude. The light monitor (LM) indicates the timing of the 200 msec light flash and was positioned at zero voltage. In *a*, with the polarizer oriented to optimally stimulate the cell at rest, the reversal potential is more positive than the intracellular potential. In *b*, with the polarizer rotated 90° and the light intensity doubled, the reversal potential for all but a brief initial component is below the intracellular potential. The superposition of *a* and *b* in *c* shows that the intracellular voltage does lie between the reversal potentials. In *d*, the increased intensity at this membrane potential attenuates but does not reverse the receptor potential.

Because the reversal voltage of the membrane response is not necessarily the intracellularly recorded reversal potential, it is not easy to isolate the intracellularly recorded e.r.g. If a negative-going extracellular receptor potential is in series with the transmembrane potential, then the intracellularly recorded reversal potential will be the voltage at which the transmembrane receptor potential and the negative-going extracellular

potential are equal and opposite. Such an operational reversal potential is more negative than the true, transmembrane reversal potential.

The influence of the extracellular potential on the reversal potential is seen as follows. If the polarization sensitivity of the cell in isolation were 2, for example, then rotating the polarizer 90° from a position of maximal to one of minimal stimulation and doubling the light intensity should produce equal transmembrane receptor potentials at any given transmembrane voltage. An extracellular potential which is in part produced by



Text-fig. 11. Polarization sensitivity can be dependent upon the intracellular voltage. Each voltage trace (*V*) shows the receptor potential at rest (*a*, *b*, and *d*), or when superimposed on a positive shift of potential produced by a pulse of constant current (*a*, *b*, *c*, and *e*). The light monitor (LM) indicates the timing of the light and was positioned at zero voltage. The position of the polarizer and the attenuation of the light in log units is given below each voltage trace. The responses at rest in *a* and *d* are equal and indicate a polarization sensitivity of 2. Changing the intracellular voltage to a value near the reversal potential caused the polarization sensitivity to increase. During the injection of current the response in *b* is no larger than in *a* (superimposed in *c* for comparison). The polarization sensitivity increased to over 4. The effect of increased intensity alone, without rotating the polarizer, is shown in *e*.

cells of the opposite polarization sensitivity, however, will become larger when the polarizer is thus rotated and the light intensity doubled. An extracellular receptor potential which previously exactly cancelled a small transmembrane receptor potential to produce an apparent reversal potential will now be larger than the transmembrane potential. The apparent reversal potential will shift to a more negative value. In several

cells the response to light polarized for maximal stimulation did in fact reverse at a higher voltage than did the response to light polarized at right angles (Text-fig. 10) (although in some cells no dependence of reversal potential upon polarizer orientation was seen).

A similar analysis leads to the prediction that the polarization sensitivity ratio might be dependent upon membrane potential. In other words, as the receptor potential is made smaller by changing membrane potential, the relative importance of the voltages produced by extracellular currents is increased. At reversal potential the polarization sensitivity ratio is not easily defined because the response to light with the polarizer at a position for maximal stimulation cannot be matched by more light with the polarizer at a position for minimal stimulation. By holding the membrane voltage just below the reversal potential for minimal stimulation, however, a polarization sensitivity greater than that at rest was measured (Text-fig. 11) in those cells with reversal potentials which were sensitive to the angle of polarization of light. The closer the membrane potential is to the reversal potential, the higher is the polarization sensitivity until at reversal it becomes immeasurably high.

DISCUSSION

Coupling between cells. Polarized light falling on a crayfish ommatidium can differentially stimulate two classes of cells. Information about the plane of polarization of the light is sensed because each cell is dichroic; this information is preserved because cells having orthogonal directions of maximal absorption are not electrically coupled. On the other hand, cells with the same spectral sensitivity and the same sensitivity to angle of polarization are well coupled electrically.

Colour information is also preserved at the retinular cell level. It is not known if each ommatidium contains both blue- and orange-sensitive cells. However, cells which are maximally sensitive to blue light are split about equally into two classes on the basis of their sensitivity to the angle of polarized light. 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.

