Electrophysiological Organization of the Eye of *Aplysia*

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ABSTRACT The eye of *Aplysia californica* was studied by electrophysiological and histological methods. It has a central spheroidal lens which is surrounded by a retina composed of several thousand receptor cells which are replete with clear vesicles, pigmented support cells, neurons which contain secretory granules, and glial cells. The thin optic nerve that connects the eye to the cerebral ganglion gives a simple "on" response of synchronized action potentials. Tonic activity occurs in the optic nerve in the dark and is dependent on previous dark adaptation. Micropipette recordings indicate that the ERG is positive (relative to a bathelectrode) on the outer surface of the eye and negative in the region of the distal segments of the receptors. Intracellular recordings show that receptor cells have resting potentials of 40–50 mv and respond to illumination with graded potentials of up to 55 mv. Dark-adapted receptors exhibit discrete bumps on the graded response to brief light flashes. Other elements in the retina that do not give large graded responses fall into two classes. One class responds to illumination with action potentials that are in synchrony with the extracellularly recorded compound optic nerve potentials. The other class is tonically active and is depolarized or hyperpolarized and inhibited upon illumination. It is apparent that complex excitatory and lateral inhibitory interactions occur among the elements of the retina.

INTRODUCTION

The central nervous system of *Aplysia* has been extensively studied by neurophysiologists (1–4) principally because its relative simplicity of organization and the large identifiable neurons offer some hope of understanding the detailed physiology of specific neuron interactions. Few electrophysiological details are available on the contribution of sensory structures to the electrical activity of the central neurons of *Aplysia* and no studies have been made on the relevance of the eye in modifying or initiating central nervous system activity. Photoperiod has been demonstrated to influence both the locomotory activity of the animal (5, 6) and the activity of single cells in the central nervous system (7). Yet, the manner in which this influence is exerted remains

obscure. Therefore this study of the electrical activity of the eye and the information passed down the optic nerve to the central neurons was initiated to gain a better understanding of the photoperiodic effects.

The receptor cells of the retina of *Aplysia* are unusual in that they contain large numbers of vesicles (8).[1] The cytoplasm of these cells, including the distal segments and neurites, is replete with 500 A clear vesicles which are often so densely packed that they assume a paracrystalline array when seen in electron micrographs. Vesicles of the same type have also been demonstrated in the receptor cells of *Helix* by Eakin and Brandenburger (9) and they (10) have discussed the physiological significance of these vesicles.

The *Aplysia* eye is interesting for visual physiology studies because (as this paper will show) intracellular recordings can be made from the light-receptive elements and inhibitory synaptic interactions occur at the receptor neurite level. Its thousands of receptors exceed the simplicity of the five visual cells of another gastropod (*Hermissenda*) eye (11, 12) but the long optic nerve allows routine simultaneous recording of the optic nerve potentials and intracellular events at the retina.

MATERIALS AND METHODS

Eyes of the marine tectibranch gastropod, *Aplysia californica*, were dissected from more than 30 animals on a light schedule of either a light/dark cycle of 12:12, or constant light. The animals were kept in seawater tanks at 14°C and most of the experiments were done at this temperature. For intracellular recording the isolated eye and optic nerve were pretreated with two enzymes, collagenase and elastase (2 mg of each for 1 ml seawater) to soften the capsule connective tissue (13) and thus ease the penetration of the fine pipettes. This treatment alters neither the electrical activity of the eye nor that of the central neurons of *Aplysia* (13). The isolated eye and optic nerve were placed in a 100 ml seawater chamber that was temperature-regulated by a Haake-Brinkman cooler. Glass pipettes filled with 0.6 M K_2SO_4 with tip resistances of 5–35 MΩ were used in conjunction with an ELSA-1 electrometer for recording the interreceptor cell potentials and surface ERG potentials. Glass pipettes with tip resistances of 40–80 MΩ were used to record the intracellular responses of receptor cells and other neuronal elements. The cells were penetrated by advancing the electrode into the retina of the intact eye from the outer surface toward the lens. The optic nerve potentials were recorded by drawing up the cut end of the optic nerve into a polyethylene suction electrode and amplified by a Tektronix Type 122. Records were obtained continuously on a Grass Model 7 polygraph and by photographing the oscilloscope trace. White light of about 600 lux from a tungsten filament bulb was used to illuminate the whole eye. Intensity was varied by Wratten neutral density filters. All experiments were performed in a darkened room with about 5 lux of background light. Successive 5 sec test pulses of light were given at 10 min intervals in most experiments since this interval allowed replication of the ERG amplitude as a control.

[1] Jacklet, J. W., R. W. Alvarez, and B. Bernstein. Data in preparation.

