Photoreceptor Potentials of Opposite Polarity in the Eye of the Scallop, *Pecten irradians*

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ABSTRACT Intracellular recordings were obtained from single visual cells of the scallop, *Pecten irradians*. Two types of units are found. One type gives a graded, depolarizing response to light and the other a graded, hyperpolarizing response. The depolarizing cells are 2–3 log units more sensitive to light and have a longer latency than the hyperpolarizing type. At high light intensities the depolarizing cells are inactivated while the hyperpolarizing cells maintain their responses. When action potentials are seen they occur during illumination in depolarizing cells ("on" response) and after illumination in hyperpolarizing cells ("off" response). The evidence suggests that the depolarizing responses are from the microvilli-brearing proximal cells, and the hyperpolarizing responses from the ciliary-type distal cells of the retina, and that both responses are directly produced by light.

INTRODUCTION

Photoreceptors from a variety of invertebrates respond to a flash of light with a graded, depolarizing receptor potential (Hartline et al., 1952; Fuortes, 1959; Naka, 1961; Walther, 1965; Dennis, 1967). In contrast, vertebrate receptors respond to light with a hyperpolarizing potential change (Bortoff, 1964; Tomita, 1965; Kaneko and Hashimoto, 1967; Werblin and Dowling, 1969; Toyoda et al., 1969; Baylor and Fuortes, 1970). There is a difference in the structure of these two groups of visual cells; the outer segments of vertebrate photoreceptors are derived from a ciliary base, whereas the depolarizing, invertebrate receptors have specialized areas composed of microvilli. Although invertebrate photoreceptors of the ciliary type have been studied anatomically (Eakin, 1963, 1965), little is known about their responses to light.

Both types of receptor structure are found in the eye of the scallop, *Pecten*.

The scallop retina has two layers of visual cells; the cells of the proximal layer contain microvilli while the cells of the distal layer bear modified cilia (Dakin, 1910; Miller, 1958, 1960; Barber et al., 1967). Furthermore, the nerve fibers from the two layers mediate different responses to light. Hartline (1938) showed that the proximal nerve fibers discharged when light was turned on ("on" response) while the distal nerve fibers were inhibited during illumination and fired at the end of the light stimulus ("off" response). These findings raised the question of whether the distal cells were capable of generating an "off" response directly on being darkened, or whether the "off" response was produced by a synaptic action from other photoreceptor elements which were excited by light. Although early microscopists reported a remarkable variety of connections in the scallop retina (reviewed by Küpfer, 1916), more recent light microscopic (Dakin, 1910, 1928; Küpfer, 1916; Schoepfle and Young, 1936) and electron microscopic studies (Miller, 1958; Barber et al., 1967) have failed to find any evidence for synaptic connections between the two layers. It thus seems likely on anatomical grounds, as well as from physiological evidence provided by optic nerve recording (Land, 1966) that the "off" responses of the optic nerve arise directly from the distal cells, and represent an example of "primary inhibition."

Toyoda and Shapley (1967) reported finding cells in the retina of *Pecten* which hyperpolarized upon illumination. We have confirmed this finding (Gorman and McReynolds, 1969) and shown that a second group of cells is depolarized by light. The purpose of this paper is to show that both responses are directly produced by light and that the depolarizing responses are from proximal cells, whereas the hyperpolarizing responses are from distal cells. The following paper (McReynolds and Gorman, 1970) is concerned with mechanisms involved in producing these responses.

METHODS

The lamellibranch mollusc, *Pecten irradians*, has 50–100 eyes, the largest of which are about 1.5 mm in diameter, situated on the ends of short stalks around the edge of the mantle. The major anatomical features of the eye were described by light microscopists (Dakin, 1910, 1928; Küpfer, 1916; Butcher, 1930). Each eye (Fig. 1 A) contains a cornea, a lens, a fibrous septum, and a double-layered retina containing approximately 5000 receptors (Land, 1968). The proximal surface of the retina is loosely covered by a reflecting argentea (tapetum) and a pigment layer. The retina has two layers of visual cells (proximal and distal cells), whose axons form separate branches of the optic nerve. Glial elements are interposed between the two layers of cells. Electron microscopical studies (Miller, 1958, 1960; Barber et al., 1967) show that the distal cells contain a number of modified cilia directed toward the lens (Fig. 1 B). In contrast, the presumed photoreceptor surfaces of the proximal cells are situated on the portion of the cell near the argentea and are composed of irregular arrays of microvilli. The