Several lines of evidence indicate that coupled cells were in some cases two distinct cells, although direct anatomical proof with dye injection is lacking. Micro-electrodes penetrated nearly as many 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 each electrode penetrates cells at random, rather than

a particular cell or cells of one class preferentially. The simultaneous impalement of blue- and orange-sensitive cells in a single ommatidium further shows that it is possible to impale two separate cells of the same polarization sensitivity in an ommatidium. That this happened only once is not surprising because of the paucity of recordings from blue-sensitive cells.

Artificial coupling of cells through a hole made by the micro-electrode must have been rare in this study. If cells of the same polarization sensitivity and spectral sensitivity were not normally coupled, then occasionally uncoupled pairs of such cells should have been impaled; no such cases were found. The only case in which cells of the same polarization sensitivity were not coupled was when the cells had different spectral sensitivities. One cannot exclude the possibility that artificial coupling occurred for some pairs of coupled cells with no measurable polarization sensitivity and having weak responses (although a twisting of the rhabdom during penetration might have produced the same result).

The membrane regions responsible for coupling between crayfish retinular cells are unknown. Coupling between pairs of cells on opposite sides of the ommatidium could be either through junctions at the ends of the microvilli or in the region of the nuclei, distal to the rhabdom. Specialized junctions have not been looked for between microvilli at the mid line of the crayfish rhabdom, but no specialized contacts have been seen between microvilli in the *Limulus* lateral eye (Lasansky, 1967; Fahrenbach 1969), where retinular cells are known to be electrically coupled.

What is the value to the crayfish of coupling between cells which have the same polarization sensitivity, spectral sensitivity, and angle of view? At bright levels of illumination the responses of the cells would be expected to be identical, hence there should be no effect of coupling. At extremely dim levels the differences in timing of quantum capture are likely to produce responses of individual cells which are not identical. Coupling would increase the area of photon-sensitive membrane, thus increasing sensitivity while smoothing or averaging the output of a single retinular cell. This could be especially desirable in crayfish, for which the axons of different retinular cells from a single ommatidium apparently have different points of termination in the first ganglion of the eyestalk (Parker, 1897).

Extracellular potentials. Outside the photoreceptor membrane in response to light one can record an extracellular voltage change of at least -10 mV. This potential is presumably generated by inward currents at the microvillar membrane. The voltage recorded by the micro-electrode inside the cell is measured with respect to a reference electrode in the bath. A voltage directly outside the photoreceptive membrane will hence be in

series with the transmembrane potential and will add algebraically to it in producing the recorded intracellular potential. A negative e.r.g. component in series with the photoreceptive membrane will attenuate the positive receptor potential that is recorded intracellularly. Such an interaction of the transmembrane receptor potential with the e.r.g. has previously been proposed for the fly *Calliphora* (Burkhardt, 1962). The local e.r.g. which one records in the crayfish eye is produced by both classes of cells sensitive to polarized light. Can one class of cells therefore affect the amplitude of the responses measured in cells of the other class?

The present experiments have produced evidence that extracellular voltages produced by other cells can influence the voltages recorded intracellularly. By recording from a cell in which the normal light response was irreversibly reduced or abolished, a negative-going voltage response was seen which resembled the local e.r.g. The maintained resting potential and the membrane charging time constant indicated that the recording was indeed intracellular. Because the negative-going response was independent of membrane potential, it is likely that the signal was not generated across the photoreceptive membrane. Unlike the normal receptor potential but like the e.r.g. the intracellular negative-going response was equally sensitive to orthogonal polarizer orientations.

Compared with the local e.r.g. and with the normal receptor potential, the negative-going potential that is recorded intracellularly is small. The negative-going potential, though masked by the receptor potential, should be greater in the normal photoreceptor. This can be explained as follows. Since the normal light response is abolished in the cell from which the negative-going potential is recorded, the resistance of the photoreceptor membrane remains high even when under illumination. The intracellular voltage of an isolated photoreceptor in the bathing solution is determined by the balance of ionic conductances of the passive and light sensitive membranes and by the Nernst potentials of the conducting ions across those membranes. If the voltage outside the cell membrane is not zero, as might happen for a cell in a resistive medium, the membrane conductances will similarly determine the intracellular effects of the extracellular voltages with which the conductances are in series. Therefore when the conductance of a patch of membrane increases, the extracellular voltage in series with that patch will make a greater contribution to the intracellularly recorded voltage. If the photoreceptive membrane resistance remains high, the intracellularly recorded magnitude of the extracellular potential might be expected to be small, but should increase as the photoreceptive membrane conductance increases.