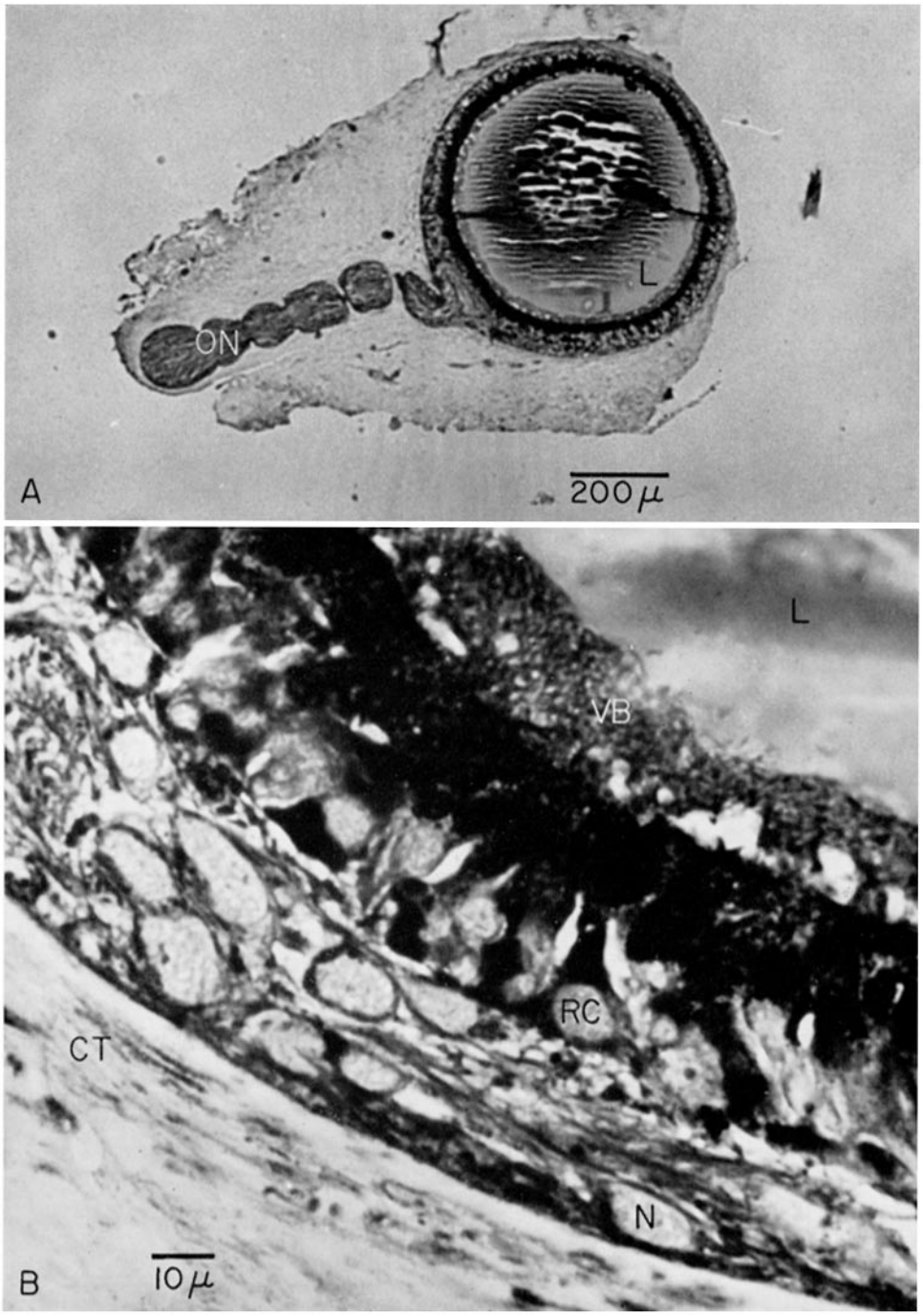
RESULTS

I. *Anatomy*

The paired cephalic eyes of *Aplysia* are located just anterior to and at the base of the rhinophores (14). The eyes are small (600 μ in diameter) and inconspicuous and can be withdrawn into the folds of the skin whenever the animal is molested.

The eye is a closed vesicle type (Fig. 1). It is composed of a central spheroidal lens surrounded by a retina made up of thousands of small (15 μ in diameter) receptor cells, pigmented support cells, glial cells, and neuronal elements (Fig. 1 B). The retinal area is less than 1 mm^2 and contains approximately 7000 receptors (15). The retina is thickest at the base where the optic nerve arises and becomes progressively thinner toward the "corneal" area where the receptors are less densely packed. The receptors are distributed in a layer around the lens; beneath them numerous neuronal cell bodies (Fig. 1 B) are interdispersed among the fibers converging toward the optic nerve. The thin optic nerve (>1 cm long) arises at the back of the eye and connects to the cerebral ganglion. Electron micrographs[1] show that the optic nerve is composed of several thousand small fibers <3 μ in diameter.

Some of the details of the receptor cells are shown in the diagram and electron micrographs of Fig. 2. Each receptor cell has a distal segment that projects to the vitreous body area. The term vitreous body is used to define that homogeneous matter between the lens and the retina. This body is presumed to be analogous to that found in the eye of *Littorina*, a prosobranch, by Newell (16). The distal segments of *Aplysia* usually consist of two or more fingerlike processes interdigitated with the pigmented support cells. The tips of the distal segment support a microvillous cone rhabdomere which penetrates the vitreous body adjacent to the lens. A neurite projects from the nuclear region of the receptor to the fiber layer below the receptors. Receptor cell cytoplasm, including the distal segments and neurites, contains numerous 500 A clear vesicles (Fig. 2 B), mitochondria, and other organelles. The 500 A profiles shown in Fig. 2 B and C are described as vesicles principally because they appear round regardless of the orientation of the electron micrographic sections. (Microvilli, on the other hand, can be readily distinguished by sections in the plane of their longitudinal dimension.) In addition, the 500 A profiles have been described as vesicles in related gastropod receptor cells by other authors (9). The vesicles frequently take on a paracrystalline array orientation especially in the cell body cytoplasm (see Fig. 2 B). The neuron processes at the base of the receptor layer contain electron-dense vesicles 1000–1500 A in diameter (Fig. 2 C) and are often in intimate association with the receptor cell processes.


