Electrophysiological Properties of Cells in the Median Ocellus of *Limulus*

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ABSTRACT Two types of photoreceptors are found in the median ocellus of *Limulus*. One type is maximally sensitive to ultraviolet (UV) light, the other to green light; they are called *UV* and *VIS* cells, respectively. Biphasic receptor potentials, consisting of a small initial hyperpolarizing phase and a later slow depolarizing phase, can be recorded from both receptor types. These biphasic responses are elicited in *UV* cells in response to long-wavelength light, and in *VIS* cells in response to ultraviolet light. Another type of hyperpolarizing response can be recorded in *UV* cells: after a bright ultraviolet stimulus, the cell remains depolarized; long-wavelength light rapidly returns the membrane potential to its value preceding ultraviolet illumination (this long-wavelength–induced potential change is called a "repolarizing response"). Also, a long-wavelength stimulus superimposed during a UV stimulus elicits a sustained repolarizing response. A third cell type (arhabdomeric cell) found in the median ocellus generates large action potentials and is maximally sensitive to UV light. Biphasic responses and repolarizing responses also can be recorded from arhabdomeric cells. The retina is divided into groups of cells; both *UV* cells and *VIS* cells can occur in the same group. *UV* cells in the same group are electrically coupled to one another and to an arhabdomeric cell.

INTRODUCTION

Many arthropods are known to possess photoreceptors sensitive to near-ultraviolet light (e.g. Autrum and von Zwehl, 1962, 1964; Goldsmith, 1960; Goldsmith and Fernandez, 1968; Wald and Seldin, 1968). In *Limulus polyphemus*, electroretinogram (ERG) studies showed that the median ocellus contains photopigments with maximum absorption at 360 nm and at 530–535 nm (Wald and Krainin, 1963). In contrast, the lateral eye of *Limulus* seems to contain a single photopigment with maximum absorption at 520 nm (Chapman and Lall, 1967; Wald, 1968). Using intracellular recording techniques, we re-

cently demonstrated the presence of two populations of photoreceptors in the median ocellus, one (called "*UV* cells") maximally sensitive to UV light and the other (called "*VIS* cells") maximally sensitive to green light.

We also recorded some responses from photoreceptors of the median ocellus which are unlike any reported for the other eyes of *Limulus*. In both *UV* cells and *VIS* cells, stimuli of specific wavelengths (UV for *VIS* cells, long wavelength for *UV* cells) evoke biphasic receptor potentials consisting of a small, brief, initial hyperpolarization and a larger slow depolarization. In addition, we recorded a second type of novel response in *UV* cells. After an intense UV stimulus, the light-evoked depolarization of *UV* cells declines very slowly. The rate of decline (i.e., the rate of return toward the original resting potential in the dark) can be greatly increased by a long-wavelength stimulus. Furthermore, during an intense UV stimulus (which depolarizes the cell), long-wavelength stimuli can elicit a negative-going response (called a "repolarizing response").

In this report, we describe the types of light responses which can be recorded in the median ocellus, and discuss the physiological organization of the different cell types. In a companion paper, we present a further investigation of the properties of the repolarizing response (Nolte and Brown, 1971). Some preliminary results of this study have appeared in earlier reports (Nolte et al., 1968; Jones et al., 1971).

METHODS

The biological preparation, the recording system, and the optical system have been described previously (Nolte and Brown, 1969). The procedures for experiments involving dye-filled microelectrodes are the same as those described elsewhere (Jones et al., 1971), with the following addition. In some experiments a 4% solution of Procion navy blue M3RS (I.C.I. Organics, Inc., Stamford, Conn.) was used for filling the electrodes. This dye was injected most successfully using 50-100–na current pulses, in contrast to the 5-10–na pulses used for Procion yellow.

In some experiments, a more complex artificial seawater ("Instant Ocean," Aquarium Systems, Inc., Eastlake, Ohio) than the one specified in our earlier report was used to bathe the preparation. There were no noticeable differences in the physiology of photoreceptors bathed in the two types of seawater.

To depolarize membrane voltage by more than 10 mv we inserted two microelectrodes into a single photoreceptor cell. One electrode was used to pass current and the other to monitor membrane voltage. By this technique, we were able to search for reversal voltages for the various components of the light responses.

RESULTS

A. *Receptor Cells*

As we previously reported (Nolte et al., 1968; Nolte and Brown, 1969), two types of ocellar receptor cell can be distinguished on the basis of the spectral

sensitivities of their depolarizing receptor potentials. One type (*UV* cell) is maximally sensitive at about 360 nm, the other (*VIS* cell) at about 525 nm. The relative numbers of *UV* and *VIS* cells vary widely from one ocellus to another, but we estimate that, on the average, the receptor population is 60–70% *UV* cells and 30–40% *VIS* cells.

1. MEMBRANE PROPERTIES Both receptor cell types have resting membrane potentials of 35–65 mv (inside negative) in the dark-adapted state. We measured cell resistances with small (< 1 na) constant current pulses injected through the recording electrode by means of a bridge circuit (Frank and Becker, 1964). The resistance value obtained depends on the direction of cur-

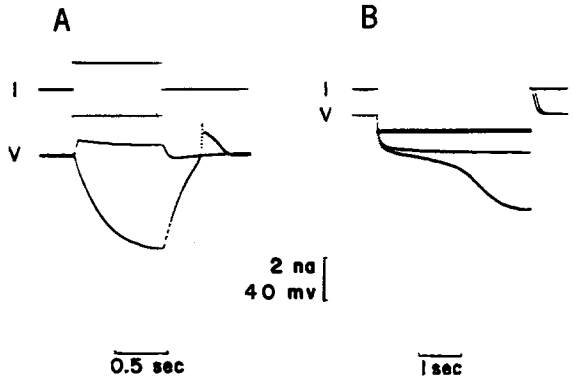

FIGURE 1. (A) Voltage changes (*V*) produced in a *UV* cell by constant current pulses (*I*) injected through the recording electrode. At the end of the hyperpolarizing pulse there is a spikelike "anodal break" response. (B) Hyperpolarizing response in a *UV* cell. A subthreshold constant current pulse produces a simple exponential charging curve, while a slightly greater current pulse produces a two-step hyperpolarization. At the end of both hyperpolarizing pulses there is a spikelike "anodal break" response.

rent flow. With hyperpolarizing current pulses we measure values of 20–100 MΩ; with depolarizing current pulses the resistance is 5–20 MΩ (Fig. 1 A). The voltage change produced by a hyperpolarizing current pulse is a simple exponential charging curve whose time constant sometimes exceeds 200 msec. Cell capacitances, calculated from resistance and time constant values, are typically $1–5 \times 10^{-9}$ farad. These resistances and capacitances are close to those determined for *Limulus* ventral photoreceptor cells by Millecchia and Mauro (1969 *a*).

