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ELECTRICAL ACTIVITY AND STRUCTURE OF RETINAL CELLS OF THE *APLYSIA* EYE: II. PHOTORECEPTORS

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SUMMARY

1. Photoreceptors of the eye of *Aplysia* were studied by intracellular recording and Lucifer yellow injection.

2. Two basic photoreceptor types were observed, R and H. Two other types of cells were occasionally encountered: one was neurone-like, giving only a slight depolarization but large action potentials (APs) in response to light; the other was presumably glial.

3. Type R photoreceptors were found in the pigmented layer of the retina, had large distal (photoreceptor) processes extending toward the lens and an axon in the optic nerve. They are probably the large, microvillous receptor type with vesicle-filled cytoplasm observed previously in electron microscope studies. Action potentials were observed in the axon but not the cell body of the R receptor. The light response was an increasing conductance, 2 component depolarization followed by hyperpolarization. All 3 components were affected by light adaptation. Electrical coupling between R receptors and secondary neurones was apparent and the system produces the synchronous compound action potentials (CAPs) in the optic nerve.

4. Type H photoreceptors gave a slight depolarization to light with APs, followed by a hyperpolarization, followed by a late depolarization and more APs. They were in the pigmented layer of the retina and had smaller cell bodies and distal processes, but larger axons than R receptors. They may correspond to the photoreceptors with short microvilli and occasional cilia described previously in electron microscope studies. Electrical and dye coupling occurred between the receptors. The H receptors do not contribute to the CAP, but produce separate unitary potentials in the optic nerve.

INTRODUCTION

Several types of neurones and photoreceptors are known in the eye of *Aplysia* (Jacklet, 1969, 1976; Luborsky-Moore & Jacklet, 1977; Jacklet, Alvarez & Bernstein, 1972; Strumwasser *et al.* 1979). In the preceding paper (Jacklet, Schuster & Rolerson, 1982), electrical activity of secondary neurones, marked with Lucifer yellow, was correlated with compound action potential (CAP) activity in the optic nerve. In this paper a similar study is made of the photoreceptors.

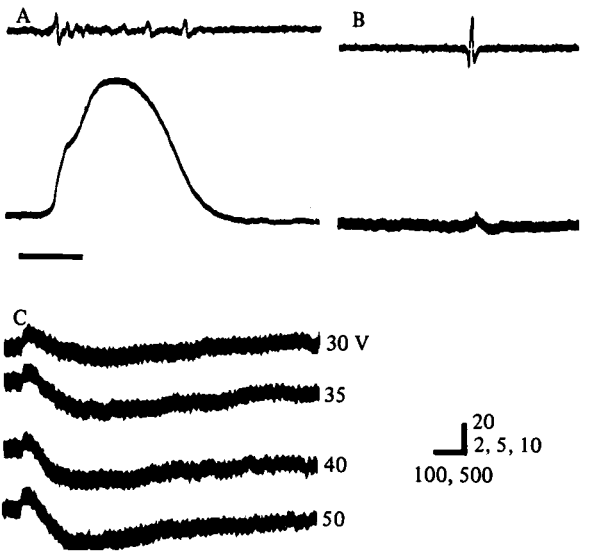

Fig. 1. Photoreceptor light-evoked responses and spontaneous inputs. (A) Graded depolarization to a 1 s light pulse; resting potential 60 mV. (B) A biphasic potential was correlated 1:1 with each spontaneous dark CAP (upper trace), but it followed the CAP. (C) Electrical stimulation of optic nerve at 30, 35, 40 and 50 V, 0·5 ms evoked a potential similar to (B). Larger responses were obtained with higher voltages. Scales: 20 μV, 10 mV, 500 ms in (A); 20 μV, 5 mV, 500 ms in (B); 2 mV, 100 ms in (C).

METHODS

The basic methods employed are described in the preceding paper (Jacklet *et al.* 1982).

RESULTS

Photoreceptors were the cells most frequently encountered when the retina was probed with a micropipette electrode. The results here are gathered from hundreds of stable impalements made over the course of several years from about 80 eyes. Two types were found: (1) those that were inactive in darkness and responded to light with a graded depolarization, consisting of several components, but rarely with evoked action potentials (APs), (2) those that occasionally fired APs, not correlated with the CAPs in the optic nerve, in darkness, and responded to light with a triphasic wave of depolarization, hyperpolarization and depolarization accompanied by large overshooting AP. Occasionally recordings were made from two other cell types. One, presumed to be neuronal, gave a slight depolarization to light and produced large APs not correlated with the optic nerve CAPs. Another, presumed to be glial, had stable resting potentials of 50–70 mV but it failed to respond to light, even after long dark adaptation.