L
ON
A
200 μ
L
VB
RC
CT
N
B
10 μ

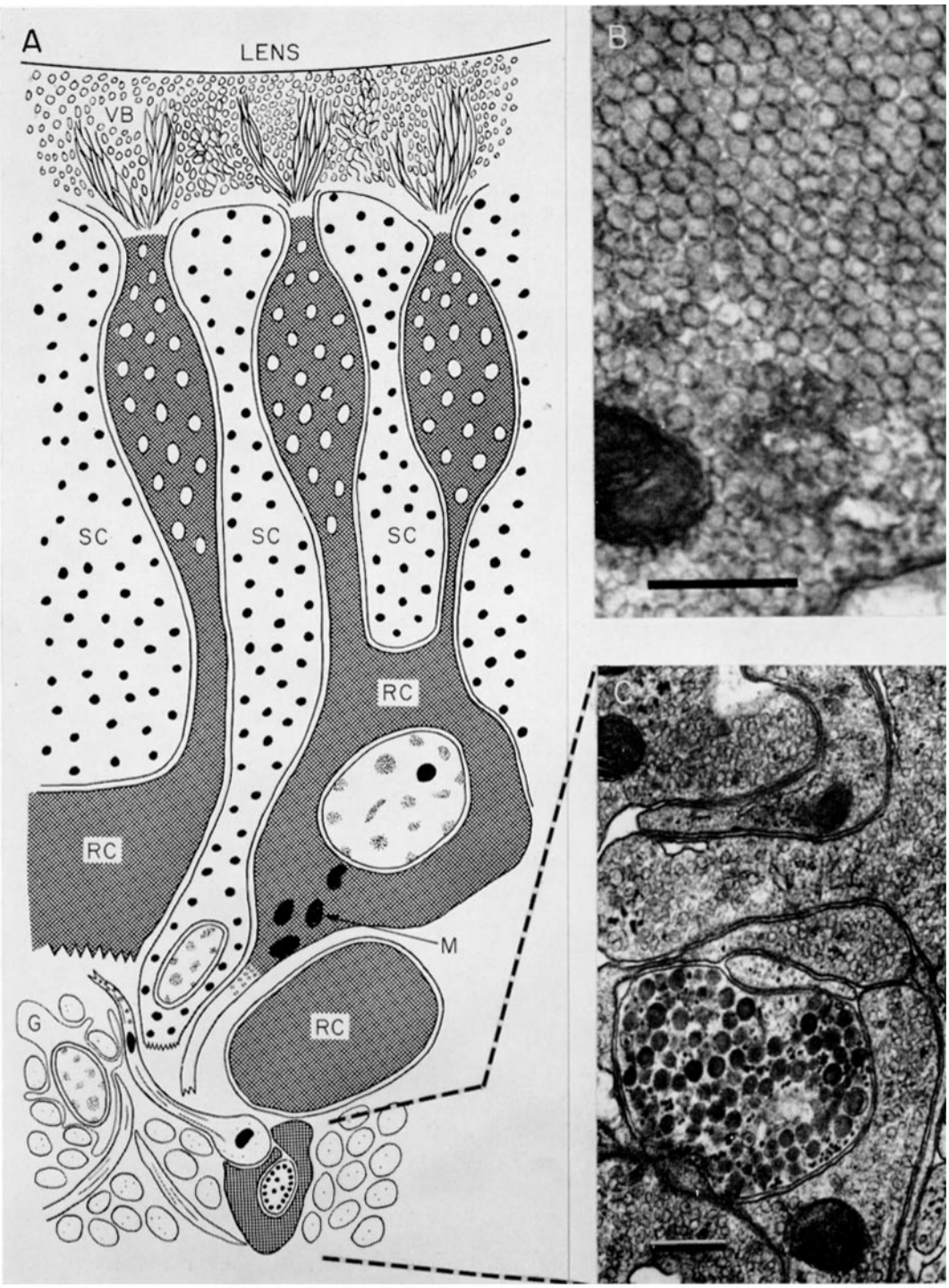
II. *Penetration of the Retina*

Penetration of the retina of the intact eye with a micropipette, recording extracellularly, indicates that the wave form of the light-evoked potential (ERG) changes with increasing depth. Fig. 3 shows the potentials recorded at three levels. On the outer connective tissue surface of the eye (Fig. 3 A) the ERG is positive (usually 1–2 mv) with respect to an indifferent electrode in the chamber and outlasts the stimulus duration. Advancing the electrode tip to the midretina in the approximate region of the receptor cell bodies reveals a reversal of the ERG polarity (Fig. 3 B). Further penetration to the approximate area of the distal receptor segments and the vitreous body shows a transient negative potential that may be as large as 12 mv followed by a sustained small potential (Fig. 3 C). Thus, recording at different levels of the retina from the outer surface towards the lens reveals an initial positivity, a reversal of potential, and a maximum negativity. The retina was penetrated at several positions along the surface of the intact eye from the proximal basal area where the optic nerve arises to the "cornea." The magnitude of the ERG is largest at the basal portion of the eye where the retina is thickest and the receptor density is highest; and smallest at the thin distal retina near the cornea where the receptors are less densely packed. Apparently, the ERG is composed principally of the summated potentials of the receptors.

III. *Optic Nerve Potentials*

The isolated eye shows regular tonic activity in the optic nerve at about 0.1/sec. The potentials may occur at regular intervals or in bursts of four or five. Tonic activity can be recorded in complete darkness inside a sealed black box for longer than 24 hr after isolation of the eye and optic nerve. Eyes from animals on a light/dark cycle of 12:12 hr invariably show tonic activity after minimal dark adaptation (½ hr) whereas eyes from animals on a constant light schedule require longer periods of dark adaptation before tonic optic nerve activity appears. Fig. 4 shows tonic activity preceding a test illumination and its return after the illumination period. Prolonged exposure to high intensity light (Fig. 4, line 0) causes a longer silent period following the illumination period than exposure to low intensity light (Fig. 4, line −3). Longer durations of exposure to constant intensity light also increase the silent period.

FIGURE 1. Structure of the eye. The whole eye and distal optic nerve (*ON*) are shown in longitudinal section in A. The large central lens (*L*) is disrupted by chatter in cutting. B is an enlargement of a segment of the retina. The receptor cells (*RC*) have distal segments that are screened by pigment cells and project to the vitreous body (*VB*) layer adjacent to the lens (*L*). Beneath the receptor cell layer numerous fibers and neuronal (*N*) cell bodies are visible near the outer layer of connective tissue (*CT*). Tissue was fixed in Alcoholic Bouin and silver-stained according to Fraser-Rowell (42).


A
LENS
VB
SC
SC
SC
RC
RC
M
RC
G
B
C

Thus, the silent period appears to be a function of the intensity and duration of the test illumination.

The light-evoked response in the optic nerve is also shown in Fig. 4. It is a typical "on" response of action potentials. The frequency of firing is dependent upon the light intensity. High intensities (600 lux in line 0) evoke an initial burst of activity followed by a pause and then resumption of activity at a lower frequency. The burst of activity becomes less pronounced with less intense light until very weak illumination (Fig. 4, line −3) barely modulates the tonic activity.

The optic nerve potentials appear to be compound action potentials as determined from observations on naturally occurring potentials, electrical

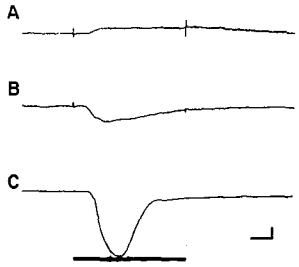


FIGURE 3. Penetration of the retina. Changes in the ERG with successive depth of penetration of the eye from the outside toward the lens. A, surface of eye near optic nerve. B, penetration of about 50 μ to area of receptor. C, penetration to area of distal segments of receptor; this is the maximum negative response. A, B, and C from same preparation. Bar beneath C is the duration of illumination for all traces. Blips on traces at on and off are contact artifacts. Calibration, 1 mv, 1 sec.

stimulation of the optic nerve, and hypotonic seawater treatment. These observations are illustrated in Fig. 5. The normal response to illumination is shown in Fig. 5 A in which the optic nerve potentials and summed receptor potentials (ERG) are recorded simultaneously. The top line shows five triphasic action potentials of various amplitudes from the optic nerve. The long dura-

FIGURE 2. Diagram and micrograph of receptor cells and processes. The diagram was constructed by referring to montages of electron micrographs of tissues fixed in glutaraldehyde, postfixed in OSO_4, and stained with lead citrate. A receptor cell (*RC*) usually has more than one distal process interdigitated with pigmented support cells (*SC*). The distal segments terminate in a microvillous rhabdomere in the vitreous body (*VB*) next to the lens. The receptor cells are packed with numerous clear vesicles (indicated by the cross-hatched areas in the receptors of Fig. 2 A) 500 A in diameter (Fig. 2 B) and often contain numerous mitochondria (*M*) at the base of the neurite. Glial cells (*G*) are seen mingled with the fiber at the base of the receptor layer. Receptor cell processes are often in intimate association with processes containing large (1000–1500 A diameter) dense granules (Fig. 2 C). The scales in Fig. 2 B and 2 C are 0.3 μ.

tion of these potentials and the amplitude variation suggest that they are either the responses of different units or are composed of several units. Fig. 5 B shows the response of this preparation in hypotonic seawater. The optic nerve potentials are desynchronized but the receptor potentials are unchanged by