Occasionally the response to a hyperpolarizing pulse occurs in two steps (Fig. 1 B). The second step of hyperpolarization has a fairly well-defined threshold. This phenomenon resembles the "hyperpolarizing response" described by others in *Limulus* lateral eye retinular cells (Smith et al., 1965; Wasserman, 1968). We have not searched carefully for these hyperpolarizing responses, but all five cells in which we found them were *UV* cells.

At the end of a hyperpolarizing pulse, a spikelike "anodal break" response is observed (Fig. 1), like that found in *Limulus* lateral eye retinular cells (Smith and Baumann, 1969) and ventral eye photoreceptors (Millecchia and Mauro, 1969 *a*). The same spikelike response can usually be elicited by a depolarizing pulse, particularly if the cell is held slightly negative to resting potential with hyperpolarizing current (Millecchia and Mauro, 1969 *a*).

2. DEPOLARIZING RECEPTOR POTENTIALS The responses to light of both receptor cell types consist of an initial transient phase followed by a "steady phase" which is maintained for the duration of the stimulus (Fig. 2 A, B, E, F). For bright lights, the transient frequently overshoots zero membrane voltage (e.g. Fig. 2 A, E) and the steady phase may reach zero (e.g. Fig. 10 C). A single spikelike response can usually be seen on the leading edge of the transient as a small notch.

A long intense flash delivered to a dark-adapted cell elicits a response with a long transient, a small dip between transient and steady phase, and a "steady phase" which slowly declines from its maximum value (Fig. 2 A, E). A flash of the same intensity delivered to a moderately light-adapted cell elicits a response with a briefer and smaller transient, a deeper dip between transient and steady phase, and a faster decline from the steady-phase maximum (Fig. 2 B). The maximum of the steady phase of the light response in a light-adapted cell may be more depolarized than that in the same cell when it is dark-adapted (Fig. 2 B).

The "steady phase" is difficult to quantify. When elicited by bright stimuli it declines slowly from its initial maximum value; this decline may last many minutes (Fig. 3). Furthermore, dim stimuli delivered to a dark-adapted cell elicit a steady phase which appears to be the temporal summation of a number of discrete responses or "bumps," as in lateral eye retinular cells (Adolph, 1964; Fuortes, 1959; Yeandle, 1958). We use the term "steady phase" with these qualifications in mind.

With prolonged stimuli, we sometimes see a response with a more complex waveform, in which the steady phase seems to develop in two parts (Fig. 2 F). Following the transient phase, the membrane potential reaches an approximately steady value; after some delay, the membrane depolarizes further to a new steady level. The transition to the more positive voltage can be considerably more delayed and more abrupt than in the example shown in Fig. 2 F. We have seen such responses only in *UV* cells, and usually only when they were light-adapted. They are elicited by stimuli in a restricted intensity range, roughly 5–6 log units above threshold.

The response to a brief flash of light resembles the transient phase of the response to a long flash. With intense stimuli, it may overshoot zero potential (e.g. Fig. 2 C, G). In dark-adapted cells, the response declines over a broad and distinct shoulder (Fig. 2 C, G). If such responses are elicited repetitively,

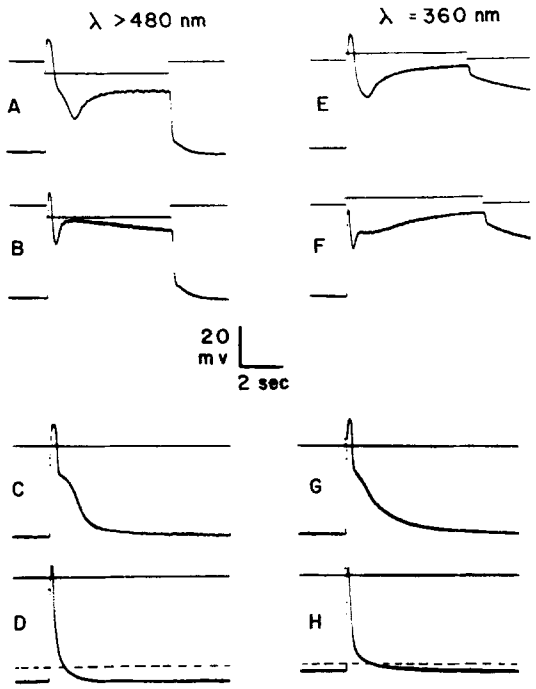

FIGURE 2. (A)–(D): responses of a *VIS* cell to intense long-wavelength (λ > 480 nm) light. Stimulus intensity is the same for all responses. The upper trace in each figure is a light monitor positioned at zero membrane voltage when the light is off. (A) Response of a dark-adapted cell. (B) Response elicited 15 sec after the end of the stimulus in (A). (C) Response of the same cell to a 50 msec flash after it had dark-adapted again. (D) Response to the same stimulus as in (C). Flashes were repeated every 10 sec until the membrane voltage and receptor potential amplitude reached constant values, at which time this response was recorded. The dashed line is positioned at the membrane voltage preceding the stimulus in (C); the cell hyperpolarized between repetitive stimuli. (E)–(H): responses of a *UV* cell to intense 360 nm light. Stimulus intensity is the same for all responses. The upper trace in each figure is a light monitor positioned at zero membrane voltage when the light is off. (E) Response of a dark-adapted cell; the cell remained depolarized at the end of the stimulus. (F) Response elicited 15 sec after the end of the stimulus in (E); the cell remained depolarized at the end of the stimulus. An intense long-wavelength stimulus (λ > 480 nm, not shown) was interposed between (E) and (F) to repolarize the membrane (see text for further explanation). (G) Response of the same cell to a 50 msec flash after it had dark-adapted again. (H) Response to the same stimulus as in (G). Flashes were repeated every 10 sec until the membrane voltage and receptor potential amplitude reached constant values, at which time this response was recorded. The dashed line is positioned at the membrane voltage preceding the stimulus in (G); the cell hyperpolarized between repetitive stimuli.