Photoreceptors with graded depolarization but no APs

This cell type was the one most frequently encountered and it gave the most stable recordings. It is therefore probably the largest receptor cell in the eye, which is about

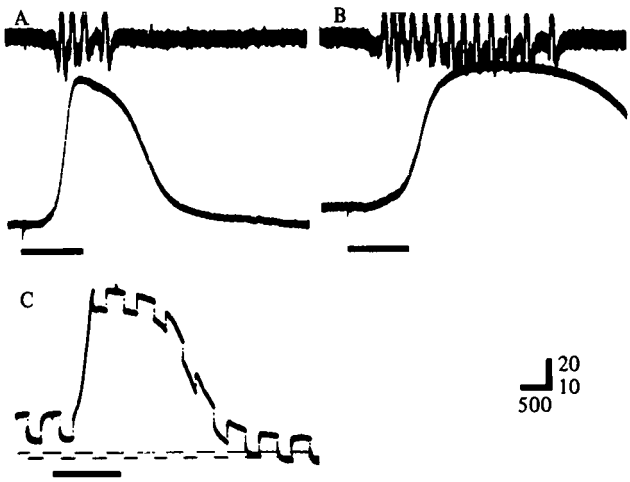

Fig. 2. Photoreceptor light responses. (A) A depolarization of 45 mV was evoked by a 1 s light pulse; resting potential 45 mV. Low Ca^{2+}-high Mg^{2+} for 30 min prolonged the photoreceptor response and the evoked CAP activity, as shown in (B). In (C), 0·1 nA current pulses were applied during the light pulse and the amplitude of the resultant voltage decreased to 60% during the light-induced depolarization. Scales: 20 μV, 500 ms in (A); 20 μV, 10 mV, 500 ms in (B); 10 mV, 500 ms, 0·1 nA pulses at 2/s in (C). Dark adapted 10 min.

15–30 μm in diameter and 50–80 μm long (Jacklet *et al.* 1972). This type had the most uniform characteristics and impalements could often be held for 0·5 h. An average ratio during a recording session was 5 good impalements of graded receptors encountered for each one of the other type. Resting potentials were usually 50–65 mV. General characteristics are shown in Fig. 1. Bright light evoked a large depolarization that approached or exceeded the OV level. Two components of the depolarization could often be observed, a rapidly rising initial depolarization followed by a slower larger depolarization, which outlasted the 1 s light pulse used most frequently. This cell type was almost always completely inactive during dark CAP activity recorded from the optic nerve. On some occasions small (< 2 mV) biphasic potentials occurred about 20 ms after the spontaneous dark CAP (Fig. 1B), suggesting that they were a consequence of the CAP. Electrical stimulation of the optic nerve evoked a similar biphasic potential and increasing the stimulus voltage enhanced the amplitude of the potential as if it was a compound potential (Fig. 1C). It was not possible to apply low Ca^{2+}-high Mg^{2+} solutions to test whether the potentials were chemically mediated, because many receptors gave no response or only a tiny response to stimulation of the optic nerve. Hyperpolarizing the receptor failed to enhance the depolarizing portion of the potential or diminish the hyperpolarizing portion. These results suggest that the potential is originating from a remote site or an electrical synapse.

Low Ca^{2+}-high Mg^{2+} sea water enhanced the frequency and number of CAP in response to a standard light pulse (Fig. 2A, B). It also caused a slight ($\sim$ 5–10 mV) depolarization of this receptor type and usually prolonged the light evoked depolarization (Fig. 2B), but did not block it. Therefore the depolarization appears to be a primary response to light and it, as well as the CAP, is not mediated by chemical

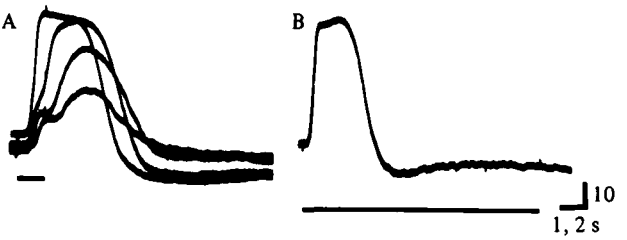

Fig. 3. Photoreceptor light responses and adaptation. (A) Photoresponses evoked successively after 15 min in dark (largest, fastest rising), 3 min in dark, 1 min in dark and 30 s in dark; resting potential 55 mV. (B) Response is to an 18 s light pulse after 15 min in dark. Scales: 10 mV, 1 s in (A); 10 mV, 2 s in (B).

synapses. During the light evoked depolarizations, resistance was measured by hyperpolarizing currents and it most often decreased to 60% as shown in Fig. 2C. This indicates the response is due to an increase in ionic conductance.

The photoreceptors showed pronounced adaptation to repeated light pulses. If the receptors were dark adapted for 10 min they usually gave responses similar to those of Fig. 2. The depolarization rose steeply and monophasically from the baseline and some degree of hyperpolarization followed the depolarization (especially Fig. 2C). A measure of the short term adaptation was obtained from responses to successive 1 s pulses of light (Fig. 3). In Fig. 3A the first (largest and fastest-rising) potential was evoked after 15 min of darkness, the second after 3 min, the third after 1 min and the last after 30 s of darkness. Both the early, fast and late, slow depolarizations adapted as well as the hyperpolarization. Also note that the hyperpolarization following the first pulse was still present after 3 min, at the onset of the second pulse. The receptor recovered fully in 15 min after the last pulse in Fig. 3A and responded to light as shown in Fig. 3B with a depolarization and hyperpolarization similar to the first response in Fig. 3A. The light pulse in Fig. 3B was prolonged but the receptor still hyperpolarized after the initial depolarization while the light was on. During the prolonged light pulse in Fig. 3B a secondary depolarization developed, which decayed when the light was terminated. The light response was complex involving an initial depolarization (with 2 components), a hyperpolarization and a secondary depolarization. The first two components are mediated by increased conductances (see Fig. 2C).