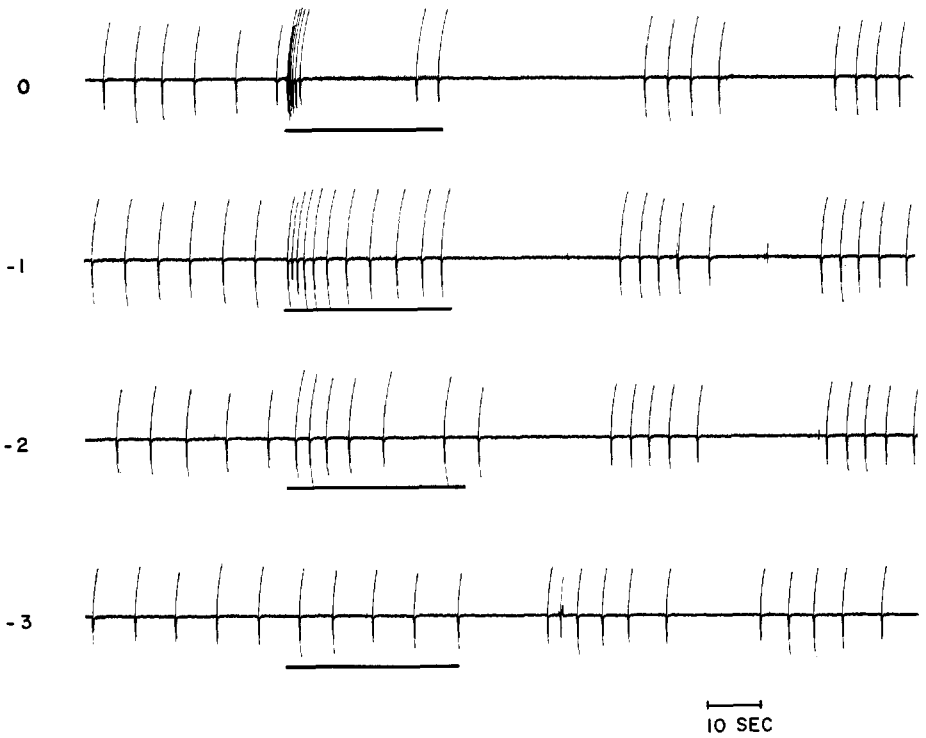


FIGURE 4. Spontaneous and evoked compound optic nerve activity. Optic nerve potentials in response to illumination of the eye at four intensities of white light are shown (the black bars indicate illumination). The 0 intensity is about 600 lux and the remaining intensities successive log units less intense. The spontaneous activity is interrupted by the on response to illumination and resumes after cessation of illumination. The potentials were recorded with a suction electrode on the optic nerve. Spike amplitude is 50 μv, negative upward.

the treatment. The effect is reversible on restoration to normal seawater. The treatment breaks the large action potentials down into many smaller units.

Optic nerve potentials, whether they occur tonically or are light-evoked, often have double or triple negative peaks as illustrated in Fig. 5 C. The duration of these multiple-peaked potentials is considerably longer (twice) than the single-peaked potentials (100 msec) usually seen and may be due to subpopulations of fibers that conduct at slightly different velocities. The conduction velocity of an electrically stimulated optic nerve is 0.1 m/sec. The ampli-

tude of optic nerve potentials evoked by electrical stimulation is graded according to the stimulus voltage (Fig. 5 D). The response is riding on the stimulus artifact, which is large because of the short length of nerve (5 mm) separating the stimulating and recording sites and also the method of stimulating by suction electrode in a volume conductor. The higher voltages effectively stimulate a greater number of fibers, thereby producing a larger summed response than lower voltages, but short duration unitary potentials have not been seen in response to electrical stimulation.

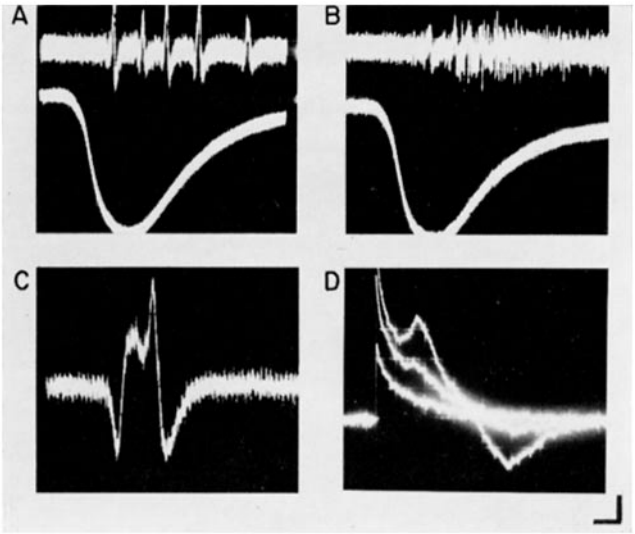


FIGURE 5. Evoked optic nerve potentials. A, normal interreceptor ERG on the bottom trace and simultaneous recording of optic nerve potentials on the top trace. B, effect of 60% seawater on the optic nerve potentials. They are desynchronized, revealing the compound nature of the potentials. C, tonic nerve potential with the double negative peak often seen. D, further evidence for the compound nature of the optic nerve potentials is the response to three successive electrical stimulations of the optic nerve at increasing voltages (7, 9, 11 v). The response is superimposed on the large stimulus artifact and increases with stimulus strength. Negative is upward on all traces except D and the bottom traces of A and B where negative is downward. Calibrations, A and B, 20 μv (upper), 1 mv (lower), 500 msec; C, 10 μv, 10 msec; D, 20 μv, 50 msec.

The desynchronization of the potentials into individual units by hypotonic seawater, the voltage-dependent amplitude, the multiple-peaked potentials, and the variation in amplitude of light-evoked responses are all evidence for the compound nature of the optic nerve potentials. Electron micrographs of the optic nerve[1] reveal several thousand small ($<3\ \mu$) fibers; this is anatomical confirmation of the conclusion drawn from physiological evidence that a compound optic nerve potential is made up of many small single units.

IV. *Intensity Effect*

The relationship between the ERG (amplitude and latency) and the frequency of optic nerve spikes as a function of the logarithm of light intensity is shown in Fig. 6. The number of spikes is directly proportional and the latency of the

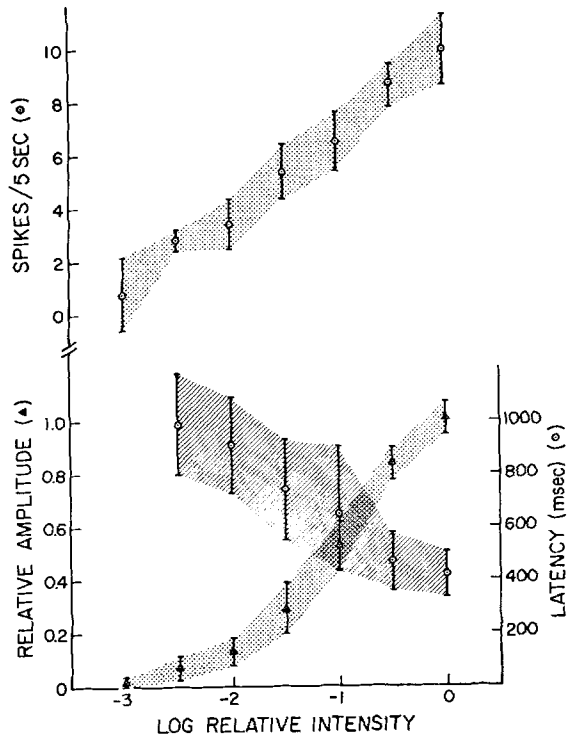


FIGURE 6. Relationship between the logarithm of intensity of white light and the amplitude and latency of the interreceptor ERG and the number of action potentials in the optic nerve. Data are averaged for five experiments. Vertical bars are one standard deviation above and below the mean. Illumination was for 5 sec with 10 min between trials. The latency was measured as the time from light on to the first deflection of the oscillograph. This measurement was necessarily somewhat arbitrary at low intensities.