they become slightly smaller (measured from the dark-adapted resting potential), briefer, and the shoulder is lost. In addition, the membrane slowly hyperpolarizes between flashes (Fig. 2 D, H). This hyperpolarization may exceed 20 mv (Figs. 4 and 5). Sometimes, a large hyperpolarization can be seen fol-

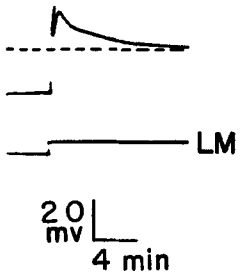

FIGURE 3. Chart record of the response of a *UV* cell to an intense 360 nm stimulus. The lower trace is a light monitor (*LM*). After the transient phase and an initial steady-phase maximum, the membrane voltage very slowly approached a steady value. Dashed line is at −20 mv.

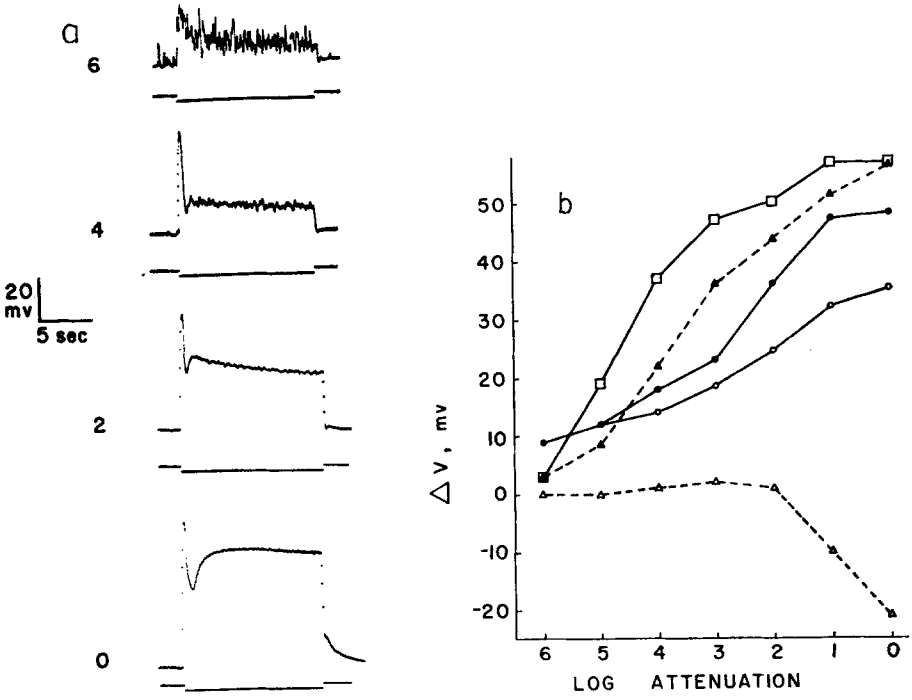

FIGURE 4. (*a*) Responses of a *VIS* cell to long-wavelength stimuli ($\lambda > 480$ nm) of several different intensities. The number to the left of each trace indicates the log of the stimulus attenuation for that response. (*b*) Stimulus intensity vs. response amplitude for the same cell as in (*a*). Responses are the voltage changes measured from the dark-adapted membrane voltage. Squares indicate the response of the dark-adapted cell to a 50 msec flash. Filled triangles indicate the responses to 50-msec stimuli after they had been repeated every 10 sec until the response amplitude and membrane voltage reached constant values; open triangles indicate these constant membrane voltage values. Filled circles indicate the maximum value of the "steady phase" of the response; opened circles indicate the value of the steady phase after about 1 min of illumination, when the membrane voltage had become approximately constant.

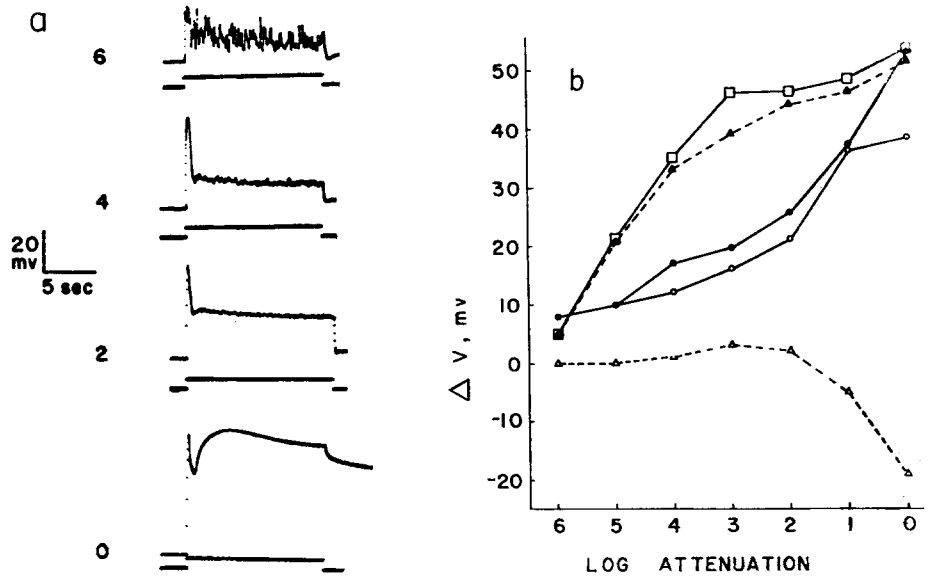

FIGURE 5. (*a*) Responses of *UV* cell to 360-nm stimuli of several different intensities. The number to the left of each trace indicates the log of the stimulus attenuation for that response. (*b*) Stimulus intensity vs. response amplitude for the same cell as in (A). All symbols and procedures are the same as in Fig. 5 *a*.

lowing the delivery of a single bright flash to a dark-adapted cell. Such hyperpolarizations following intense stimulation have been found in retinular cells of *Limulus* lateral eye (Benolken, 1961; Stieve, 1965), in *Limulus* ventral photoreceptors,[1] and in barnacle photoreceptors (Koike et al., 1970), as well as in other types of receptor cells (e.g. Eyzaguirre and Kuffler, 1955; Nakajima and Takahashi, 1966).