The spectral sensitivity of two receptors of this type were checked at three wavelengths, 420, 500 and 660 nm. For a response of 3 mV depolarization after 10 min of dark adaptation the least intensity was required at 500 nm. One hundred times the intensity was needed at 660 nm and 3 times as much was needed at 420 nm.

Injecting this type of photoreceptor with Lucifer yellow demonstrated that it is in the innermost (near the lens) receptor layer of the retina. It has a prominent photoreceptor distal segment that extends toward the lens as shown in the two examples of Fig. 4. Each of the eight cells successfully injected was tested before injection to be sure it responded to light in the characteristic way (see inset to Fig. 4A). Both cells shown in Fig. 4 had very bright fluorescent cell bodies suggesting that the cell was injected there. The distal segments were quite prominent and especially in Fig. 4A it

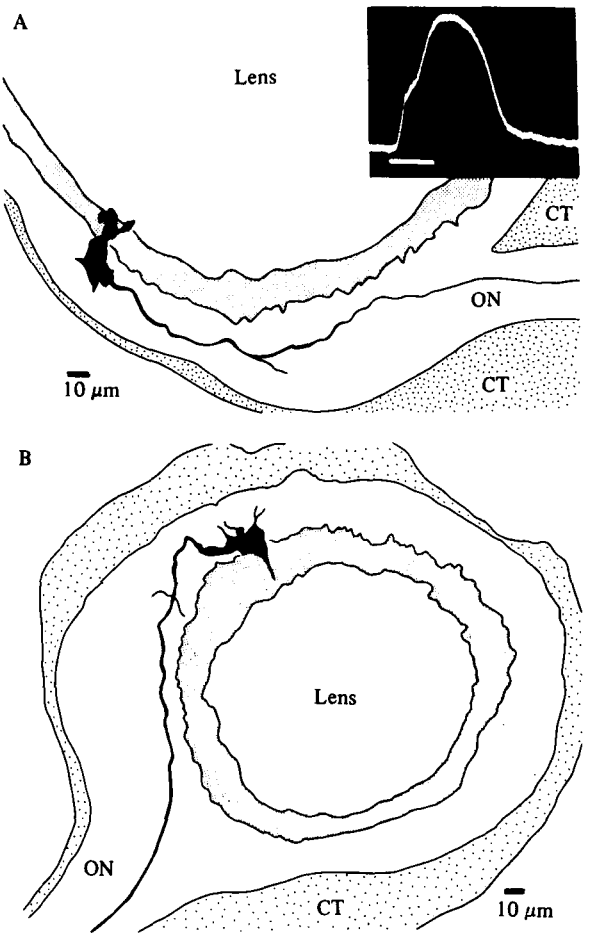

Fig. 4. Lucifer yellow injected photoreceptors of the graded depolarization type. Two examples are shown. The receptor in (A) gave the 50 mV graded depolarization shown in the inset to a 1 s light pulse. It had a large distal segment and a single axon in the optic nerve. A similar receptor in (B) had more visible processes extending from the soma. Fine stipple is pigmented retina, coarse stipple is connective tissue (CT).

extended well beyond the pigmented layer of the retina and into the vitreous body adjacent to the lens. The large vesicle-filled microvillus photoreceptors of the eye have a similar morphology (Jacklet, 1969). Each filled receptor had numerous fibre processes extending from the soma and one of these was the axon of the cell which could be traced to the optic nerve.

Photoreceptors with graded depolarization and superimposed APs

Large graded depolarizations in response to light accompanied by APs were recorded from a few rarely encountered cells. These cells had resting potentials of 50–60 mV and did not generate APs during spontaneous dark CAP activity. Figure 5 shows responses recorded from that type of cell after successive dark adaptation

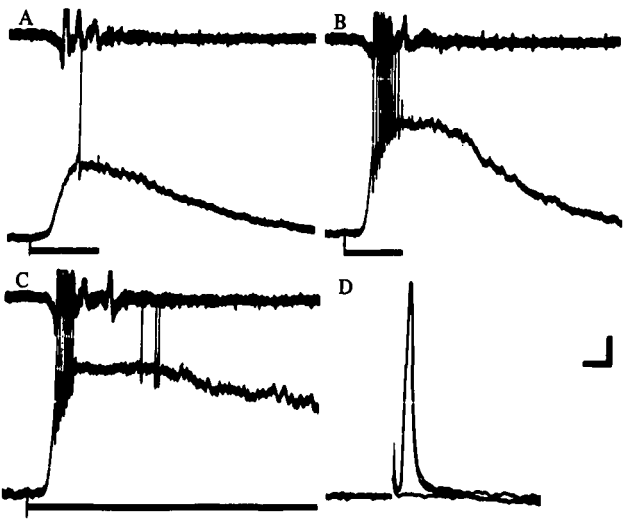

Fig. 5. Photoreceptor light response with graded depolarization and superimposed action potentials (APs). Responded to 1 s light pulses (A) after 5 min in darkness; resting potential 60 mV; and (B) after 10 min in dark. (C) Cell received a 10 s light pulse. (D) Optic nerve stimulation at 20 and 30 V, 0·5 ms evoked an antidromic AP but not at 10 V. No AP activity occurred during spontaneous dark CAP activity. Scales: 20 μV, 10 mV, 500 ms in (A), (B) and (C); 10 mV, 20 ms in (D).