ERG inversely proportional to the logarithm of intensity. The amplitude of the ERG is directly proportional over much of the intensity range but departs at low and high intensities from the straight line. This relationship is similar to that found in another gastropod (17) and many visual responses (18). The frequency of optic nerve potentials in *Aplysia* is also proportional to the log of intensity for monochromatic wavelengths between 400 and 660 mμ (19). Low intensities of light appear to have only a transient effect on the tonic optic nerve activity (Fig. 3). Higher intensities progressively lengthen the pause

after the initial burst of spikes upon illumination and the silent period after cessation of illumination. Thus, clearly there is information about intensity and duration of illumination available in optic nerve coding.

V. *Temperature Effect*

The ERG is temperature-sensitive as shown in Fig. 7. The amplitude decreases at temperatures above and below the optimum of 15–16°C. This is the normal water temperature at which the animal lives. The latency is shortest at 22°C,

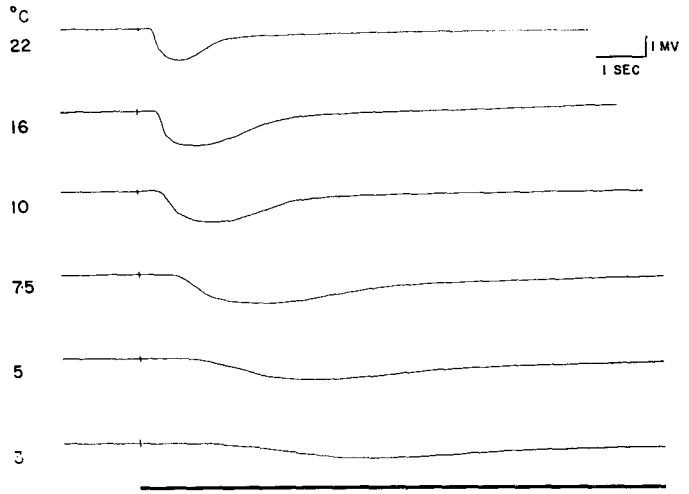


FIGURE 7. Temperature effect on the shape of the interreceptor ERG. The black bar shows the illumination duration. Normal seawater temperature for the animal is close to 15°C. Latency is inversely proportional to temperature but maximum amplitude is obtained about 16°C. All responses were recorded at the same electrode position in a single preparation and are typical of responses from other preparations.

about 200 msec, and progressively increases with decreasing temperature. It is measured from the stimulus artifact to the first significant departure of the response from the base line and is thus somewhat arbitrary at low temperatures and at low intensities (see above). The peak of the ERG occurs progressively later with decreasing temperature but is still evident at 3°C. The Q_{10} of the latency is 2–2.5 over the range studied and is indicative of a chemical process in the events leading to receptor potential initiation.

VI. *Intracellular Receptor Potentials*

Several kinds of intracellular responses to illumination are recorded from the retina. The type described in this section has several distinguishing characteristics and is referred to as a receptor potential. These cells have resting potentials of 40–50 mv and show graded depolarization of as much as 55 mv in response to illumination. Large-amplitude potentials frequently have a step

or notch in the rising phase (Fig. 8 A, B) and usually overshoot the resting potential level by a few millivolts. Typically neither the amplitude nor the notch could be duplicated on the second trial illumination (see Fig. 8 A). The notch and overshoot may depend on a large resting potential of the cell or a particular dark adaptation condition that is yet not well-defined. Spikes are not typically superimposed on the graded potential. However, infrequently a graded potential does give rise to a single spike which is not correlated with the

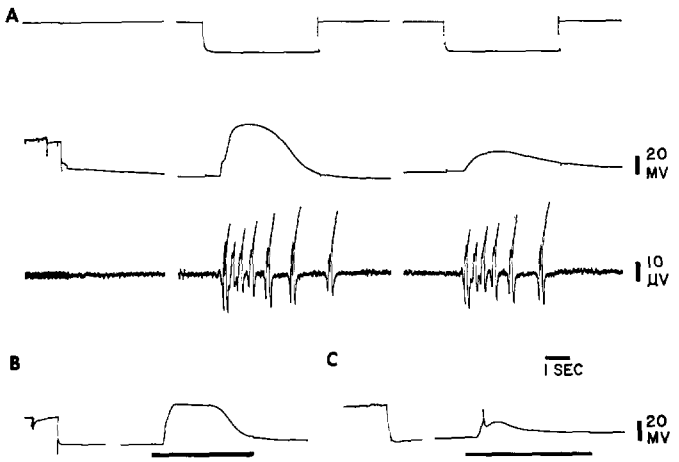


FIGURE 8. Intracellular receptor potentials. A, two successive test illuminations of a receptor cell at 10 min intervals. The top line is a monitor of light; downward deflection indicates illumination of the eye. The second line is a DC record of penetration, 2 min gap, test illumination, 10 min gap, and second test illumination. Line 3 is a simultaneous extracellular record of optic nerve potentials. Note the notch in the rising phase and the apparent overshoot of the graded potential in the first test. B, another receptor cell showing apparent overshoot and a notch in the rising phase of the potential. C, another type of potential, possibly receptor, sometimes recorded.

optic nerve potentials (Fig. 8 C). These few exceptions are arbitrarily included in this type. Some variation in the receptor potential wave form occurs as exemplified in Fig. 9. Although the small size of the retinal cells makes prolonged intracellular recordings difficult, short-term recording of 5–30 min is possible with minimal deterioration of the cell. The most frequently occurring potential is composed of the transient wave that lasts for 2 sec, followed by a lower amplitude sustained depolarization (Fig. 9 A). It is similar to extracellular potentials (Fig. 3 C) recorded near the distal segments of the receptors but, of course, of the opposite polarity. Other potentials do not have a clear transient wave (Fig. 9 B) or they may be contaminated by a secondary depolarization (Fig. 9 C) or they may have a very slow rising depolarization (Fig. 9 D).