If the responses of one class of cells can influence the intracellular potential of a cell in the other class, the polarization sensitivity of the cell

can be increased as follows. Consider a cell that is illuminated with polarized light oriented for maximum response. If the plane of polarization is then rotated by 90°, the response of the cell is reduced. Now more light is required to produce an equivalent response and an equivalent contribution to the extracellular potential. However, when the plane of polarization was rotated 90° the responses of cells of the other class increased, thus raising their contribution to the local e.r.g. Such increased extracellular voltages can subtract from the transmembrane response of the cell, thereby further attenuating the intracellularly recorded receptor potential. The amount of light required to match the original response is thus greater than it would be in the absence of an extracellular potential. Such a mutual interaction could therefore enhance the polarization sensitivity of cells of each class.

If the polarization sensitivity of a photoreceptor is increased by extracellular interactions with other cells, then the polarization sensitivity ought to be raised further by decreasing the size of the receptor potential relative to the extracellular potential, if in so doing the associated conductance changes of the cell's membrane are not altered. That is, polarization sensitivity increases as the relative importance of the extracellular potential increases. This can be accomplished by making the intracellular potential more positive by passing current through the electrode. As the intracellular potential becomes more positive, the receptor potential becomes smaller and is finally abolished at reversal potential. In several cases the polarization sensitivity ratio increased as the intracellular potential was made more positive (Text-fig. 11). Also, the reversal potential shifted to more negative values when the polarizer was rotated 90° from optimum stimulation and the light intensity increased. This negative shift of reversal potential is the predicted effect of extracellular currents, as argued above. In contrast if there were an *intracellular* contribution from coupled cells of the opposite class, the reversal potential would shift to a more positive value.

This paper has proposed that electrical interactions between cells can increase the sensitivity of retinular cells to polarized light. While inhibitory synaptic interactions could similarly increase polarized light sensitivity, one would then expect that voltage changes produced in one cell would affect cells of the other class. As the coupling experiments demonstrate, no such effects are seen. There are several mechanisms which conceivably could make the polarized light sensitivity ratio larger than the dichroic absorption ratio. For example, a larger polarization sensitivity ratio than the dichroic absorption ratio could result if the dioptric apparatus selectively polarizes the light. This appears to be unlikely because the rhabdom acts as a waveguide (as shown for the fused rhabdom of the honeybee by

Varela & Wiitanen, 1970), and because the polarization sensitivities of cells in the two classes can be equally large (Text-figs. 6 and 8). Also to be considered are the possibilities that the microvilli *in vivo* are elliptical rather than circular in cross-section, that rhodopsin molecules which absorb light polarized parallel to the microvillar axis are preferentially coupled to the conductance change mechanism in the membrane, or that rhodopsin molecules in intact microvilli are oriented along the microvillar axis. The results presented here suggest that a cell's extracellular interactions can produce a sensitivity to polarized light which is greater than the dichroic absorption ratio of 2 measured by Waterman *et al.* (1969). This being so, one might find even larger polarization sensitivity ratios than have been so far reported.

The physiologically significant potentials are presumably the transmembrane potentials at the axon terminals of the receptor cells and not the intracellular voltages recorded near the photoreceptive membrane. Even if the voltage in the cell body is accurately followed in the axon terminals, the polarization sensitivity ratio across the terminal membrane remains unknown. The analysis of the transmission of information from receptor cells to second-order neurones in the crayfish visual system must consider not only the intracellular voltages of the pre- and post-synaptic neurones, but extracellular potentials as well.