periods. In Fig. 5A the response was obtained after 5 min in darkness soon after the cell was impaled. The cell had a resting potential of 60 mV and responded to light with a prolonged depolarization and one overshooting AP. After 10 min of dark adaptation the cell responded (Fig. 5B) with a larger graded potential and a volley of APs, which were asynchronous with the optic nerve CAPs. Prominent bumps appeared on the graded depolarization. After 10 min more of dark adaptation the cell responded in much the same way (Fig. 5C) but remained depolarized as long as the illumination continued. Note that the CAP activity was phasic even though the depolarization of this cell was prolonged during the long light pulse of Fig. 5C. This receptor lacked the pronounced late hyperpolarizing response seen in the graded photoresponse in non-spiking receptors (see Fig. 3). Electrical stimulation of the optic nerve evoked a large AP (Fig. 5D). Increasing the stimulus voltage above threshold did not enhance the large antidromic AP but it did produce some smaller late potentials similar to the bumps in Fig. 5B, C.

One cell of this type was successfully injected with Lucifer yellow. The response it gave to light before injection and the morphology of the cell are shown in Fig. 6. The response to light was similar to Fig. 5 but it had a slightly more pronounced late component depolarization after the light pulse. The brightest fluorescent spot in the cell was not the soma, which was faintly stained, but the large spot some distance from the soma along the axon of the cell. This was probably the injection site. The cell did have a distal segment and the cell body was in the pigmented layer. The morphology of the cell and its response to light imply it is a primary receptor cell.

A cell with a response similar to that shown in Figs. 5 and 6 was studied in further

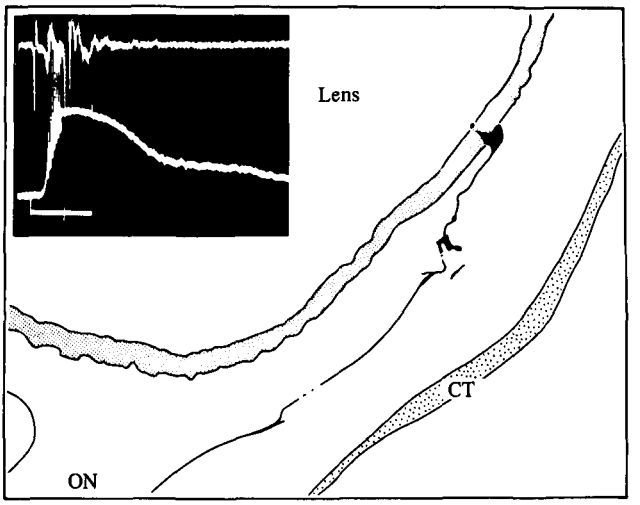

Fig. 6. Lucifer injected photoreceptor, which gave a graded light response and APs (inset). Photoreceptor had distal segment, axon in optic nerve, and was apparently injected in the axon (stain blob on axon) not in the soma. Highest stain intensity was at axon site. Depolarization in inset is 30 mV and light pulse 1 s. Top trace is CAP activity and first CAP is not light evoked.

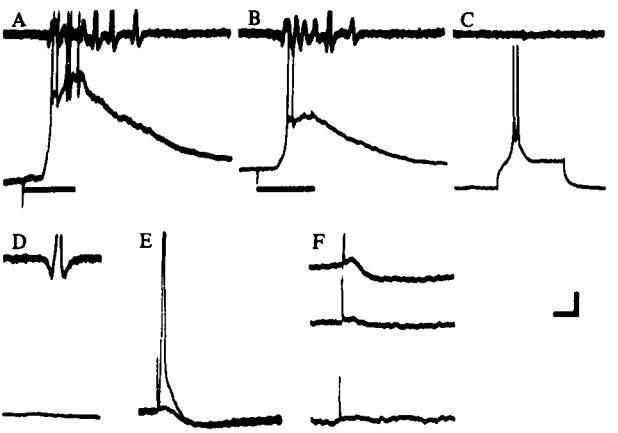

Fig. 7. Graded photoreceptor responses with APs. Responses in (A) after impalement and (B) after 5 min in the dark, resting potential 60 mV. (C) Depolarizing the cell with current evoked 2 APs but no CAPs in the optic nerve. (D) Spontaneous dark CAP did not have a correlated AP in the cell. Stimulation of optic nerve at 30 V, 0·5 ms evoked a biphasic potential; 40 V evoked an antidromic AP too, in (E). (F) Hyperpolarizing the cell with 0·1 and 0·2 nA, while stimulating the optic nerve at 30 V, eventually blocked the evoked biphasic response. Scales: 20 μV, 10 mV, 500 ms in (A); 20 μV, 20 mV, 500 ms in (B); 20 μV, 20 mV, 200 ms in (C); 20 μV, 20 mV, 200 ms in (D); 10 mV, 50 ms in (E) and (F).

detail (Fig. 7). It had a 60 mV resting potential and in response to light gave very large overshooting APs superimposed on a large graded depolarization. It was completely inactive during spontaneous dark CAP activity. Depolarization of the receptor by current injection through the microelectrode depolarized the cell and evoked a characteristic doublet of APs (Fig. 7C). No CAP was evoked in the optic nerve during