Discrete bumps are seen in receptor potentials recorded from eyes that have

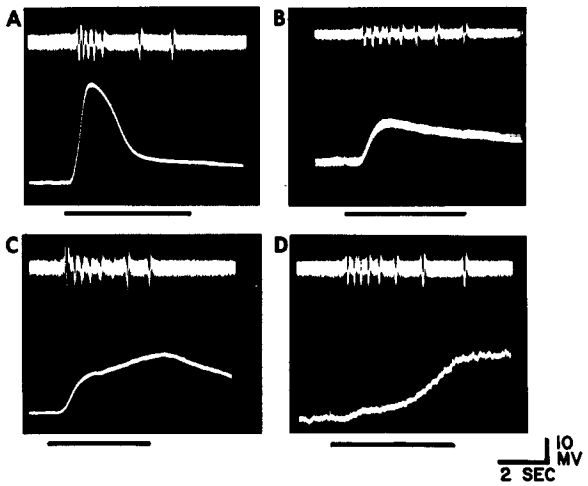


FIGURE 9. Intracellularly recorded receptor potentials. A, the most frequently recorded receptor potential has a sharply rising transient phase and then a sustained phase. B, sustained type. C, complex type possibly representing contamination from another receptor or support cell at the secondary depolarization. D, very slow rising response. Upper traces are optic nerve spikes simultaneously recorded. Voltage scale for D is 5 mv. Black bars indicate the illumination period.

been dark-adapted for long periods. Fig. 10 shows the responses of a receptor that had been in darkness overnight. The typical graded receptor potential response to a prolonged illumination is shown on the top line of Fig. 10 A and the simultaneously recorded optic nerve potentials on the second line. 4 min later the cell was subjected to brief flashes of light (indicated by the horizontal black bars) as shown in B of Fig. 10. The evoked discrete bumps are brief and of low amplitude. They appear to be similar to events seen in *Limulus* visual

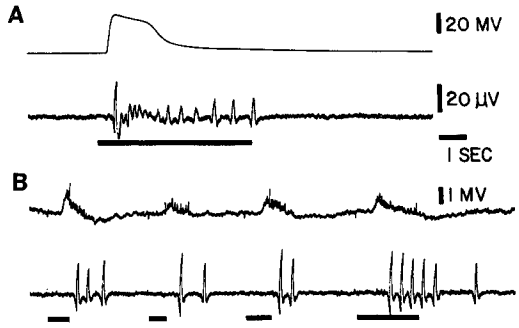


FIGURE 10. Discrete bumps in the receptors. A is the response of a dark-adapted receptor to a prolonged illumination indicated by the black horizontal bar. The second line of A is the simultaneously recorded optic nerve potentials (negative upward). B (AC-coupled) follows 4 min after A and is the response to brief flashes of light. Discrete bumps appear in the receptor potential.

cells which were first called quantum bumps by Yeandle (20) but more recently they are referred to as discrete potentials (21). The discrete bumps in *Aplysia* (under these conditions of prior illumination) do not lead to regenerative events. They do appear to summate (Fig. 10 B) although they may be superimposed on the receptor potential.

A point of some interest is the nature of the optic nerve potentials in Fig. 10 A and B. These potentials occur in Fig. 10 A at a higher frequency but lower amplitude than those in B. Since these potentials are compound, the lower amplitude indicates that fewer units are contributing to the activity. As the

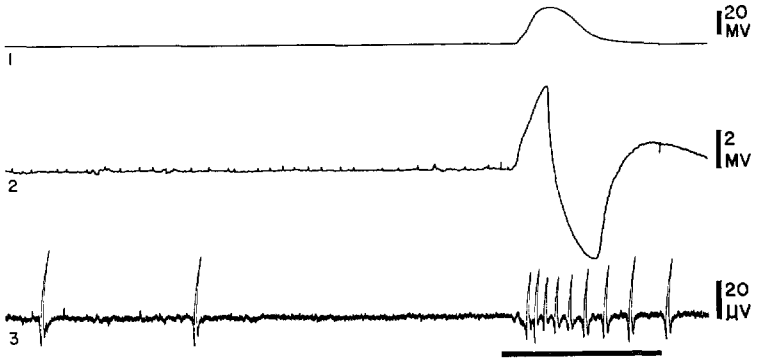


FIGURE 11. Tonic rhythmical potentials in a receptor cell. Line 1 is DC-recorded and shows the response of the cell to illumination. Line 2 is an AC high gain of line 1 showing the tonic potentials in the receptor. Line 3 is simultaneously recorded optic nerve activity. The tonic optic nerve activity is not correlated with the tonic potentials in the receptor. The black bar indicates the illumination duration of 7 sec.

receptor potential amplitude diminishes the compound optic nerve potentials get larger, suggesting that there may be inhibition of units during the transient wave of the receptor. Inhibition in the retina will be more fully discussed in the next section.

Cells that give a receptor potential response to illumination may exhibit tonic rhythmical potentials as shown in Fig. 11. Line 1 is an intracellular recording showing the typical receptor potential. Line 2 is a high gain AC record of line 1 and shows rhythmic potentials. Note that the small potentials are not correlated with the optic nerve activity simultaneously recorded in line 3. The frequency of the potentials suggests that they are initiated by other elements that are typically spiking at that frequency (see Fig. 13 B). Thus, receptor cells may possibly receive synaptic input from other elements. Alternatively, the potentials may be electrotonically invading the recording site from a spike zone in the cell neurite.

VIII. *Intracellular Responses Other Than Receptor Potentials*

Responses of two types other than receptor potentials are evoked from elements of the retina that have resting potentials of 20–50 mv. One type consists of depolarizations that lead to firing in synchrony with the optic nerve potentials. The other type consists of hyperpolarizations that inhibit the tonically occurring activity. They both typically exhibit synaptic and spike activity and do not give large graded receptor potentials.

Responses of the first type are shown in Fig. 12. The tonic activity seen in Fig. 12 A occurs with background illumination. The intracellular spikes on

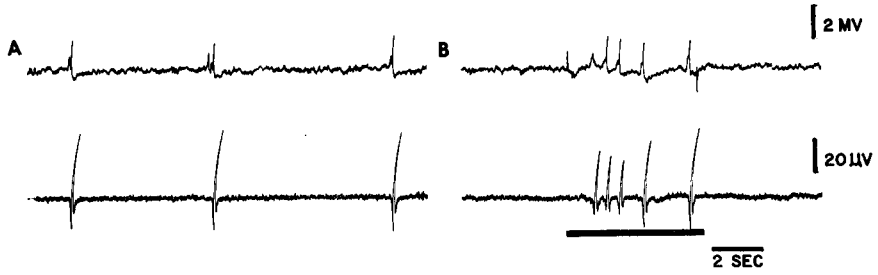


FIGURE 12. Tonic and evoked retinal element activity. A, simultaneous recording of intracellular activity (upper trace) in a retinal element and extracellular optic nerve potentials during dark adaptation. B, illumination of eye causes intracellular spikes correlated with optic nerve potentials. Note the one-to-one relationship in both tonic dark activity and response to illumination.

the upper trace are preceded by prepotentials and are in synchrony with the extracellularly recorded optic nerve potentials on the bottom trace. Fig. 12 B shows the response of the same element to illumination. The intracellular spikes appear to arise from the prepotential activity evident in the record. Experimental hyperpolarizations of these cells to determine whether the prepotentials are postsynaptic or decremented spikes were inconclusive. Prepotentials and spikes are in one-to-one accord with the optic nerve potentials which suggests that the axonal processes of these elements are optic nerve fibers. The intracellular spikes seldom exceed 5 mv (see Fig. 15 D) and are usually 2 mv in amplitude even in cells with large resting potentials. The spikes are apparently electrotonically invading the recording site with some decrement as has been suggested for the variation in size of spikes in *Limulus* eccentric cells (22). Some elements respond to illumination with a similar burst of impulses, but the spikes are not correlated in a one-to-one fashion with the activity in the optic nerve (see Fig. 13 A). The impulses in these cells contribute to only a few of the compound optic nerve potentials.

