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Hyperpolarizing photoreceptors in the eyes of the giant clam *Tridacna*: physiological evidence for both spiking and nonspiking cell types

Lon A. Wilkens*

Department of Neurobiology, Research School of Biological Science, Australian National University, Canberra, A.C.T. 2601, Australia

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Summary. Intracellular studies on photoreceptors in the eyes of the giant clam *Tridacna* give evidence for two types of light-sensitive cells, both of which are hyperpolarized by light. These cells are distinguished by the presence or absence of spikes and corresponding characteristics of the receptor potential. In non-spiking (NS) receptors, the average resting potential in the dark is low (−15 mV) and peak receptor potentials are large (to 100 mV) and adapt rapidly to light. Spiking (S) receptors have higher average resting potentials (−45 mV), but receptor potentials do not exceed 20 mV and also do not adapt to light. The spikes in S-receptors are small (3–8 mV), occur spontaneously at low levels of illumination and are inhibited by light. Bursts of spikes arise on the repolarizing off-component of the receptor potential. Light adaptation increases the excitability of S-receptors in terms of a higher frequency and shorter latency of the off response burst. The receptor potential in both cells is due to a light-activated increase in membrane conductance to potassium ions. Membrane conductance decreases in NS-receptors in relation to light adaptation. Unlike the scallop eye, no depolarizing photoreceptors are present.

Introduction

Bivalve molluscs respond primarily to shadows with reflex behaviors such as siphon retraction and/or shell closure (Land 1968; Wilkens 1986). The receptors for these protective responses are located peripherally in the mantle (as opposed to the largely vestigial cerebral eyes inside the shell), are innervated by the pallial nervous system, and vary from individual photoreceptors clustered around the siphon apertures to well organized retinal arrays, as in the eyes of scallops. Bivalve photoreceptors are well adapted for the detection of shadows as suggested by the 'off' response of spikes in the optic (pallial) nerves whenever stimulus intensities decrease (Hartline 1938; Kennedy 1960; Barber and Land 1967; Wiederhold et al. 1973; Mpitsos 1973). Light rapidly inhibits this activity as well as spontaneous discharges which occur in the dark. The scallop and file clam also have receptors excited by light that give rise to an 'on' response (Hartline 1938; Mpitsos 1973).

The receptor physiology of both off and on cells has been examined in intracellular studies on the scallop (beginning with McReynolds and Gorman 1970). Ciliary receptors in the distal retina have hyperpolarizing receptor potentials which account for the spike inhibiting effects of light. Axon spikes in these photoreceptors arise in response to membrane repolarization[1] at the end of a light stimulus. Microvillar photoreceptors in the proximal retina of the scallop are similar to other invertebrate photoreceptors which produce axon spikes in response to depolarizing receptor potentials (Järvilehto 1979).

The similarity of shadow response activity in the siphon nerves of *Mercenaria* with that of the off response in the scallop led Wiederhold et al. (1973) to predict that *Mercenaria* photoreceptors are also hyperpolarizing. It is reasonable to suggest that hyperpolarizing photoreceptors are common to all bivalve visual systems in that the predominant or exclusive neural and behavioral responses

Abbreviations: *NS* non-spiking photoreceptors; *S* spiking photoreceptors: *SW* seawater

* Present address: Department of Biology, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121, USA

[1] Since the receptor potential is hyperpolarizing, membrane repolarization, as it will be referred to hereafter, involves a decrease in potential

to light are associated with dimming. In this report, intracellular studies from a third bivalve species, the giant clam *Tridacna*, support this hypothesis. Interestingly, however, there is evidence for two types of hyperpolarizing photoreceptors, one of which generates spikes – the S-receptor – and is comparable to the distal photoreceptors of the scallop. *Tridacna* eyes also contain non-spiking (NS) receptors which are distinguishable on the basis of receptor potential characteristics. A preliminary account of these results has been published, along with a description of the unique (for molluscs) sensitivity of *Tridacna* photoreceptors to ultraviolet light (Wilkens 1984).

Materials and methods

Electrophysiological studies were conducted primarily on the eyes of *Tridacna maxima*, animals collected on the Great Barrier Reef and shipped to Canberra. A few experiments were also performed on the eyes of *T. squamosa* and *T. crocea* with entirely comparable results. Animals were maintained in sunlit marine aquaria and remained in viable condition for several months without feeding. For electrophysiological recording, one or two eyes were extirpated from the margin of the siphon using Shearer rongeurs. Since only small pieces of mantle tissue were removed (3 mm^2), a small animal of only 7–8 cm in shell length could supply eyes for 20–30 experiments. Therefore, all of the data was taken from only 7 clams. Animals appeared to remain robust and within 1 month thin sections of mantle tissue containing the rudiments of new eyes regenerated at each wound site.

Dissection procedures. The eyes were pinned to the Sylgard lining of a small dish containing filtered seawater. Most of the surrounding tissue was dissected away to restrict movements of the muscularized mantle tissue. Otherwise, movements occurred frequently, either spontaneously or in response to shadow stimuli. The retina was then exposed to permit microelectrode penetration by removal of the epithelial surface, causing a slight bulging of the lens from the eye capsule, and subsequent gentle expression of the lens using blunt forceps. Although some retinal damage may have resulted, the isolated lens appeared to be homogeneous suggesting that the retina was largely intact.