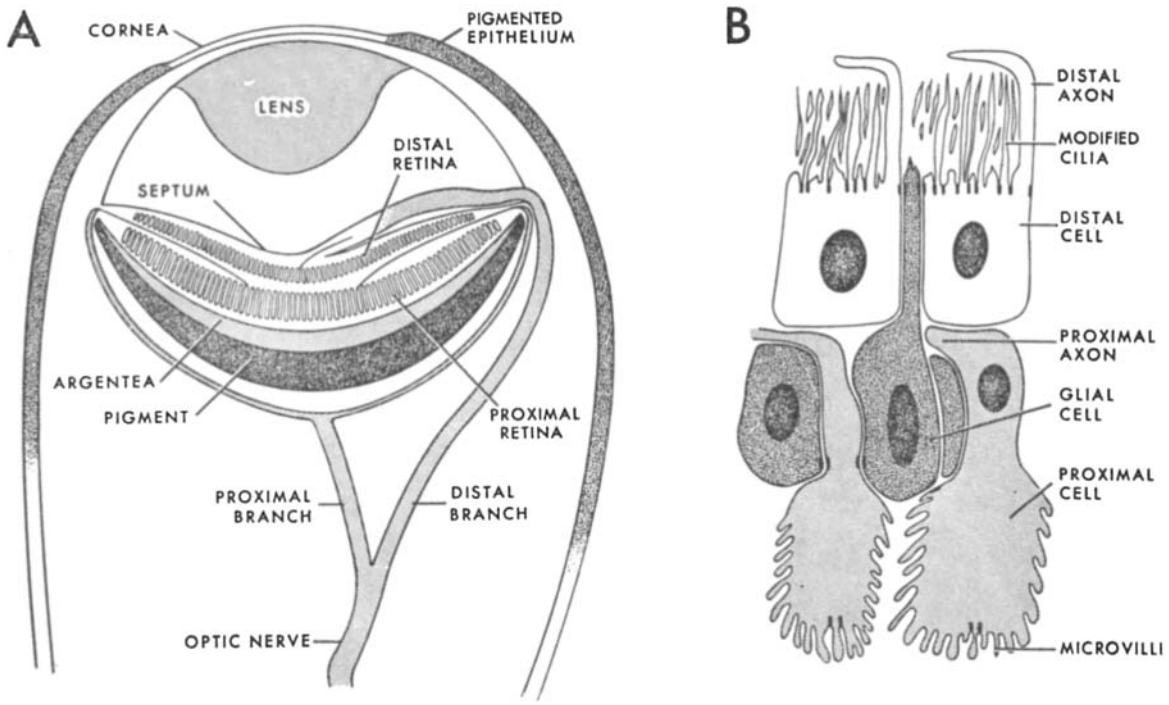

FIGURE 1. Anatomy of *Pecten* eye. A, diagram of longitudinal section through eye showing double-layered retina in relation to other elements. The axons of one distal and two proximal cells are shown leading to the respective branches of the optic nerve. (Modified from Dakin, 1928.) B, schematic diagram of an enlarged section of the retina showing relationship of proximal, distal, and glial cells. Desmosomes shown joining some cells. (Modified from Barber et al., 1967.)

proximal and distal cells are approximately 5 μ in diameter (V. C. Barber, personal communication).

Two different methods of dissecting and mounting the eye were used. One method, described in a previous report (Gorman and McReynolds, 1969), consisted of severing the eye stalk transversely with a razor blade through the pigment layer located behind the argentea. The dissection was performed under seawater and when the cut was made at the right place, the pigment layer and argentea then floated off during the next few minutes, exposing the proximal surface of the retina without further manipulation. These preparations were pinned down with the exposed proximal surface of the retina facing up.

A second method of dissection was to pin down an excised section of the mantle containing one of the larger eyes and its stalk, with the corneal surface facing up. An incision around the edge of the cornea was made with fine scissors, and the cornea and lens removed. With this method the microelectrode had to penetrate the fibrous septum overlying the distal surface of the retina, which usually resulted in breaking the fine electrode tip. An application of a mixture of 1% pronase and 0.5% collagenase in seawater (Hafeman and Miller, 1967) for 2 min, followed by rinsing in seawater for several minutes, greatly facilitated penetration of this barrier. Although more difficult to obtain, essentially the same responses were recorded in preparations without enzymatic treatment. Both methods of dissection gave preparations which remained in good condition for several hours, but action potentials were more often recorded from units when the second method was used.

Intracellular recordings were made with glass capillary micropipettes filled with 3 M KCl; the DC resistance of the electrodes in seawater was 60–100 megohms. Electrodes filled with 4 M potassium acetate were used on some occasions, with no difference in results. All recordings were made against a Ag-AgCl reference electrode in the filtered seawater bath which covered the preparation. The recording electrode was connected through a capacity-compensated electrometer to a DC amplifier. Signals were simultaneously displayed on an oscilloscope and on a rectilinear pen-recorder. A Wheatstone bridge circuit was used to pass constant currents through the recording electrode (see Fuortes, 1959). The bridge was balanced to eliminate the voltage drop across the electrode, leaving the slower charging membrane response. Current was monitored as the voltage drop across a 10^9 Ω resistor which formed the high impedance arm of the bridge.

Although it was possible to record from occasional cells for 10–20 min, most cells were lost within 1–2 min after impalement. In later experiments penetration was aided by the use of an electromagnetic jolting device, which advanced the electrode approximately 1 μ in less than a millisecond (Fish et al., in preparation).

White light from a tungsten quartz-iodine lamp passed through an electrically operated shutter and a field aperture which was focused to an evenly illuminated spot that covered the entire retina (approximately 750 μ in diameter). The intensity of the light stimulus was controlled by calibrated neutral density filters placed in the beam beyond the shutter. A photocell monitored the light stimulus at a point between the shutter and the neutral density filters. The unattenuated light intensity, measured at the level of the retina, was equivalent to 10^{15} photons/cm^2 per sec at 500 nm. This

value was obtained from the spectral distribution of the light energy and the spectral sensitivity of the photoreceptors (McReynolds and Gorman, 1970). Intensities of light stimuli are expressed in log units relative to this value, e.g. for a full intensity flash, $\log I = 0$; for a flash attenuated by a factor of 1000, $\log I = -3.0$.

All experiments were carried out at room temperature (21–23°C). Unless otherwise noted, all recordings were made from eyes which had been dark-adapted for at least half an hour.