The shapes of the intensity-response curves for both transient and steady phases of the light response are the same for both *UV* and *VIS* cells (Fig. 4 *b* and 5 *b*). The size of the response of a dark-adapted cell to a brief flash increases linearly with the logarithm of the flash intensity for about 3 log units above threshold intensity. For brighter stimuli, the response amplitude slowly saturates over at least 3 log units more. The responses are somewhat smaller (measured from the dark-adapted resting potential) when a cell is partially light-adapted by repetitive flashes, especially for moderate intensities. Measurement of the amplitude of the steady phase is complicated by the phenomena mentioned above; namely, dim lights elicit a very noisy response and bright lights elicit a steady phase which is not constant in time. (Figs. 4 *a* and 5 *a*). In the plots of Figs. 4 *b* and 5 *b* we have estimated the amplitude of the steady phase for dim lights. For bright lights we have measured (with

[1] Brown, J. E., and J. E. Lisman. An electrogenic sodium pump in *Limulus* ventral photoreceptor cells. Manuscript in preparation.

respect to the dark-adapted resting potential) both the highest point of the response after the transient and the potential after about a minute of illumination; by this time the response had become approximately constant. The curves are sigmoid and extend over at least 6 log units of intensity without saturating. We seldom find curves in which the steady phase amplitude saturates completely.

3. BIPHASIC RECEPTOR POTENTIALS In addition to a depolarizing receptor potential, we record a biphasic receptor potential from both *UV* and *VIS* cells, consisting of a small transient hyperpolarization and a later slow depolarizing phase (Fig. 6). Biphasic responses are similar in *UV* and *VIS* cells, except that the slow depolarizing phase is generally larger in *VIS* cells. The spectral sensitivity curves for the transient hyperpolarizations are the same as those for the depolarizing receptor potentials described above (Nolte and Brown, 1969), but UV-elicited biphasic responses occur in *VIS* cells and long-wavelength–

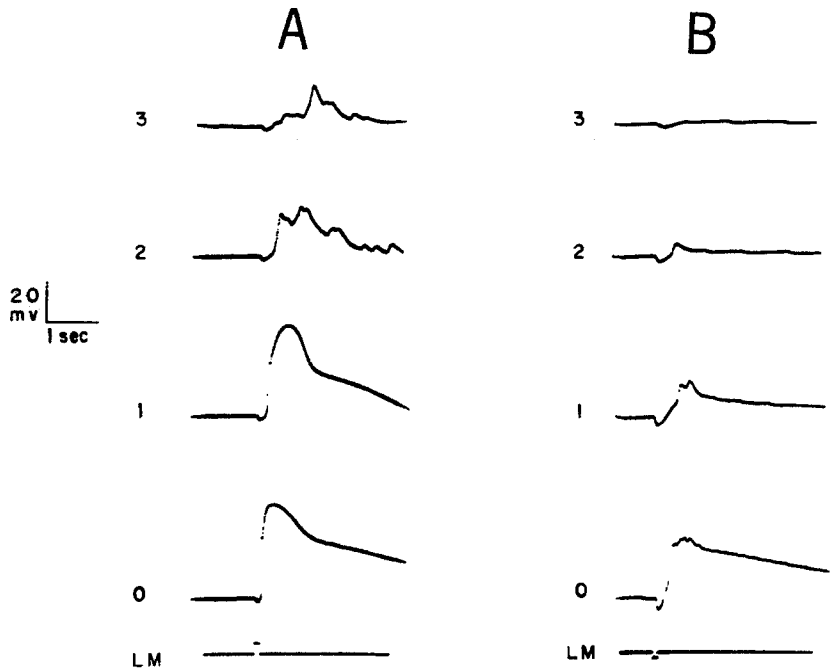

FIGURE 6. (A) Responses of a *VIS* cell to 360-nm stimuli of several different intensities. The cell was allowed to dark-adapt between stimuli. The number to the left of each trace is the log of the stimulus attenuation for that response. The bottom trace is a light monitor (*LM*). (B) Responses of a *UV* cell to long-wavelength ($\lambda > 480$ nm) stimuli of several different intensities. The cell was allowed to dark-adapt between stimuli. Small spikes can be seen during the depolarizing phases of the responses. The number to the left of each trace is the log of the stimulus attenuation for that response. The bottom trace is a light monitor (*LM*).

elicited biphasic responses occur in *UV* cells. The spectral sensitivities of the slow depolarizing phase of these biphasic responses have not been determined.

The threshold intensity for a biphasic response in *VIS* cells, measured with 360-nm stimuli, is about the same as, or slightly higher than, the threshold for a depolarizing receptor potential measured with 525-nm stimuli. The threshold for a biphasic response in *UV* cells, measured with 525-nm stimuli, is about 10^4 times greater than the threshold for a depolarizing receptor potential measured with 360-nm stimuli. The latencies of both depolarizing and biphasic responses are about the same at their respective thresholds and change in a parallel fashion with stimulus intensity (Fig. 7 A–C). Thus, in *UV* cells, 360-nm and 525-nm stimuli will elicit depolarizing and biphasic responses with approximately equal latencies if the intensity of the 525 nm stimulus is 10^4 times that of the 360 nm stimulus. In *VIS* cells, depolarizing and biphasic re-