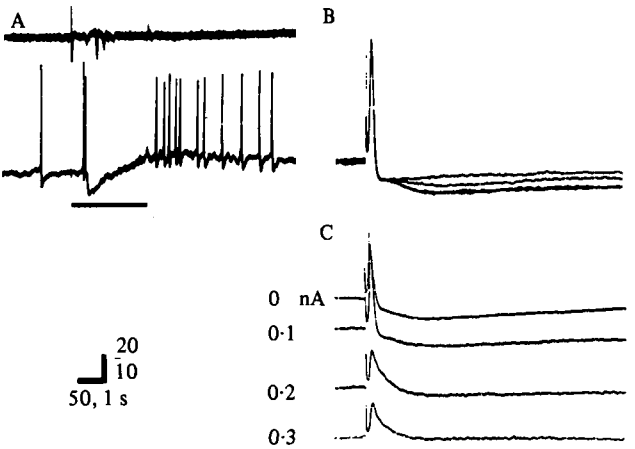

Fig. 8. Receptor with a triphasic response to light: depolarization, hyperpolarization and depolarization. The cell was spontaneously active (overshooting AP: RP = −35 mV) in dark, and responded to light. (A) Stimulation of the optic nerve at 25 V, 0·5 ms produced an AP (B) and increasing stimulus voltage, 30 V, 35 V caused increased hyperpolarization following the AP, but 50 V had same effect as 35 V. With progressive hyperpolarization of cell by injected current (C), the response was diminished. Scales: 20 μV, 10 mV, 1 s in (A); 10 mV, 50 ms in (B) and (C). Blip in (A) at light-on is artifact.

the selectively evoked AP and when spontaneous CAP did occur the cell showed no sign of activity (Fig. 7D). Electrical stimulation of the optic nerve produced a compound input. Lower voltages evoked a biphasic response (depolarization-hyperpolarization) and higher voltages produced a large antidromic AP plus the biphasic potential. Selective hyperpolarization of the receptor caused both the depolarizing phase and the hyperpolarizing phase of the biphasic potential to disappear (Fig. 7F). This suggests that the biphasic potential was electrotonic and hyperpolarizing the receptor caused the invading potential to be blocked before it reached the recording site.

Photoreceptors with graded depolarization/hyperpolarization/depolarization and APs

Another type of photoreceptor, previously called H (Jacklet, 1976), was frequently encountered. Its usual response to light was triphasic, comprising an initial brief depolarization and AP, followed by a hyperpolarization and then a later depolarization and APs as shown in Fig. 8.

After the light was turned off the cell fired APs at a higher rate (Fig. 8A), similar to an 'off' response in other photoreceptors. However, this was not a true 'off' response because in other cases if the light was prolonged the firing occurred at about the same time after light onset even though the light was still on (not shown). This suggests that the receptor undergoes depolarizing and hyperpolarizing changes evoked by light and the balance of these influences changes with time after light onset. Optic nerve stimulation evoked an antidromic AP followed by a late and separate hyperpolarization, which could be increased in depth and duration by increasing the stimulus voltage, suggesting a compound inhibitory postsynaptic potential input. Pro-

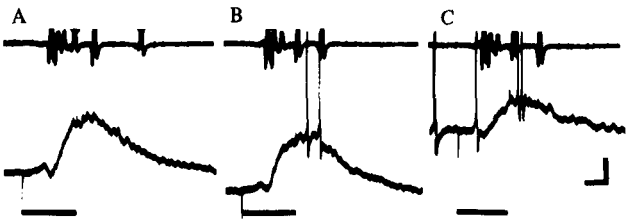

Fig. 9. Receptor with triphasic response to light. Pulses of light (1 s) were given 1/min in a series. Just after impalement, A was obtained and 4 min later B. When the cell was depolarized by current injection (C), APs were evoked in dark and during depolarizing phases of light response, but the hyperpolarizing phase was not enhanced. Scales: 50 μV, 10 mV, 500 ms.

gressive hyperpolarization of the cell with injected current was successful in diminishing the antidromic AP and abolishing (but not reversing) the hyperpolarizing component (Fig. 8C).

A receptor with a more pronounced prolonged depolarization following the initial depolarization-hyperpolarization is shown in Fig. 9. In a series of eight light pulses given at 1 min intervals this receptor showed a reproducible slow wave response with or without superimposed action potentials (Fig. 9A, B). Steadily depolarizing the cell with intracellular current injection (Fig. 9C) was sufficient to cause an AP in darkness and to allow the light evoked early depolarization to produce an AP. However, the light-evoked hyperpolarization was not enhanced as expected if it had a reversal potential more negative than the resting potential (Fig. 9A, B).

Type H receptors were injected with Lucifer yellow. All those injected with sufficient dye ($n = 7$) had axons in the optic nerve and several points on the axon where branching occurred (Fig. 10). Dye coupling between receptors was observed on two occasions (e.g. Fig. 10A). The larger triangular-shaped receptor shown in Fig. 10A was probably injected because it was intensely fluorescent compared to the smaller cell on its left. Only one axon extended to the optic nerve and a complex web of fibre processes was observed immediately below the filled receptors. The injected receptor clearly had a distal process extending towards the lens but the faintly injected cell did not. The injected receptor gave the response to light shown in the inset. A receptor which gave a similar response is shown in Fig. 10B. This cell was intensely fluorescent in the soma and along the total length of the axon, which branched at two distinct levels in the retinal neuropile.