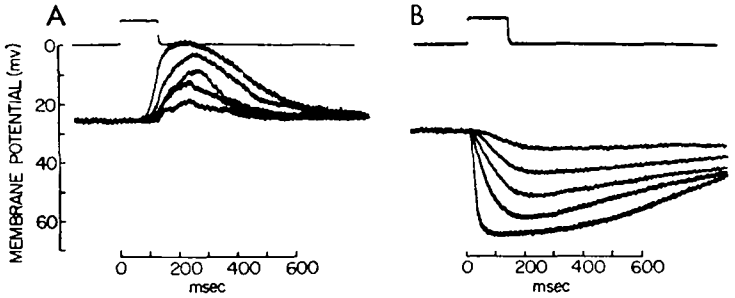

FIGURE 2. Responses of depolarizing and hyperpolarizing cells to brief light flashes of varying intensity. Superimposed oscilloscope traces of responses to five light flashes for each cell. A, depolarizing cell. Intensity of successive flashes increased from $\log I = -5.7$ (smallest response) to $\log I = -3.6$ (largest response). B, hyperpolarizing cell. Intensity of successive flashes increased from $\log I = -3.0$ (smallest response) to $\log I = 0$ (largest response). Both cells from the same eye. Upper beam in A and B indicates zero membrane potential level and shows signal from photocell monitoring the light flash. Time measured from beginning of light flash.

RESULTS

Depolarizing and Hyperpolarizing Responses

When visual cells were penetrated with a microelectrode they showed steady resting potentials of −20 to −40 mv. Two types of units were distinguished by their response to light (Fig. 2). One type responded to a dim light flash with a transient, depolarizing potential change. Superimposed responses from a unit of this type to five light flashes ranging in relative intensity from −5.7 to −3.6 are shown in Fig. 2 A. The amplitude of the depolarization was graded with the intensity of the light flash, and at intensities of about −3.6 the response often overshot the zero membrane potential level by several millivolts. Increasing the intensity above this value, however, usually produced no further increase in response amplitude, and unless a relatively long time for recovery was allowed, resulted in responses of decreased amplitude due to light adaptation of these units.

The other type of cell was unresponsive to flashes in this range of intensities (−5.7 to −3.6). It responded to brighter light flashes with a graded, hyperpolarizing potential change, which could be as large as 40 mv. Superimposed

responses of a unit of this type to five flashes ranging in intensity from −3.0 to 0 are shown in Fig. 2 B. The two cells shown in Fig. 2 were from the same eye, yet the smallest response of the hyperpolarizing cell was obtained at a light intensity 0.6 log unit brighter than that which gave the largest response of the depolarizing cell.

Fig. 3 shows a plot of the response amplitude vs. the logarithm of the intensity of a 100 msec light flash (V–log I curve) for five depolarizing and five

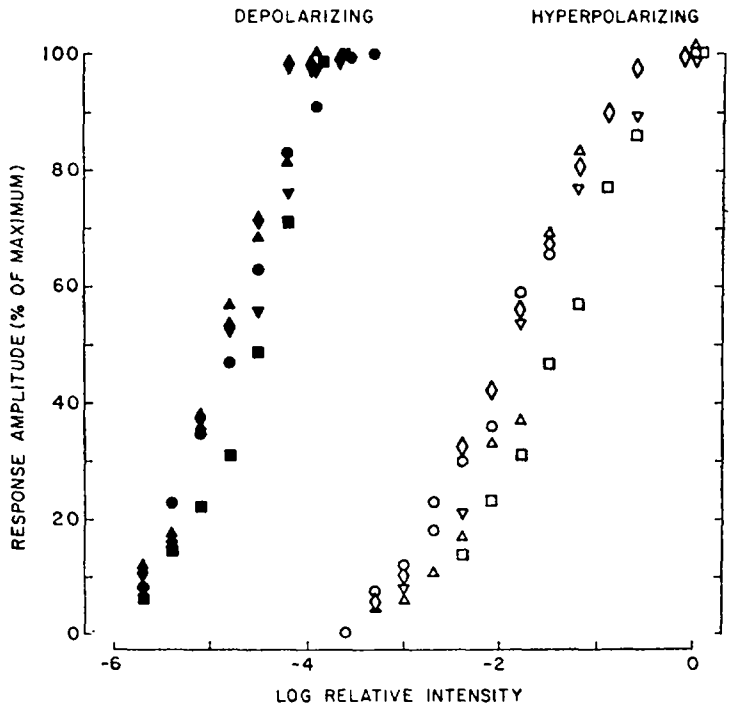

Figure 3. Plot of peak response amplitude vs. flash intensity (V–log I relation) for five depolarizing (filled symbols) and five hyperpolarizing (open symbols) cells. Responses are expressed as % of the maximum response for each cell. All depolarizing responses from eyes dark-adapted at least 30 min; hyperpolarizing responses from both light- and dark-adapted preparations. All responses obtained with 100 msec flashes.

hyperpolarizing units. Since the maximum response amplitudes were not the same in all cells, the responses are expressed as a percentage of the maximum response for each unit. The maximum response for the depolarizing cells is less well-defined because of the adaptation that occurred following any flash brighter than approximately −3.9. A more accurate V–log I curve could have been obtained by letting the eye dark adapt after each test flash, but this was not possible because of our inability to hold most units for long periods of time. However, the values shown in Fig. 3 are reasonably accurate, since when

a dark-adapted depolarizing unit was given a bright flash (log $I \gg -3.6$) early in the series, the response was not much larger (about 10%) than that obtained at -3.6 in other cells. In the dark-adapted eye the depolarizing cells were approximately 2–3 log units more sensitive to light than the hyperpolarizing cells.