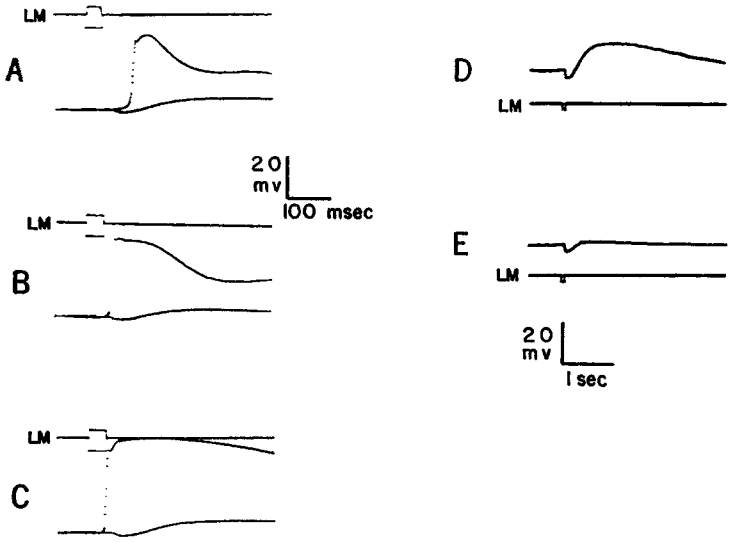

FIGURE 7. Latencies of the depolarizing and biphasic receptor potentials in a *VIS* cell. Upward deflections of the light monitor (*LM*) indicate UV light; downward deflections indicate long-wavelength ($\lambda > 480$ nm) light. UV stimuli elicited biphasic receptor potentials; long-wavelength stimuli elicited depolarizing receptor potentials. The intensity of the UV stimulus was the same for all three responses, about 2 log units greater than threshold intensity. (A) Long-wavelength intensity about 1.3 log units greater than threshold. (B) Long-wavelength intensity about 2.3 log units greater than threshold. (C) Long-wavelength intensity about 3.3 log units greater than threshold. In (B), biphasic and depolarizing receptor potentials have approximately the same latency; the stimuli eliciting these receptor potentials are both 2–2.3 log units more intense than threshold intensity for the respective responses. (D)–(E) Light adaptation of the biphasic receptor potential. (D) Response of a dark-adapted *UV* cell to a long-wavelength ($\lambda > 480$ nm) stimulus. (E) Response to an identical stimulus delivered 5 sec after that in (D). The hyperpolarizing phase is about the same as that in (D), but the depolarizing phase is greatly reduced.

sponses of comparable latencies are elicited by long-wavelength ($\lambda > 480$ nm) and 360-nm stimuli of the same intensity.

The hyperpolarizing and depolarizing phases of the biphasic receptor potential differ markedly in three respects. First, the hyperpolarizing phase is relatively less sensitive to light adaptation than is the depolarizing phase. This is particularly noticeable in *UV* cells, where the second of two bright flashes delivered less than 15 sec apart may elicit only the hyperpolarizing phase (Fig. 7 D, E). Second, we have carefully withdrawn the microelectrode from inside a cell, and recorded the receptor potentials extracellularly; in such a case the hyperpolarizing phase does not change its sign, while the depolarizing phase disappears (Fig. 8). Third, we have been unable to find a reversal voltage for

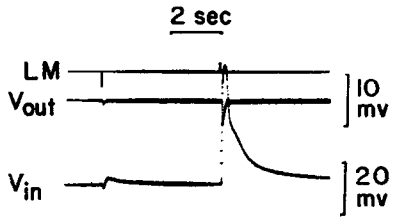

FIGURE 8. Intracellular and extracellular recordings of receptor potentials from a *UV* cell. The upward deflection of the light monitor (*LM*) indicates UV light; the downward deflection indicates long-wavelength ($\lambda > 480$ nm) light. After the biphasic and depolarizing receptor potentials of the V_{in} trace were recorded, the electrode was withdrawn slightly until the membrane voltage was lost. Then the responses of the V_{out} trace were recorded at higher gain. The polarity of the response elicited by UV light was reversed, while the polarity of the hyperpolarizing phase of the biphasic receptor potential did not change. The depolarizing phase of the biphasic receptor potential could no longer be recorded. The position of the V_{out} trace corresponds to zero voltage for the V_{in} trace.

the hyperpolarizing phase, while the depolarizing phase usually reverses between -20 and -30 mv (Fig. 9).

4. INDUCED LONG-WAVELENGTH SENSITIVITY IN UV CELLS In *UV* cells, after the cessation of a bright UV stimulus, the membrane potential returns to its original (prestimulus) value exceedingly slowly (Fig. 10 A); complete return sometimes takes many minutes. In contrast, after a stimulus of any wavelength, the membrane potential of *VIS* cells returns quickly to the original resting level (compare the responses to the highest intensity stimuli in Figs. 4 and 5). If, during the course of such a long depolarizing afterpotential in a *UV* cell, the cell is stimulated with bright, long-wavelength ($\lambda > 480$ nm) light, the membrane potential quickly returns to the resting level (Fig. 10 B). We call this return of membrane potential a "repolarizing response." Repolarizing responses also can be elicited during a UV stimulus: a long-wavelength stimulus superimposed on a bright UV background (which has depo-

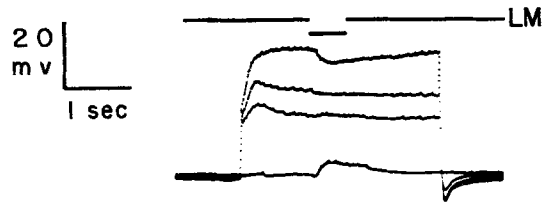

FIGURE 9. Reversal of the depolarizing phase of the biphasic receptor potential. Identical long-wavelength (λ > 480 nm) stimuli were delivered every 60 sec and were timed to occur during a depolarizing current pulse. The light monitor (*LM*) is positioned at zero membrane voltage when the light was off. The depolarizing phase of the biphasic receptor potential reverses its polarity around −25 mv.

larized the cell) produces a response which repolarizes the cell by as much as 30–40 mv and lasts as long as the long-wavelength stimulus (Fig. 10 C). These repolarizing responses are discussed in greater detail in a companion paper (Nolte and Brown, 1971).

5. IMPULSE ACTIVITY We frequently find small (< 2 mv) spikes superimposed on the receptor potentials of *UV* cells (e.g. Figs 6 A, 7 B, 9). They seem analogous in all ways to the small spikes recorded in retinular cells of the lateral eye (Behrens and Wulff, 1965; Smith et al., 1965; Tomita et al., 1960). We have observed such small spikes only once in a *VIS* cell.