Some cells were only slightly depolarized/hyperpolarized by light but produced large overshooting AP (e.g. Fig. 11). The APs that were evoked by the depolarization were not correlated with the CAPs in the optic nerve but they were correlated with smaller unitary potentials in the optic nerve for both light evoked (Fig. 11A) and spontaneous (Fig. 11C) APs. This receptor readily produced overshooting AP when the cell was initially impaled (Fig. 11C) and when the cell was depolarized with intracellular current injection (Fig. 11B). The light-evoked depolarization was diminished when the receptor was depolarized with applied current and it was enhanced, but the AP was blocked, revealing the underlying depolarization when the receptor was hyperpolarized (Fig. 11B). The light-evoked hyperpolarization was diminished

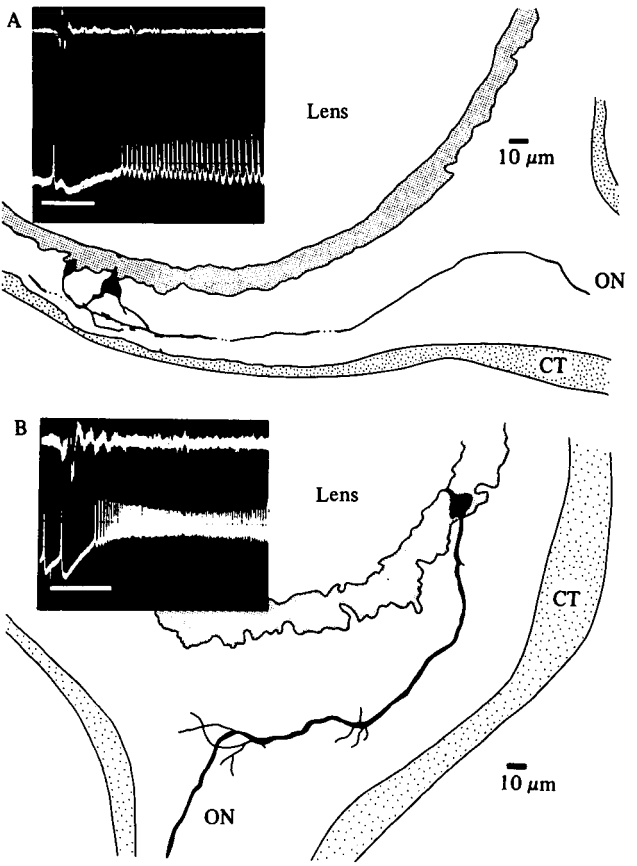

Fig. 10. Lucifer injected receptors that gave triphasic light responses. Two cells were stained (A) but only one (on right) was injected. It gave the light response in the inset. In (B), the receptor response is shown in the inset. The injected receptor was intensely fluorescent and had a large axon with several branch points before it reached the optic nerve (ON). Scales for insets: 10 mV, 500 ms. CAPs were not correlated with cell APs.

by hyperpolarizing the cells (Fig. 11B) and possibly reversed. Antidromic activation of the cell by electrically stimulating the optic nerve also evoked APs (Fig. 11D), but there was no evidence of a compound input in this particular cell. This cell type appears to be different from the H type photoreceptors shown in Figs. 8–10 and more nearly like the neurone type described below.

A neurone having a weak monophasic response to light of the type shown in Fig. 12 was rarely encountered. This cell was very active when impaled (probably due to injury) but became quiet with a stable resting potential of −70 mV and overshooting APs of up to 100 mV in amplitude (Fig. 12B). When it was actively firing in darkness it received frequent input that resembled IPSPs. The response to light was a brief depolarization with APs (Fig. 12A). Later on, when the cell was completely silent, it responded to a light pulse with depolarization and an AP only. APs in this cell were not correlated with CAPs, regardless of whether APs were evoked by light or by applied intracellular current (Fig. 12B). Stimulation of the optic nerve produced an