The responses of depolarizing and hyperpolarizing units to long flashes of light are shown in Fig. 4. At low intensities the depolarizing responses showed irregular fluctuations, but with brighter lights the responses became smoother and developed an initial peak which decayed to a steady-state level. In these respects the responses are similar to those of other depolarizing photoreceptors. At a moderate intensity (Fig. 4, log $I = -3.3$) the peak response overshot the zero potential level and decayed rapidly to a lower value. With flashes of

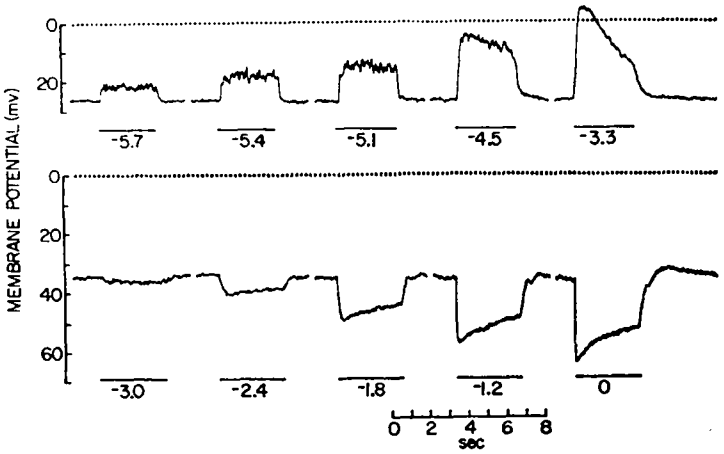

FIGURE 4. Responses of a depolarizing cell (above) and a hyperpolarizing cell (below) to long flashes of light. Duration and relative intensity of each flash shown below response. Dashed lines indicate zero membrane potential level. The response to flash intensity -3.3 was the maximum response for the depolarizing cell.

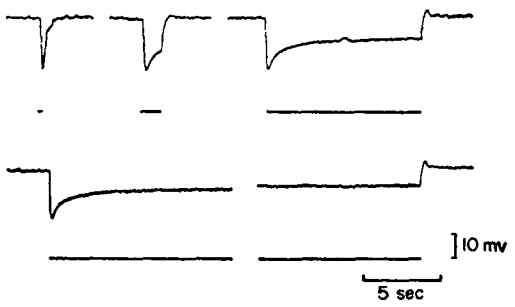

FIGURE 5. Effect of flash duration on "off" transient in a hyperpolarizing cell. Responses to 0.01, 0.1, 1, and 34 sec flashes of the same intensity (log $I = -1.2$). Duration of flash indicated by line under each response. 12.5 sec cut out of bottom record at gap.

higher intensity the response fell from the initial peak to resting potential during continued illumination (Fig. 6 C).

Hyperpolarizing units were unresponsive until intensities were reached which caused adaptation of the depolarizing response. Even the smallest responses (Fig. 4) showed little evidence of the fluctuations that occurred in the depolarizing responses. With increasing intensity of illumination the hyperpolarizing responses also developed a peak and a steady-state portion which was maintained as long as the light was on.

At the cessation of illumination the hyperpolarizing response was often characterized by a notch and a transient rebound above resting potential. The amplitude of the depolarizing rebound was graded in proportion to the size of the response, and, over a limited range, with the duration of the stimulus. Fig. 5 shows the response of a hyperpolarizing cell to flashes of the same intensity but different durations. Although the depolarizing rebound initially became larger with longer flashes, a further increase of stimulus duration beyond a few seconds had no additional effect.

Adaptation of Depolarizing Responses

Individual cells could not be held long enough to study the effect of different levels of background illumination on the V–log I relation, but a striking difference in the adaptation of depolarizing and hyperpolarizing units following a bright stimulus was apparent. Fig. 6 B shows superimposed responses of a hyperpolarizing cell to two flashes of full intensity. The first flash produced a 35 mv response; the same intensity flash given 5 sec later produced an equally

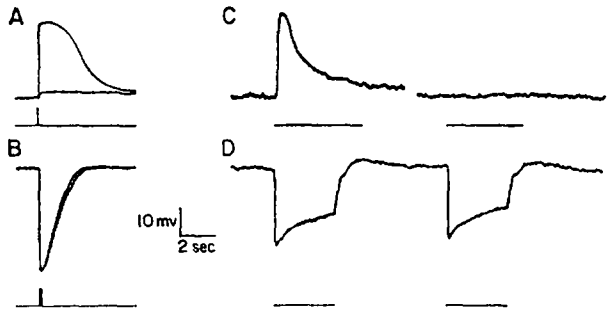

Figure 6. Differences in adaptive properties of depolarizing and hyperpolarizing cells. A, superimposed responses of a depolarizing cell to two 10 msec flashes (log I = 0) spaced 25 sec apart. B, superimposed responses of a hyperpolarizing cell to two 100 msec flashes (log I = 0) spaced 5 sec apart. C, response of a depolarizing cell to a long flash (log I = −1.2) followed by response of same cell to a full intensity flash (log I = 0) 45 sec later. D, continuous recording of a hyperpolarizing cell's responses to two long flashes (log I = 0) separated by 6 sec darkness. Different cells in A, B, C, and D. Light flashes indicated by photocell monitor in A and B, and by lines under responses in C and D.

large response which differed only in its slightly faster return to the base line. The effect of a bright flash on a dark-adapted depolarizing unit was quite different (Fig. 6 A). The cell responded initially to a full intensity flash with a large depolarizing potential, whereas a second flash given 25 sec later produced only a very small response. In another depolarizing cell (Fig. 6 C), although a long flash of intensity -1.2 elicited a large initial transient, the steady state was not maintained, and after 45 sec of darkness a second long flash of full intensity gave no detectable response. In contrast, all components of the hyperpolarizing response were less affected by adaptation (Fig. 6 D).