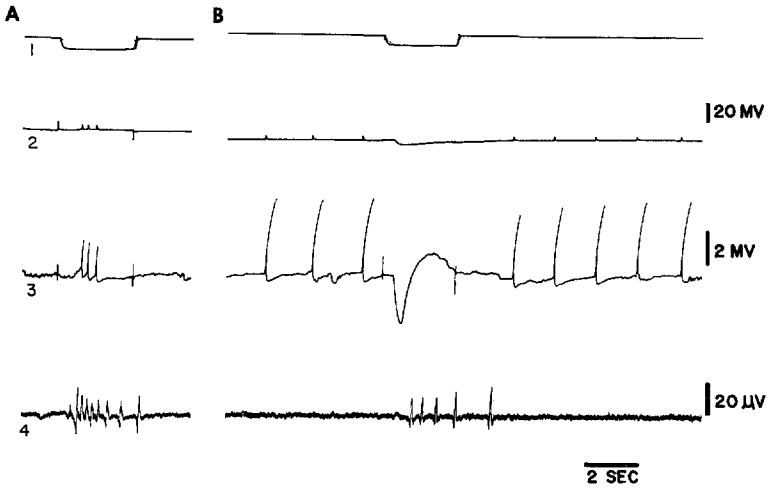


FIGURE 13. Intracellularly recorded responses. A, illumination (1) of eye evokes intracellular on response (2 and 3) in neuron that is not correlated with optic nerve (4) activity. B, hyperpolarization on illumination (1) of an element in the eye (2 and 3). Note this cell's activity does not appear in the optic nerve which shows the typical on response and the increase in optic nerve activity amplitude as the hyperpolarization diminishes. Line 3 is AC-coupled high gain of the DC record in line 2.

Elements of the hyperpolarizing type are typically tonically active but this activity is not seen in the optic nerve. Although some of the spike activity on penetration is undoubtedly injury discharge, it is significant that this is the only type that consistently spikes following penetration. This element is hyperpolarized and the spiking inhibited upon illumination. At cessation of illumination the spiking rebounds at a slightly higher frequency (Fig. 13 B) and slowly adapts. The hyperpolarization may be long-lasting (several

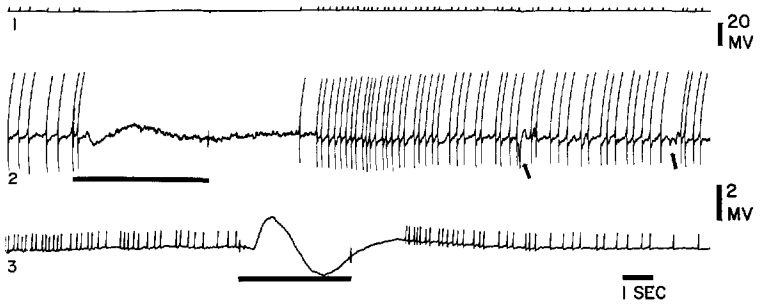


FIGURE 14. Tonically active cell inhibited by illumination. Line 2 is high gain AC-couple duplication of the DC activity in line 1. Illumination of the eye caused hyperpolarization and inhibition of the spike activity which rebounds on cessation of illumination. Ipsp's can be seen at the arrows. Line 3 shows a different preparation (AC-coupled) that is depolarized on illumination but nevertheless inhibits spiking in the cell. Amplifier time constant 0.45 sec.

seconds), up to 10 mv in amplitude, and does not show any obvious summation of individual postsynaptic potentials although these cells do receive random inhibitory postsynaptic potentials (ipsp's) as shown in Fig. 14 (lines 1 and 2). The different amplitudes of these ipsp's further suggest that these elements receive several different ipsp's. The multiplicity of inputs to these elements is demonstrated by the variation of response. Fig. 14 (line 3) shows a tonically active element that is depolarized but the spike activity is suppressed and rebounds. These elements may receive multiple inputs at several sites on the element. An inhibitory potential at the spike-generating zone could suppress spikes

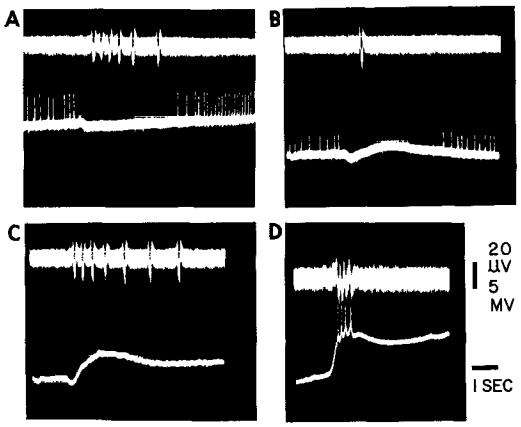


FIGURE 15. Illumination-evoked hyperpolarization and depolarization (DC-recorded). A, a neuron shows hyperpolarization preceded by depolarization. B, depolarization preceded by hyperpolarization. C, similar response but with absence of spiking. D, element spiking synchronized with optic nerve activity (upper trace).

but an excitatory input at a site closer to the recording electrode could cause a net depolarization. Fig. 15 shows more variations in the evoked responses; hyperpolarization may be preceded by depolarization as in A or depolarization may be preceded by hyperpolarization. The response may be obtained from quiet cells also as shown in Fig. 15 C. These hyperpolarizing responses are contrasted with a depolarizing response (Fig. 15 D) which generates spikes in one-to-one relationship with the optic nerve potentials.

DISCUSSION

The retina of the *Aplysia* eye contains thousands of cells but only a few cell types. This is apparent from the histology[1] which shows receptor cells and pigmented support cells in a layer next to the central lens. The receptors contribute processes to the fiber plexus beneath the receptor layer, which also contains other nonreceptor elements and their processes. These nonreceptor elements include glial cells and other elements that contain elementary neurosecretory granules. The fibers of the plexus contribute to the several thousand

optic nerve fibers. The small number of cell types is reflected in the physiological results, which will be discussed in some detail with emphasis on the composition of the ERG, the several types of intracellular responses, the organization of the retina, and finally some thoughts on the function of the eye.

The principal contributor to the *Aplysia* ERG is the summed graded potentials of the receptor distal segments since the ERG amplitude is largest when the recording electrode is in the area of greatest receptor density and at the distal segments. The ERG shows a maximum negativity at the distal segments, neutrality near the cell body layer, and positivity on the outside of the eye capsule. The change in shape and polarity of the potential at different levels of the retina is compatible with the observations on the *Octopus* retina (23) and the squid retina (24). It was concluded that the receptor cell acted as a dipole with the outer segment the current sink and the cell body the source. In the absence of more refined measurements on the *Aplysia* retina, it is concluded that the receptors act in a manner consistent with the dipole hypothesis. The contribution of graded (electrically inexcitable) receptor potentials to the *Aplysia* ERG is further demonstrated by the observation that tetrodotoxin abolishes the optic nerve potentials without changing the ERG (8). Tetrodotoxin blocks electrically excitable responses but does not affect electrically inexcitable responses (25) although it blocks part of the graded transient response of the *Limulus* eye (26).