B. *Cells Producing Large Action Potential*

Occasionally we have penetrated cells which respond to light by producing relatively large (10–40 mv) action potentials superimposed on relatively small slow potentials. Such recordings are rather unstable, and seldom can be maintained for more than 10 min. These units are generally encountered in distal portions of the retina. The axons of the receptor cells are known to arise from the proximal ends of the cells (Demoll, 1914); this geometry suggests that the large spikes which we record do not arise in the axons of receptor cells.

We recorded from more than 50 of these cells which generated large spikes. In all but two we observed a depolarization and increased firing frequency in response to a UV stimulus, and a biphasic response to a long-wavelength stimulus (Fig. 11 A and B). In addition, intense UV stimuli often elicited a sustained after-depolarization and firing which outlasted the stimulus. Both the after-depolarization and the firing could be abolished by long-wavelength (λ > 480 nm) light (Fig. 11 C). In the presence of a UV stimulus which caused the cell to depolarize and fire, long-wavelength light partially repolarized the cell and the firing frequency decreased (Fig. 11 C).

The two exceptional cases were cells which responded with depolarization and firing to light of any wavelength; both were considerably more sensitive to UV than to long-wavelength stimuli.

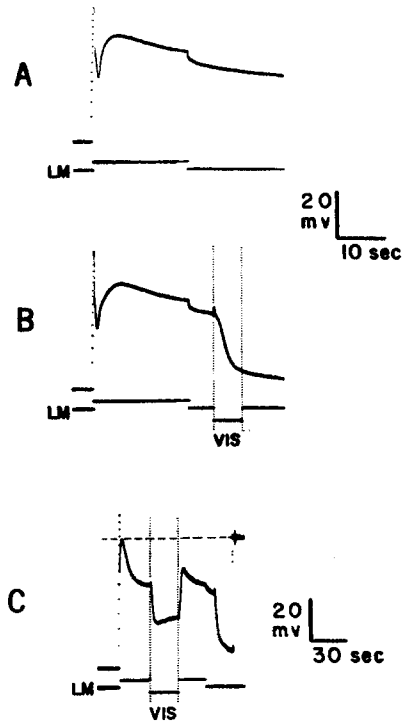

FIGURE 10. UV-induced long-wavelength sensitivity in *UV* cells. In all cases, the lower trace (*LM*) is a light monitor; upward deflections indicate 360 nm light, and downward deflections indicate long-wavelength ($\lambda > 480$ nm) light. (A) Response to a bright 360 nm stimulus; after the end of the stimulus, the membrane voltage slowly returns to the original resting value. (B) Same cell and stimulus as in (A). After the 360 nm stimulus the membrane voltage rapidly returned to the original resting value in the presence of bright long-wavelength light. Preceding this "repolarizing" response, a biphasic receptor potential was elicited. (C) Another *UV* cell. A long-wavelength stimulus applied during a 360 nm stimulus elicits a repolarizing response which lasts as long as the long-wavelength stimulus. After the 360 nm stimulus, the membrane voltage began to return slowly to resting voltage; the rate of return was greatly increased by long-wavelength light, as in (B). The electrode was then withdrawn, showing that the steady-phase maximum of the response to 360 nm light just reached zero membrane voltage.

C. *Retinal Organization*

We used pairs of microelectrodes, each filled with a different dye solution, to investigate the physiological and anatomical relationships of pairs of cells situated near each other in the retina. Whenever the waveforms or spectral sensitivities of the light responses recorded by the two electrodes were significantly different, it was assumed that the electrodes were in two different cells. In every case in which both dyes could be found histologically, this assumption was borne out. Hence we use the term "pair of cells" to denote any situation in which two intracellular electrodes record light responses which differ significantly in waveform or spectral sensitivity. It was not possible, using this

dye injection technique, to demonstrate unequivocally whether two cells were actually in contact, since cells in the median ocellus are often separated from each other by sheets of glia too thin to be resolved by light microscopy (Jones et al., 1971). However, when two cells appeared, by light microscopy, to be in

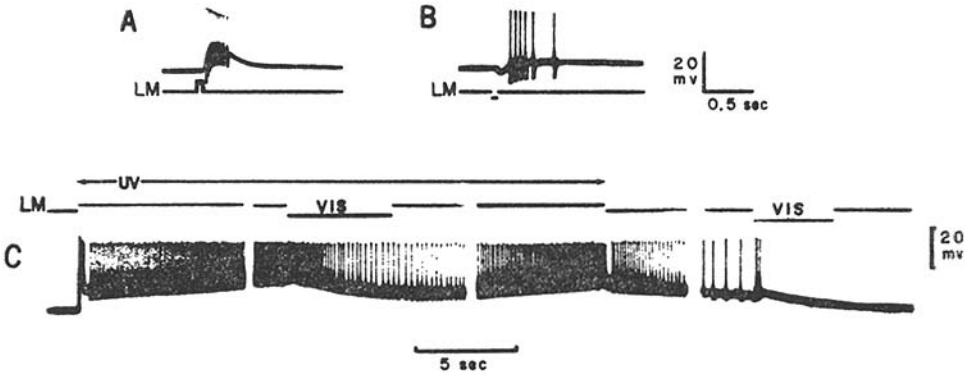

FIGURE 11. Responses of cells which generated large action potentials. In all cases, the cell was slightly hyperpolarized with extrinsic current in order to suppress spontaneous firing. Upward deflections of light monitor (*LM*) indicate 360 nm light, downward deflections indicate long-wavelength ($\lambda > 480$ nm) light. (A) Response to a 50 msec flash of 360 nm light. (B) Response to a 50 msec flash of long-wavelength ($\lambda > 480$ nm) light. Same cell as in (A). (C) In another cell, long-wavelength light applied during a 360 nm stimulus causes a hyperpolarization and decreased impulse frequency. After the end of the 360 nm stimulus, the cell remains depolarized and continues to generate impulses. A long-wavelength stimulus applied at this time causes an initial burst of impulses, followed by repolarization and cessation of spikes.