Time Course of Responses

The hyperpolarizing responses had a much shorter latency of onset and time-to-peak than the depolarizing responses. The earliest latency of onset recorded for a hyperpolarizing unit was 7 msec whereas that for depolarizing units was 20 msec. Because of the differences in sensitivity ranges and adaptation, it was not practical to compare responses to flashes of equal intensity between most cells, but if responses of equal amplitude are compared a marked difference in latency of onset of the potential change is apparent (inset, Fig. 7). For a given cell of either type the latency of onset of the response decreased with increasing flash intensity. In spite of these variations, there is a significant

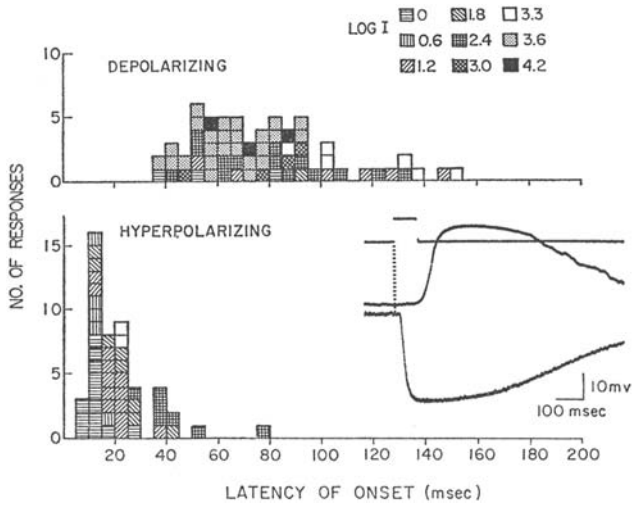

FIGURE 7. Latency histogram for onset of depolarizing and hyperpolarizing responses: Responses are of equal amplitude but were to flashes of various intensities (indicated by hatching). Each response is from a different cell. Inset, responses of equal amplitude from a depolarizing and a hyperpolarizing unit. Light intensity same for both responses (log $I = -1.2$). Photographically superimposed oscilloscope traces. Uppermost beam indicates zero membrane potential level and shows signal from photocell monitoring light flash. Vertical dashed line indicates time from which latencies were measured.

difference between the two groups when responses to a wide range of light intensities are compared (Fig. 7).

Discharge Characteristics of Depolarizing and Hyperpolarizing Units

Spikes were infrequently recorded in both hyperpolarizing and depolarizing cells. They were always of small amplitude (5–30 mv) and were usually seen only during the first minute of a penetration, after which time they became smaller and disappeared completely. This usually occurred with no change in resting potential or sensitivity to light; consistently large receptor potentials were obtained long after all traces of impulse activity had vanished. It appears that impalement with a microelectrode can easily damage the impulse-generating mechanism without affecting other properties of these cells.

In depolarizing units, spikes were superimposed on the receptor potential (Fig. 8 A) and firing frequency increased with amplitude of the depolarizing response. In cells in which light caused a hyperpolarizing potential change, a burst of firing occurred at the end of illumination. When background firing was present, it was inhibited during the light-induced hyperpolarization. It should be noted that the transient increase in firing at the end of illumination was not necessarily associated with a depolarizing rebound of membrane potential and, in fact, could occur before the membrane fully recovered from its hyperpolarized state (Fig. 8 B).

Responses to applied currents were similar in both types of cells. For example, "on" discharges during depolarizing current and "off" discharges after hyperpolarizing current occurred in both types of cells. These findings suggest that the firing pattern of a given unit in response to illumination depends upon the direction in which light drives the membrane potential rather than on intrinsic differences in the impulse-generating mechanisms. In two cells, a large depolarizing "off" transient was seen following release of hyperpolarizing current; this response was graded with the amount of previous hyperpolarization. Similar transients following release of hyperpolarizing current have been seen in other photoreceptors (Smith and Baumann, 1969).

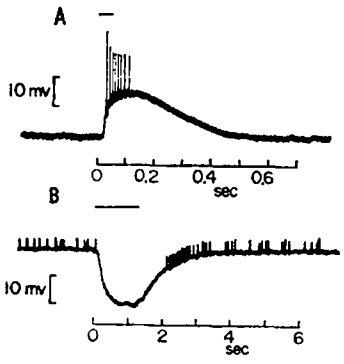

FIGURE 8. Action potentials in depolarizing and hyperpolarizing cells. A, response of depolarizing cell to brief flash (log I = −4.2). B, response of hyperpolarizing cell to longer flash (log I = −2.4). Duration of light flash indicated by horizontal line above each response.

Localization of Depolarizing and Hyperpolarizing Units

The depolarizing and hyperpolarizing units were found at different levels within the retina. When the retina was approached from the corneal (distal) side hyperpolarizing units were encountered near the distal surface, while depolarizing cells were found deeper. The opposite sequence was found in experiments in which the retina was approached from its proximal surface. The exact depth at which the two types of response occurred could not be reliably measured because of the thinness of the retina, and because the angle of the electrode track was not always the same with respect to the curved retinal surface.