The large graded intracellular depolarizations referred to as receptor potentials in this report have characteristics in common with potentials recorded from other photoreceptor elements. The shape of the potential is similar to those in crayfish receptors (27) except that in *Aplysia* the potentials are of longer duration. Crayfish receptors sometimes show a spikelike potential superimposed on the rising phase of the transient response. Often a notch was seen in the rising phase of the *Aplysia* potential as has been reported for other photoreceptors (28, 29). In *Aplysia* the graded potentials with a notch overshoot the resting potential by a few millivolts; such overshoots have been reported for *Limulus* cells (30, 31). Also the *Aplysia* receptors exhibit discrete bumps that may be similar to discrete potentials reported in the visual cells of *Limulus* most recently (21, 32, 33) under conditions of low illumination and dark adaptation. These slow potentials were first considered as quantum bumps by Yeandle (20), and the idea was put forward that each bump represented the absorption of a single photon. The discrete bumps seen in *Aplysia* receptors have some of the same characteristics as those in *Limulus*. They are best seen after prolonged dark adaptation in cells with large resting potentials and they appear to sum and fuse on illumination. However, no analysis has been made of the frequency of response or the distribution of potential types (bump and regenerative).

The responses to illumination recorded from the *Aplysia* eye are similar in

many respects to the activity from the simple eye of the nudibranch *Hermissenda*. The five primary photoreceptors (34) of that eye were shown to be synaptically coupled and possibly electrotonically connected (35) but no large graded potentials, devoid of spikes, such as occur in *Aplysia*, were found. However, the activity of the type I and II cells of that eye is like the activity in nonreceptor elements of *Aplysia*. In particular the biphasic response of a depolarization followed by a hyperpolarization seen in *Aplysia* elements is often seen in type I cells. This response is also very similar to the biphasic postsynaptic potential reported from the giant cell of the pleural ganglion of *Aplysia* (36). The hyperpolarizing responses of *Aplysia's* eye appear to be the same as type II responses in *Hermissenda*; in both the effect is long-lasting. Changes from type I to type II responses within a cell were demonstrated in *Hermissenda* and may be true of *Aplysia* responses also but they have not been clearly shown. It is apparent that there are extensive excitatory and inhibitory inputs to the elements of these eyes that can produce various responses depending on their spatial and temporal occurrence. The fact that inhibitory synaptic activity, known to occur among the five receptors of *Hermissenda*, occurs in elements of *Aplysia*, suggests that this activity may be recorded from the receptor neurites. The failure of this activity to invade the site of the graded receptor potential can be attributed to electrotonic decrement (35). The anatomical studies agree with this interpretation since receptor neurites are closely associated in the fiber plexus of the retina and synapses between fibers are evident.[1] However, other cells and fibers are seen in the plexus and some of the electrical activity may have been recorded from them. The notion that the cells in the plexus contribute fibers to the optic nerve has some support from the physiological data but is not conclusive.

Elements that exhibit tonic activity that is correlated one-to-one with optic nerve potentials have not been found to receive hyperpolarizing inhibitory potentials, whereas the tonic activity of elements that do receive hyperpolarizing potentials is not seen in the optic nerve, suggesting that there is a synapse between this element and the optic nerve fibers. Further support for a synapse is provided by the observation that acetylcholine at 10^{-8} M blocks the optic nerve potentials without influencing the evoked graded receptor potentials (8). However, the results of antidromic electrical stimulation of the optic nerve are not in complete agreement with the synapse idea.

Preliminary results of electrical stimulation of the optic nerve (Jacklet, unpublished data) while recording intracellularly from retinal elements show several kinds of responses. Depolarizing responses of several millivolts are recorded from elements that spike tonically; these are probably antidromic spikes. Some elements that give graded receptor responses to illumination are invaded by biphasic synaptic potentials when the optic nerve is stimulated. These potentials are graded in amplitude over a range of stimulus voltages,

suggesting that they are summed according to the number of optic nerve fibers stimulated. This result confirms the idea that receptors receive multiple lateral synaptic influences but still leaves in doubt the contribution of fibers, other than those from receptors, to the optic nerve.

The simplest hypothesis for the organization of the eye is that all the recorded activity is occurring at various sites (distal segments, soma, and neurites) of the photoreceptor cell. If this is so, the receptor potential is more accurately referred to as a generator potential and the organization becomes analogous to that of the *Hermissenda* eye but with many more photoreceptor elements. However, the inconsistencies noted earlier and the presence of other neuronal elements in the retina that most probably contribute processes to the optic nerve add some complication to this simplicity. Most gastropod retinas have been interpreted as containing primary receptor cells whose axons make up the optic nerve (cf. reference 9) but the eye of *Littorina*, a prosobranch (16), is believed to have bipolar neurons at the base of the receptors.

The potentials in the *Aplysia* optic nerve are synchronized into compound potentials during tonic and evoked activity as a consequence of coupling among receptors and/or nerve fibers much as electrotonic coupling among the five receptors of *Hermissenda* produces synchronous spiking (35). The amplitude of the compound potentials should be proportional to the number of active nerve fibers. It is found that at high illumination intensities the initial burst of compound potentials is low in amplitude and subsequent potentials get progressively larger in coincidence with the reduced inhibitory influence among the cells, so that more and more fibers are actively contributing to the compound potential as inhibitory influences decrease (cf. Figs. 10 and 13). Such lateral inhibitory activity among photoreceptor processes has been elegantly described for *Limulus* (37).

The tonic optic nerve activity is dependent upon a prior dark adaptation period. The duration of the silent period after cessation of illumination is increased in proportion to the increased intensity and duration of the acute illumination. This silent period may be minutes long and is not accompanied by any special polarization of the receptors, as shown for *Limulus* (31), or synaptic and spike activity in the neurites. The tonic activity could be due to an endogenously active cell (possibly the receptor) whose activity depends on the buildup of a chemical substance, which may be associated with the vesicles in the receptors.

An ultrastructural feature common to many primitive eyes is vesicles about 500 A in diameter in the cytoplasm of the receptors (9, 33). The receptors of *Aplysia* contain such vesicles in very large numbers. They may contain a substance that is released during illumination (22) and tonic activity conditions or they may participate in a kind of intracellular coupling between the rhabdomere and the receptor membrane (38, 27).

In addition to the great number of 500 A vesicles in the receptor cells,

electron micrographs show that numerous 1000 A dense granules occur in the neuron processes of the retina which appear to be typical (39) neurosecretory granules. The slow uniform conduction activity, typical of many neurosecretory cells (40), of the optic nerve and the presence of vesicles and granules in the retina suggest a neurosecretory function for the eye. The amount of secretion could be controlled by light duration and intensity. This idea is not entirely unprecedented as Bern (41) has suggested there may be simple eyes that do not see. The eye of *Aplysia* may combine the aspects of conventional vision and secretion.

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