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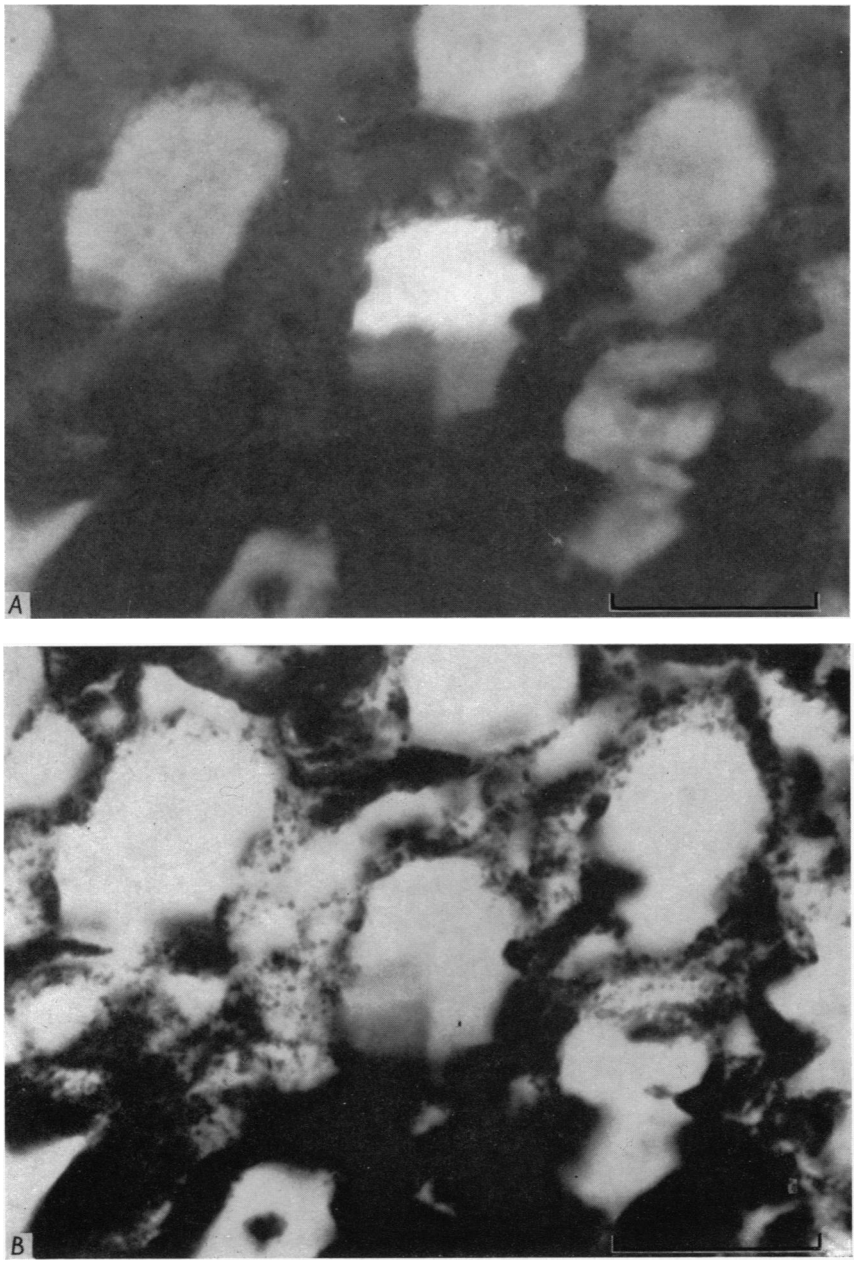

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EXPLANATION OF PLATE

Pl. 1. Light micrographs of a cross-section of a pair of cells in the same ommatidium injected with Procion Yellow M4RS and Procion Navy Blue M3RS dyes. The rhabdoms of neighbouring ommatidia are visible and are separated by dense screening pigment. The cells had orthogonal polarization sensitivities. The banding pattern of the rhabdom is suggested in this 6 μm section. In more distal sections this pattern indicated that the microvilli of the two cells occupied different layers.

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. Calibration marks are 20 μm.

B, the same section in yellow light. Only the blue dye is visible.