Biphasic Responses

Units were frequently penetrated which had much larger resting potentials than the cells described above. These cells were always encountered in the proximal part of the retina, had resting potentials of 50–60 mv, and responded to a light flash with a slow prolonged depolarization of up to 20 mv in amplitude, usually preceded by a small hyperpolarizing deflection (Fig. 9). In dark-adapted eyes, responses could be obtained with light intensities as low as -4.2, and they adapted at bright intensities, much like the depolarizing units. The depolarizing component sometimes showed a summation in response to repetitive flashing, a phenomenon which was never observed in the other cell types. Spikes were not recorded from the biphasic units in response to either light or current. The input resistance of these cells was relatively low (less than 10^6 ohms), since the maximum current that could be passed with

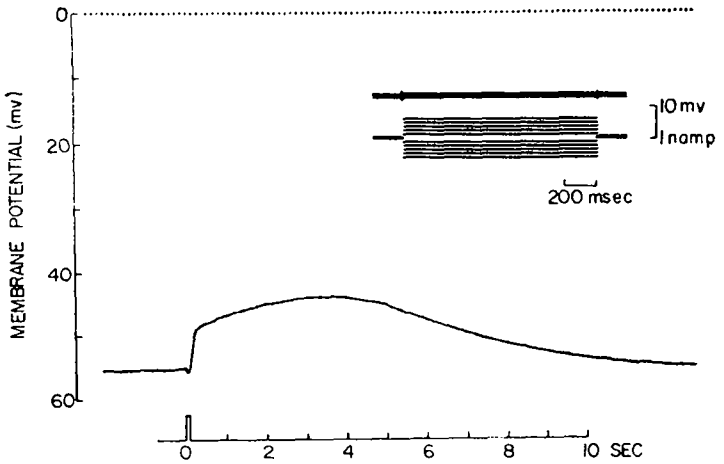

FIGURE 9. Response of a biphasic unit to a brief flash. 100 msec flash (log $I = -1.2$) given at $t = 0$. Zero membrane potential indicated by dashed line. Inset shows lack of response (above) to positive and negative constant current steps (below).

our electrodes (0.5–1.0 $\times$ 10^{-9} amp) produced less than 1 mv potential change (inset, Fig. 9). This is in marked contrast to the depolarizing and hyperpolarizing units, which showed a relatively high input resistance using the same method (McReynolds and Gorman, 1970). The large depolarizing component became much smaller and reversed polarity when the electrode was withdrawn slightly, and resting potential was lost, whereas the small hyperpolarizing deflection was unchanged in amplitude or polarity under the same conditions.

DISCUSSION

Three classes of cells in the scallop retina can be distinguished by their membrane response to illumination—depolarizing, hyperpolarizing, and biphasic units. The following discussion is primarily concerned with two questions: (*a*) can these responses be identified with anatomically defined elements in the retina, and (*b*) are these responses primary or secondary effects of light?

The sequence in which hyperpolarizing and depolarizing potentials are encountered as the electrode is advanced through the retina shows that there is a definite spatial segregation of these two response types within the retina. Hyperpolarizing responses are recorded from cells in the distal part, and depolarizing responses from cells in the proximal portion of the retina. Furthermore, depolarizing responses were associated with firing during illumination ("on" discharge) whereas hyperpolarizing responses were associated with an "off" discharge at the end of the light stimulus. The discharge patterns of the depolarizing and hyperpolarizing cells thus correspond respectively to the "on" and "off" responses of the proximal and distal nerve fibers (Hartline, 1938; Land, 1966). For these reasons we identify the depolarizing units as proximal cells and the hyperpolarizing units as distal cells.

The properties of the third class of units are different from those of the proximal and distal cells. The relatively large resting potential, low input resistance, the slowly rising, prolonged responses to a light flash, and summation of successive responses resemble more the properties of glial cells in amphibian optic nerve (Kuffler et al., 1966; Orkand et al., 1966). These responses were encountered in the more proximal portions of the retina, in a region where glial cell bodies are relatively large (Barber et al., 1967). Although glial cells are likely candidates, positive identification of these units cannot be made on the basis of our evidence. The small, initial hyperpolarizing phase of the biphasic potential could be a reflection of the local ERG from nearby receptors as it did not reverse polarity or change appreciably in size when the recording electrode was withdrawn from the cell. The long depolarizing phase of this potential may be due to changes in extracellular potassium ion concentration resulting from visual cell activity (*cf.* Orkand et al., 1966).

Several consistent findings indicate that both the hyperpolarizing and depolarizing responses are produced directly by light, and not by synaptic action. First, the depolarizing response is about 2 orders of magnitude more sensitive to light than the hyperpolarizing response at threshold. Second, the hyperpolarizing response is relatively unaffected by bright illumination which completely abolishes the depolarizing response. It is thus possible by controlling the stimulus intensity and the state of adaptation to selectively activate either proximal or distal cells. Third, the hyperpolarizing response has a much shorter latency of onset than the depolarizing response at all intensities.

The basis for these striking differences between proximal and distal cells is not clear, but in many respects they show a remarkable similarity to vertebrate rods and cones. For example, the rod system is several orders of magnitude more sensitive than the cone system. Although the greater convergence of rods, compared to cones, onto higher order neurons obviously affects the relative sensitivity of the two *systems*, it is not certain to what extent the two types of receptors differ in their threshold for producing an electrical response. The finding that proximal cells of *Pecten* are inactivated by higher light intensities which are adequate stimuli for distal cells is especially interesting in relation to the shift from rod cone vision at higher levels of intensity. Although the rod system clearly saturates at high levels of illumination (Aguilar and Stiles, 1954), it has not been clearly established that the rods cease to produce a receptor potential under these conditions. The situation in the *Pecten* retina raises the question of whether rods may become unresponsive to light at high levels of illumination.

Neither Hartline (1938) nor Land (1966) reported any difference in sensitivity of "on" and "off" discharges in *Pecten* optic nerve fibers. This apparent contradiction between their results and ours may be due to two factors. First, the 2 log unit difference in threshold between proximal and distal cells was only evident in eyes which had been sufficiently dark-adapted. Light intensities which activate distal cells result in light adaptation of the proximal cells, thereby markedly reducing the difference in sensitivity. Second, we have measured the sensitivities of the depolarizing and hyperpolarizing *receptor potentials* rather than the discharge of action potentials, and it is quite possible that factors influencing spike threshold may be different in the two cell types. Certainly, the "off" discharge is not related to the receptor potential in the same way that the "on" response is.