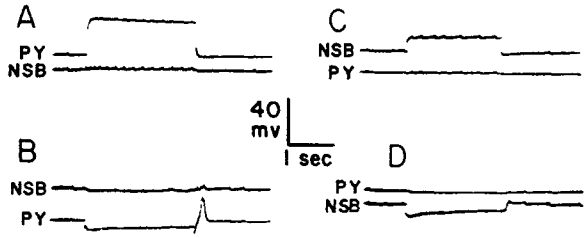

FIGURE 12. Electrical coupling between two *UV* cells. Small spikes are synchronous in both cells. We were unable to keep the bridge circuit properly balanced with the high resistance dye-filled electrodes used in this experiment. (A) and (B) Current injected through Procion yellow electrode (*PY*). (C) and (D) Current injected through Niagara sky blue electrode (*NSB*).

contact, they were considered to be neighbors and part of the same cell group (Jones et al., 1971). By this criterion we found that any receptor type could be a neighbor of any other receptor type, i.e., *UV* cells and *VIS* cells can occur mixed in the same group. In 61 pairs of cells consisting of one *UV* cell and one *VIS* cell, we found no cases of electrotonic coupling. Of 12 pairs of *VIS* cells,

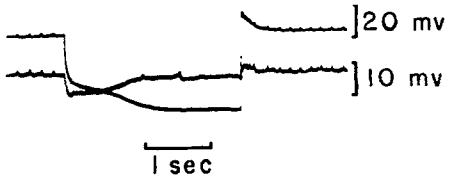

FIGURE 13. "Uncoupling" in *UV* cells. Hyperpolarizing current was passed through the electrode in one cell (upper trace) and a "hyperpolarizing response" was elicited (see Fig. 1 B). An electrotonic voltage change was recorded in the second cell, and this voltage change diminished greatly when the hyperpolarizing response occurred.

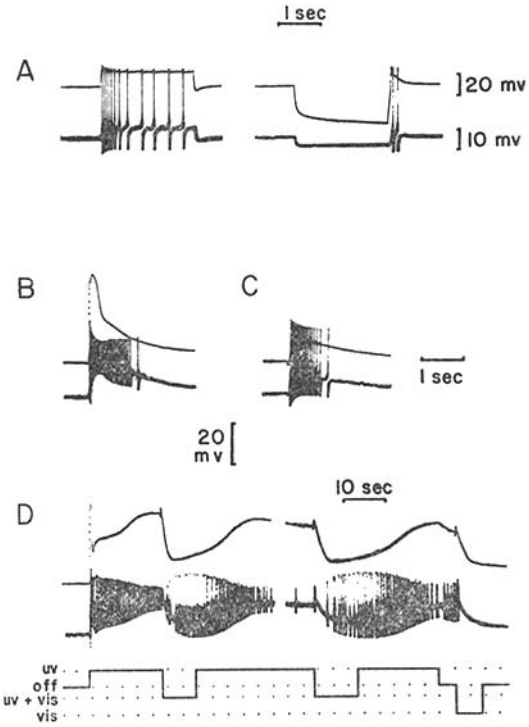

FIGURE 14. A *UV* cell (upper traces) electrically coupled to a cell which generated large action potentials ("arhabdomeric cell," lower traces). In all cases where small spikes can be distinguished in the *UV* cell, they are synchronous with large spikes in the arhabdomeric cell. The arhabdomeric cell was hyperpolarized slightly with extrinsic current to suppress spontaneous firing. (A) Current injected into the *UV* cell through the recording electrode causes an electrotonic voltage change in the arhabdomeric cell. Depolarization causes impulse generation in the arhabdomeric cell. At the end of a hyperpolarizing current pulse, there is a spikelike anodal break response in the *UV* cell and a short burst of impulses in the arhabdomeric cell. (B) A 50 msec, 360 nm flash causes a depolarizing receptor potential in both cells. (C) A long-wavelength ($\lambda > 480$ nm) flash causes a biphasic receptor potential in both cells. (D) A long-wavelength stimulus presented during or shortly after a 360 nm stimulus elicits repolarizing responses in both cells. The repolarizing responses were preceded by brief biphasic receptor potentials. The lowest trace is a light monitor.

only two were electrotonically coupled, even though some of the noncoupled pairs appeared by light microscopy to consist of neighboring cells.

On the other hand, many pairs of *UV* cells were electrotonically coupled (Fig. 12 A, B). The cells of some of these pairs appeared to be in direct contact while others appeared not to be. The small spikes frequently seen in *UV* cells were always synchronous in a coupled pair (Figs. 12 and 13). In one case, it was possible to produce a hyperpolarizing response in one member of a coupled pair by passing hyperpolarizing current pulses of sufficient magnitude into the cell. At the onset of the hyperpolarizing response, the electrotonic voltage change seen in the other member of the pair decreased, and the previously inhibited small spikes reappeared (Fig. 13).

In five cases we found *UV* cells which were electrically coupled to cells producing large action potentials. In such pairs the large spikes were synchronous with small spikes in the *UV* cell (Fig. 14). Unfortunately, we never succeeded in staining both members of such a pair. However, from these experiments and from others involving a single dye-filled electrode, we were able to show that the large action potentials seen in our experiments do not arise from receptor cell axons. They arise from another type of cell, which has been called an "arhabdomeric cell" on anatomical grounds (Jones et al., 1971). These cells occur much less frequently than receptor cells and are much smaller. They have a thin axon and a stout dendrite that branches into many fine processes which terminate in the rhabdomere regions of receptor cells.

DICUSSION

Many physiological properties of the photoreceptors of the median ocellus of *Limulus* are similar to those of the lateral and ventral eyes: for example, the nonlinear current-voltage relation of the membrane (Smith and Baumann, 1969; Smith et al., 1968; Lisman and Brown, 1971), the spikelike anodal break response (Millecchia and Mauro, 1969 *a*; Smith and Baumann, 1969), the large discrete potentials or bumps observed in dark-adapted cell (Fuortes, 1959; Millecchia and Mauro, 1969 *a*; Yeandle, 1958), and a depolarizing receptor potential whose transient component overshoots zero voltage (Benolken, 1961; Fuortes, 1958; Millecchia and Mauro, 1969 *a*).