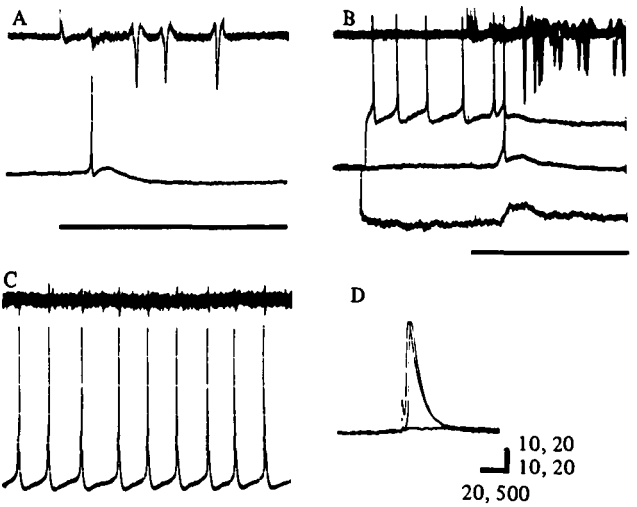

Fig. 11. Receptor with a biphasic response to light: depolarization followed by hyperpolarization. In (A), light (bar) evoked a single overshooting AP, RP = 60 mV, followed by prolonged hyperpolarization. Light pulses with the cell polarized at 3 levels (0, + and −0·1 nA) revealed (B) an underlying depolarization responsible for the AP and apparent reversal of the hyperpolarizing response. Spontaneous dark APs (C) and also light-evoked APs (A and B) were correlated 1:1 with optic nerve unitary potentials (not CAP). Stimulation of the optic nerve (D) evoked a single AP at 10 V, 50 V, 0·5 ms but not at 5 V. Scales: 20 μV, 20 mV, 500 ms in (A) and (B); 10 μV, 10 mV, 500 ms in (C); 20 mV, 20 ms in (D). Blips on records at light-on are artifacts.

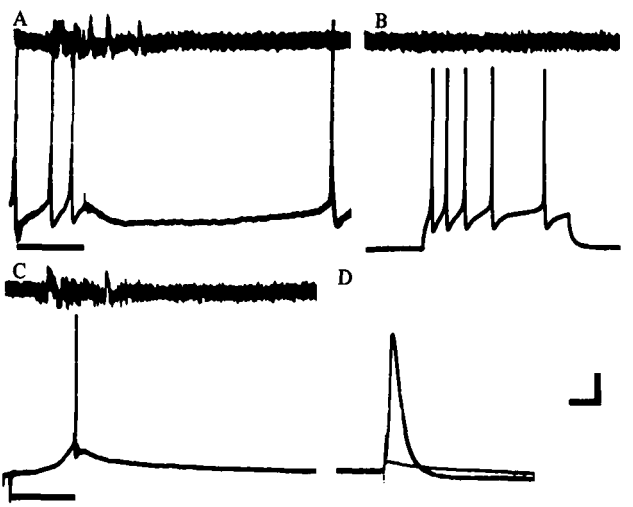

Fig. 12. Cell with a weak depolarizing response to light and large AP. The cell had a −60 mV resting potential and produced large overshooting APs when it was impaled. It was depolarized by light and APs were evoked (A). Applied currents (B) depolarized the cell and evoked APs but no correlated CAPs (upper traces). Later, when resting potential was −70 mV a light pulse depolarized the cell and evoked an AP (C). Stimulation of optic nerve (D) at 10 V, 0·5 ms evoked a small depolarization and stimulation at 15 V and 20 V evoked identical APs. Scales: 10 μV, 10 mV, 500 ms in (A); 10 μV, 20 mV, 500 ms in (C); 20 mV, 20 ms in (D).

all-or-none antidromic AP only. The cell responded to light with only a slight depolarization compared to most of the spiking receptors. This type of cell was never successfully filled with Lucifer Yellow.

DISCUSSION

R receptors were not observed to be dye-coupled to other cells, despite the presence of gap junctions between them and other R cells and secondary neurones (Strumwasser *et al.* 1979) and despite the observation of small biphasic potentials correlated with spontaneous CAP activity (Fig. 1). The junctions responsible for these potentials are probably at some distance from the R cell soma because the potentials are small and not observed in all R receptors. In fact, some cells classified as secondary neurones, on the basis of APs correlated with each optic nerve CAP, in the preceding paper (Jacklet *et al.* 1982, Fig. 9), have characteristics of both secondary neurones and R receptors. Those recordings may have been from receptor processes near the electrical junctions and so had electrical characteristics contributed by each cell type.

The depolarizing light response of R receptors had two components, a fast early depolarization followed by a slower depolarization. These two could be distinguished in an adaptation test such as Fig. 3. Both depolarizing components adapted and the break between the two became obvious. A two component depolarizing response has been seen in other gastropod receptors (*Strombus* eye) by Quandt & Gillary (1979) and conspicuous two component depolarization, sensitive to light adaptation, is also seen in *Limulus* ventral photoreceptors (Maaz *et al.* 1981). In *Aplysia* R receptors this depolarization overshoots the o potential level and becomes positive by a few mV in dark adapted receptors. After the depolarizing components a pronounced hyperpolarization of the receptor occurs, which is most conspicuous in dark adapted receptors (Fig. 3). If the receptor is light adapted the hyperpolarizing component is attenuated and the response is essentially an early two component depolarization (Fig. 3A). The transient depolarization followed by hyperpolarization in response to a bright flash after long dark adaptation is reflected in the CAP firing too. Optic nerve recordings show a burst of CAP activity at the beginning of a long bright pulse and then silence for many seconds while the light is still on before resumption of CAP firing later (Jacklet, 1969).

The spectral sensitivity of the R type cells measured in this study matches the spectral sensitivity of the CAP firing (Jacklet, 1980). Both have a peak sensitivity near 500 nm. The spectral sensitivity of the *Strombus* eye ERG peaks at 485 nm (Gillary, 1974) and is very similar to the *Aplysia* eye spectrum.