The conclusion that the depolarizing and hyperpolarizing receptor potentials are independent, primary effects of light is not completely unexpected in view of the lack of histological evidence for synaptic connections between retinal layers and the fact that anatomically both proximal and distal cells have specialized membrane regions associated with photoreceptor cells (Miller, 1958, 1960; Barber et al., 1967; Eakin, 1963, 1965). Additional physio-

logical evidence for the independence of the two layers has been provided in *Pecten maximus* by Land (1966), who showed in a series of experiments with moving light and dark stripes that the distal nerve could be made to fire independently of the proximal nerve. The basis for this difference was attributed to the fact that such responses are image dependent and in *Pecten* an image is formed only at the distal cell layer (Land, 1965, 1966).

A depolarizing transient was often seen in hyperpolarizing cells at the end of illumination, and a similar phenomenon could be produced upon release of hyperpolarizing current. An "off" rebound could certainly increase the firing frequency and enhance the initial high-frequency burst typical of "off" discharges. However, "off" discharges could occur in the absence of any depolarizing rebound, indicating that other factors must also be involved. It is possible that processes which are not reflected by a depolarizing membrane potential change play a role in generating the "off" response. Anode break responses have been explained in terms of a slow return of sodium inactivation following release of membrane hyperpolarization (Hodgkin and Huxley, 1952 *a*, *b*). If the time constants for removal and return of such an inactivation process were sufficiently long, they could account for the ability of the distal cells to integrate light over a period of time and produce the "off" discharges recorded in the optic nerve (Hartline, 1938; Land, 1966) which are much longer lasting than any slow potential changes we have recorded.

In many visual systems the generation of "off" responses is achieved by synaptic inhibition, the "off" discharge occurring only in a second (or higher) order neuron (Wilska and Hartline, 1941; Ratliff and Mueller, 1957; Ruck, 1961; Gwilliam, 1963; Dennis, 1967). The finding that the distal cells of the *Pecten* retina respond to light with a hyperpolarizing receptor potential and an "off" discharge establishes that primary inhibition can occur in a photoreceptor. Primary inhibition was inferred by Kennedy (1960) from the effects of light on the discharge patterns of the pallial nerve of *Spisula* and has also been suggested in *Cardium* (Barber and Land, 1967) and *Lima* (Mpitsos, 1969) from studies on nerve discharge patterns. In *Lima*, moreover, a hyperpolarizing response to light was recorded (Mpitsos, 1969) from a region of the eye containing numerous ciliated cells (Bell and Mpitsos, 1968).

It has been suggested (Land, 1968) that there is, at least in molluscs, a functional association between ciliary-type photoreceptors and primary inhibition. It would therefore be interesting to determine whether hyperpolarizing receptor potentials are characteristic of other ciliary photoreceptors. Aside from *Pecten* and *Lima* the only other reported intracellular recordings from photoreceptors of the ciliary type are from vertebrate cones, which also give hyperpolarizing receptor potentials (Bortoff, 1964; Tomita, 1965; Kaneko and Hashimoto, 1967; Werblin and Dowling, 1969; Toyoda et al., 1969; Baylor and Fuortes, 1970). Action potentials have not been seen in

cones, however, and since cones excite "on" responses in higher order neurons there is no reason to regard their hyperpolarizing potentials as necessarily inhibitory (cf. Grundfest, 1958). Generation of a hyperpolarizing receptor potential is thus not synonymous with primary inhibition. Furthermore, although hyperpolarizing receptor potentials so far appear to be associated with ciliary-type photoreceptors, this relationship may not prove to be of any functional significance, since the mechanisms by which the hyperpolarizing receptor potentials are generated in *Pecten* and in vertebrate photoreceptors are basically different (see McReynolds and Gorman, 1970).

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REFERENCES

AGUILAR, M., and W. S. STILES. 1954. Saturation of the rod mechanism of the retina at high levels of stimulation. *Opt. Acta.* **1**:59.

BARBER, V. C., E. M. EVANS, and M. F. LAND. 1967. The fine structure of the eye of the mollusc *Pecten maximus*. *Z. Zellforsch.* **76**:295.

BARBER, V. C., and M. F. LAND. 1967. Eye of the cockle, *Cardium edule*: anatomical and physiological observations. *Experientia (Basel).* **23**:677.

BAYLOR, D. A., and M. G. F. FUORTES. 1970. Electrical responses of single cones in the retina of the turtle. *J. Physiol. (London).* **207**:77.

BELL, A. L., and G. J. MPITSOS. 1968. Morphology of the eye of the flame fringed clam, *Lima scabra*. *Biol. Bull. (Woods Hole).* **135**:414.

BORTOFF, A. 1964. Localization of slow potential responses in the *Necturus* retina. *Vision Res.* **4**:627.

BUTCHER, E. O. 1930. The formation, regeneration and transplantation of eyes in *Pecten (Gibbus borealis)*. *Biol. Bull. (Woods Hole).* **59**:154.

DAKIN, W. J. 1910. The eye of *Pecten*. *Quart. J. Microsc. Sci.* **55**: 49.

DAKIN, W. J. 1928. The eyes of *Pecten, Spondylus, Amussium* and allied lamellibranchs with a short discussion on their evolution. *Proc. Roy. Soc. Ser. B. Biol. Sci.* **103**:355.

DENNIS, M. J. 1967. Electrophysiology of the visual system of a nudibranch mollusc. *J. Neurophysiol.* **30**:1439.