We have, however, found several ways in which the receptors of the median ocellus differ from other *Limulus* photoreceptors. The steady phase of the depolarizing receptor potential of median ocellus receptors sometimes reaches zero voltage, whereas the steady phase of lateral eye retinular cells (Benolken, 1961) or of ventral photoreceptors (Millecchia et al., 1966) apparently does not. This feature of the steady phase in photoreceptors of the median ocellus is not unique, since the receptor potential of barnacle photoreceptors reaches and even overshoots zero voltage (Brown et al., 1970). However, the two-step steady-phase response we have observed in *UV* cells of the median ocellus has not, to our knowledge, been reported for other *Limulus* photoreceptors. Also,

the repolarizing response found in *UV* cells is novel in photoreceptor physiology.

The biphasic response found in median ocellus receptors has not been described in other *Limulus* photoreceptors, but a similar response has been described in the *Pecten* retina (McReynolds and Gorman, 1970). In *Pecten*, as in *Limulus*, the hyperpolarizing phase does not reverse its polarity when the electrode is withdrawn from the cell, and the slow depolarizing phase is very sensitive to light adaptation. However, in *Pecten* biphasic responses probably are recorded from glial cells (McReynolds and Gorman, 1970), whereas in *Limulus* they are definitely recorded from photoreceptors.

In contrast to our earlier view (Nolte et al., 1968), we now think it likely that the hyperpolarizing phase of the biphasic response is a local ERG, generated by neighboring cells. In a tightly packed array of cells, the light-induced current entering a photoreceptor cell through its active membrane can flow along the extracellular channels or can flow through neighboring cells. If the resistance to the flow of current through the extracellular channels becomes relatively large, more current will tend to flow out across the membranes of neighboring cells apposed to the active region. This outward flow of current will tend to hyperpolarize these neighboring cells. Since the light-activated current during the transient phase of the receptor potential is probably much larger than during the steady phase (in ventral photoreceptors, up to 50 times greater: Millecchia and Mauro, 1969 *b*), a similar effect for the steady phase is unlikely to be observed. The same interpretation has been offered for the *Pecten* eye (McReynolds and Gorman, 1970). This hypothesis is consistent with the findings that the hyperpolarizing phase does not have a reversal potential and does not reverse its sign across the cell membrane. We assume that the reason the hyperpolarizing phase is only seen in response to stimuli from restricted regions of the spectrum is that it can be occluded by the depolarizing receptor potential. That is, in response to UV stimuli, *UV* cells generate a depolarizing receptor potential which, recorded intracellularly, is much larger than the local ERG generated by nearby receptors. Since the intracellularly recorded receptor potential and the local ERG are of opposite polarity, the net response is a depolarization. However, this local ERG generated by nearby *VIS* cells can be recorded in *UV* cells in response to long-wavelength stimuli, to which the *UV* cells are much less sensitive. Our dye electrode studies have shown that *UV* and *VIS* cells occur in close proximity to one another, as would be expected if this interpretation is correct.

The depolarizing phase of the biphasic receptor potential has a different time-course than the depolarizing receptor potential and is more sensitive to light adaptation. It also has a different reversal potential: the depolarizing receptor potential of *Limulus* photoreceptors reverses between +5 and +15 mv (Kikuchi et al., 1962; Millecchia and Mauro, 1969 *b*; Nolte and Brown,

1972; Smith, 1966; Lisman and Brown, 1971), whereas the depolarizing phase of the biphasic receptor potential reverses between −20 and −30 mv. Thus, it seems likely that the two responses are generated, at least in part, by different mechanisms. McReynolds and Gorman (1970) suggested that the slow depolarizing phase of the biphasic response recorded in the *Pecten* retina might be due to a transient extracellular accumulation of potassium ions which were released by neighboring photoreceptors. However, the sign of the change of membrane voltage produced by changing the extracellular concentration of an ion should be independent of membrane voltage (assuming the permeability of the membrane to that ion is independent of both membrane voltage and the extracellular concentration of the ion). Since the depolarizing phase of the biphasic response reverses between −20 and −30 mv, it is unlikely that a transient increase in extracellular potassium ion concentration can be the sole mechanism generating this depolarization.

Earlier anatomical studies (Demoll, 1914; Lankester and Bourne, 1883), as well as our own recent investigation (Jones et al., 1971), have shown that receptor cells in the median ocellus are segregated into groups of various sizes. The present results show that at least some of these groups contain both *UV* and *VIS* cells. They also show that *UV* cells, together with second-order neurons (which we have called arhabdomeric cells) are organized into subgroups having many of the properties of lateral eye ommatidia. The *UV* cells in such a subgroup are electrotonically coupled to each other and to the second-order neuron. Small spikes seen in the *UV* cells are synchronous with each other and with large spikes in the second-order neuron. Hyperpolarizing current pulses sometimes elicit hyperpolarizing responses in *UV* cells like those seen in lateral eye retinular cells, where such responses reflect "uncoupling" of electrically coupled cells. (cf. Smith and Baumann, 1969, and Fig. 13 of this paper). It seems likely that *VIS* cells are usually not coupled to a spike-generating mechanism, and that they are usually not coupled to one another.

We previously reported a third type of receptor cell in the median ocellus, which produced depolarizing receptor potentials in response to stimuli of all wavelengths (Nolte et al., 1968; Nolte and Brown, 1969). We called these receptors "*UV-VIS* cells." These cells are rarely penetrated (much less often than we had previously estimated) and have not been thoroughly investigated. We have noticed that the rare ocelli which contain *UV-VIS* cells usually contain more than one. On one occasion, we recorded simultaneously from two *UV-VIS* cells in the same ocellus, and these cells were electrotonically coupled. We have injected dye into several *UV-VIS* cells; in each case the dye appeared to be localized in a single photoreceptor cell. Also, *UV-VIS* cells usually have small spikes, like *UV* cells. Finally, we have recorded from two cells which produced large spikes and small depolarizing receptor potentials in response to stimuli of all wavelengths. All these data are consistent with the interpreta-

tion that "*UV-VIS* cells" are actually *UV* cells in an ommatidium-like group which contains one or more *VIS* cells.

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