Depolarizing receptor responses similar to type R responses but with superimposed APs (Figs. 5 and 6) can be recorded from the axons of R cells (Fig. 6). These responses are monophasic and depolarizing with little hint of a late hyperpolarizing component even in the very dark adapted condition (Fig. 5). If these responses are indeed recording from axons of type R receptors, as suggested by the Lucifer yellow filled receptor of Fig. 6, they correlate very well with locational differences in responses of similar receptors in *Strombus* reported by Quandt & Gillary (1979). They associated distinct depolarizing components of the response and rapid decay of the response with a presumed distal segment-soma recording site and associated monophasic long-lasting depolarizations, like Fig. 5, with an initial-axon-segment or neurite recording site.

Receptor light responses having large overshooting APs and a distinctive early hyperpolarizing potential component were formerly assigned to one type (Jacklet, 1969) called H (Jacklet, 1976). It is apparent from this study that the H type is heterogeneous and includes a spectrum of responses. The type does not include the weakly depolarized spiking neurone shown in Fig. 12, which had no distinct hyperpolarization and had APs that were not correlated with the CAPs. It may be one of the non-receptor neurones seen in the retina by Strumwasser *et al.* (1979) and noted in cobalt backfills in the preceding paper (Jacklet *et al.* 1982) but it is clearly not a secondary 'D' neurone.

The size of the hyperpolarizing component, as well as the late depolarizing component of the H type is quite variable from cell to cell but the response in general is stereotyped. It consists of an early (often brief) depolarization, causing an AP or two, then a hyperpolarization and finally a late long-lasting depolarization accompanied by APs (Figs. 9, 10). This late burst of AP resembles an 'off' response but it may still occur even if the light is still on and so it is not strictly an 'off' response. The type H response is similar to the triphasic graded potential recorded from axotomized *Hermissenda* receptors by Detwiler (1976) or intact type B photoreceptors of *Hermissenda* by Alkon & Grossman (1978). The responses given by those cells are very sensitive to light intensity and light adaptation (Detwiler, 1976). Weak light caused a monophasic depolarization and stronger light systematically changed the response to a large triphasic one. Light adaptation affected the depolarizing component more strongly than the hyperpolarizing component with the result that a triphasic response could be converted to a monophasic hyperpolarization by light adaptation. In the present study on *Aplysia* receptors, the impalements were rarely held long enough to do light adaptation, light intensity testing. It seems likely that many of the variations of the generalized triphasic response can be explained by differences in light intensity reaching the receptor and its state of light adaptation. In *Hermissenda* inhibitory chemical synaptic potentials contribute to the hyperpolarizing component of the light response as well as the light-evoked ionic conductances (Alkon & Grossman, 1978). Inhibitory chemical synapses may contribute to the *Aplysia* H receptors response too but the evidence is incomplete. Receptor responses similar to the H type are reported from the eye of *Strombus* by Quandt & Gillary (1979).

H receptors injected with Lucifer yellow were always found in the retinal receptor layer. Generally, the cell body was smaller, the distal segment less pronounced and the axon larger compared to the R type receptor. They may correspond to the upper retinal neurones described by Strumwasser *et al.* (1979) and the smaller receptors seen in the *Aplysia* retina with cobalt backfills in the preceding paper (Jacklet *et al.* 1982). Receptors in a similar retinal position with a distal segment capped with short microvilli and occasional cilia have been seen in the *Aplysia* retina (Jacklet & Colquhoun, unpublished), described in the *Aplysia punctata* retina by Hughes (1970), observed in the retina of *Strombus* (Gillary & Gillary, 1979) and discovered in the *Helix* retina (Brandenberger, 1975). The photoreceptor type in the scallop, *Pecten*, known to give a pure hyperpolarizing light response is a ciliated receptor. Microvillous receptors in *Pecten* give depolarizing responses (McReynolds & Gorman, 1970). It has been suggested by Land (1968) that in molluscs a functional association exists between

ciliary photoreceptors and primary inhibition. However, the H response of the *Aplysia* eye is not a pure inhibition and it has not been definitely associated with ciliary photoreceptors. It is interesting that photoreceptors of *Hermissenda*, which respond similarly to *Aplysia* H receptors are definitely microvillous (Eakin, Westfall & Dennis, 1967). Molluscan photoreceptors are generally microvillous and Eakin believes there are two major evolutionary lines of photoreceptors, microvillous and ciliary, but in both lines photoreceptors of the opposite type are found (Eakin, 1979).

The APs of H receptors and similar cells were observed in favourable optic nerve recordings (Fig. 11). This confirmed the presence of H axons in the optic nerve already shown by Lucifer yellow filled H receptors. The unitary APs of the H receptors, that occurred spontaneously or were light evoked, were never correlated with the CAPs produced by the secondary neurones. In an earlier study (Benson & Jacklet, 1977) 'small' potentials were observed in 10% of the extracellular recordings from the optic nerve. The cellular origin of those potentials is not clarified by this study but they could be from H cells or similar cells. The H receptors appear to be a separate light-signalling system from the synchronous CAP system. They and other neuronal types (Fig. 12) are apparently organized to convey light patterned information to the cerebral ganglion where it may possibly be used to shape the immediate visually-guided behaviour of the animal. The H system contrasts with the CAP system and its tonic activity, which is driven by the circadian pacemaker and modulated by light intensity. The CAP system consists of the secondary neurones, which directly contribute to the CAP, and the R receptors, which are electrically coupled to the secondary neurones and most likely mediate the light driven CAP activity.

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