EAKIN, R. M. 1963. Lines of evolution of photoreceptors. *In* General Physiology of Cell Specialization. D. Mazia and A. Tyler, editors. McGraw-Hill Book Co., New York. 393.

EAKIN, R. M. 1965. Evolution of photoreceptors. *Cold Spring Harbor Symp. Quant. Biol.* **30**:363.

FUORTES, M. G. F. 1959. Initiation of impulses in visual cells of *Limulus*. *J. Physiol. (London).* **148**:14.

GORMAN, A. L. F., and J. S. MCREYNOLDS. 1969. Hyperpolarizing and depolarizing receptor potentials in the scallop eye. *Science (Washington).* **165**:309.

GRUNDFEST, H. 1958. An electrophysiological basis for cone vision in fish. *Arch. Ital. Biol.* **96**:135.

GWILLIAM, G. F. 1963. The mechanism of the shadow reflex in cirripedia. I. Electrical activity in the supraesophageal ganglion and ocellar nerve. *Biol. Bull. (Woods Hole).* **125**:470.

HAFEMAN, D. R., and S. L. MILLER. 1967. Enzymatic softening of connective tissue sheaths to aid microelectrode penetration. *Comp. Biochem. Physiol.* **22**:303.

HARTLINE, H. K. 1938. The discharge of impulses in the optic nerve of *Pecten* in response to illumination of the eye. *J. Cell. Comp. Physiol.* **11**:465.

HARTLINE, H. K., H. G. WAGNER, and E. F. MACNICHOL. 1952. The peripheral origin of nervous activity in the visual system. *Cold Spring Harbor Symp. Quant. Biol.* **17**:125.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *a*. The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol. (London).* **116**:497.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *b*. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (London).* **117**:500.

KANEKO, A., and H. HASHIMOTO. 1967. Recording site of the single cone response determined by an electrode marking technique. *Vision Res.* **7**:847.

KENNEDY, D. 1960. Neural photoreception in a lamellibranch mollusc. *J. Gen. Physiol.* **44**:277.

KÜPFER, M. 1916. Sehorgane am Mantelrande der *Pecten-Arten.* Gustav Fischer, Jena.

KUFFLER, S. W., J. G. NICHOLLS, and R. K. ORKAND. 1966. Physiological properties of glial cells in the central nervous system of amphibia. *J. Neurophysiol.* **29**:768.

LAND, M. F. 1965. Image formation by a concave reflector in the eye of the scallop, *Pecten maximus. J. Physiol. (London).* **179**:138.

LAND, M. F. 1966. Activity in the optic nerves of *Pecten maximus* in response to changes in light intensity, and to pattern and movement in the optical environment. *J. Exp. Biol.* **45**:433.

LAND, M. F. 1968. Functional aspects of the optical and retinal organization of the mollusc eye. *Symp. Zool. Soc. London.* **23**:75.

McREYNOLDS, J. S., and A. L. F. GORMAN. 1970. Membrane conductances and spectral sensitivities of *Pecten* photoreceptors. *J. Gen. Physiol.* **56**:392.

MILLER, W. H. 1958. Derivatives of cilia in the distal sense cells of the retina of *Pecten. J. Biophys. Biochem. Cytol.* **4**:227.

MILLER, W. H. 1960. Visual photoreceptor structures. *In* The Cell. J. Brachet and A. E. Mirsky, editors. Academic Press, Inc., London. Pt. IV, 325.

MPITSOS, G. J. 1969. An electrophysiological analysis of the vision in a file clam (*Lima scabra* Born). Ph.D. Dissertation, University of Virginia.

NAKA, K. I. 1961. Recording of retinal action potentials from single cells in the insect compound eye. *J. Gen. Physiol.* **44**:571.

ORKAND, R. K., J. G. NICHOLLS, and S. W. KUFFLER. 1966. Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J. Neurophysiol.* **29**:788.

RATLIFF, F., and C. G. MUELLER. 1957. Synthesis of "on-off" and "off" responses in a visual-neural system. *Science (Washington).* **126**:840.

RUCK, P. 1961. Electrophysiology of the insect dorsal ocellus. II. Mechanisms of generation and inhibition of impulses in the ocellar nerve of dragon flies. *J. Gen. Physiol.* **44**:629.

SCHOEPFLE, G., and J. Z. YOUNG. 1936. The structure of the eye of *Pecten. Biol. Bull. (Woods Hole).* **71**:403.

SMITH, T. G., and F. BAUMANN. 1969. The functional organization within the ommatidium of the compound eye of *Limulus. Progr. Brain Res.* **31**:313.

TOMITA, T. 1965. Electrophysiological study of the mechanisms subserving color coding in the fish retina. *Cold Spring Harbor Symp. Quant. Biol.* **30**:559.

TOYODA, J. I., H. NOSAKI, and T. TOMITA. 1969. Light-induced resistance changes in single photoreceptors of *Necturus* and *Gekko. Vision Res.* **9**:453.

TOYODA, J. I., and R. M. SHAPLEY. 1967. The intracellularly recorded response in the scallop eye. *Biol. Bull. (Woods Hole).* **133**:490.

WALTHER, J. B. 1965. Single cell responses from the primitive eyes of an annelid. *In* Functional Organization of the Compound Eye. C. G. Bernhard, editor. Pergamon Press, New York. 329.

WERBLIN, F. S., and J. E. DOWLING. 1969. Organization of the retina of the mudpuppy, *Necturus maculosus.* II. Intracellular recording. *J. Neurophysiol.* **32**:339.

WILSKA, A., and H. K. HARTLINE. 1941. The origin of "off-responses" in the optic pathway. *Amer. J. Physiol.* **133**:491.