Recording and stimulation. Conventional intracellular microelectrode recordings were made from photoreceptor cells in the retina by advancing the pipette tip into the crater previously occupied by the lens cells. Both 3 *M* KCl (30–50 MΩ) and beveled Lucifer Yellow-filled pipettes (80–100 MΩ) were used to record receptor potentials in conjunction with an electrometer and bridge circuit for passing current (WPI Model 701). Unfiltered activity was stored on tape (Hewlett-Packard, 4-channel FM) along with the output from a stimulus monitor and played back to make permanent records (Watanabe chart recorder). Tape playback speeds were slowed to reproduce spikes. Light stimuli from a Xenon arc source and interposed interference and neutral density filters were delivered via a light guide centered above the eye. An electromagnetic shutter was positioned between the filtered light and the fiber-optic light guide. Light and/or shadow stimuli were presented by operation in either the normally-closed or normally-open position under the control of a function generator.

Intracellular staining. For marking cells, negative current pulses of 2–3 nA and 500 ms duration were delivered through Lucifer Yellow CH-filled pipettes at a rate of 1 Hz for 30–60 min. Eyes were then transferred with or without fixation to depression slides and viewed with a compound microscope (Zeiss, Photomicroscope II) using UV illumination and appropriate excitation and barrier filters (Stewart 1978). Stained cells were photographed either in whole mounts of the eye, or in 10 µm paraffin sections of fixed material (10% formalin in seawater). Tissues were dehydrated in an ethanol series and cleared in methyl salicylate. Stained axons were difficult to trace in whole mounts for more than 100–200 µm since the zooxanthellae (photosynthetic endosymbionts) did not clear well. Even in sectioned material, the migration of detectable amounts of dye seemed to be limited to a similar extent.

Ionic substitutions. Artificial seawater (SW) solutions of different ionic composition and a constant pH of 8.1 were made be mixing stocks of isotonic (to SW) salt solutions. Details of the formulae are available elsewhere (Wilkens 1972). For K^+-free SW, an equal volume of 0.54 *M* NaCl was substituted for the 0.54 *M* KCl stock solution. Na^+-free SW was made by substituting Tris-Cl for NaCl. High-K^+ SW solutions (ca. 5× and 10× normal K^+ concentration) were made by mixing K^+-free SW and 0.54 *M* KCl in various proportions: 9 to 1 for 54 m*M* K^+ SW, 4 to 1 for 108 m*M* K^+ SW. For these experiments, eyes were pinned in a narrow chamber (2.5 mm wide) and continuously superfused with seawater. Test solutions were switched by a manifold and flow rates were adjusted by gravity feed to provide a delay of approximately 5 s, as judged from the onset of depolarization in high-potassium solutions.

Results

The eyes of *Tridacna* were probed at various levels and positions in the retina with intracellular recording pipettes. Among the cell types observed were light-sensitive photoreceptors and glial or lens cells with stable −50 to −60 mV membrane potentials. Based on recordings from more than 100 photoreceptors, two types are recognizable, both of which hyperpolarize in response to light. No depolarizing receptor potentials were recorded.

Physiological characteristics of hyperpolarizing photoreceptors

Membrane and receptor potentials. The two photoreceptor types are distinguishable in terms of their membrane and receptor potentials, and these properties are correlated with the presence or absence of spikes. Thus, cells will be referred to as spiking (S) and non-spiking (NS) receptors. An approximately equal number of penetrations was made in each cell type (e.g. 52% in NS-cells), but with no apparent correlation between receptor type and area or depth of penetration in the retina. The photoreceptor properties are summarized in Table 1.

Table 1. Physiological properties of *Tridacna* photoreceptors

	Spiking (S)	Non-spiking (NS)
Membrane Potential		
Dark adapted	-43.8 ± 6.6 mV	-14.4 ± 6.7 mV
Light adapted	-57.8 ± 5.2 mV	-37.8 ± 7.3 mV
Receptor Potential		
Amplitude	-14.0 ± 5.2 mV	> -100 mV
Latency–onset	26.2 ± 1.2 ms	9.3 ± 3.2 ms
–offset	20.7 ± 1.7 ms	25.0 ± 7.3 ms
Rate of change–onset	-0.08 V/s	-0.11 V/s
–offset	0.02 V/s	0.03 V/s
Facilitation	No	Yes
Spikes		
Amplitude	3–8 mV	–
Latency (minimum from offset)	22 ms	–
Adaptation		
Receptor potential	Relatively insensitive	Increases with dark adaptation
Spikes	Increase with light adaptation	

Most values include standard deviations. The maximum rate of change in potential for on and off responses in NS-receptors is from data in Fig. 1

S-receptors have membrane potentials ranging from -34 to -58 mV in the dark. In response to bright light flashes, these cells give negative-going receptor potentials with a range in amplitude of 8 to 16 mV (Fig. 1A, B); but, seemingly, they do not adapt to steady illumination. The 5 mV partial repolarization seen in Fig. 1B occurred after 2 h of continuous recording whereas earlier records from the same cell characteristically were nonadapting (cf. Fig. 2). The insensitivity of the membrane and receptor potentials to light or dark adaptation is evident in Fig. 2 where prior exposure to neither the light or dark has any effect.

In contrast, NS-receptors have low membrane potentials, ranging from -6 to -27 mV in the dark, and hyperpolarizing receptor potentials that adapt rapidly to light (Fig. 1C, D). In cells completely dark adapted, receptor potential amplitudes up to 100 mV have been recorded. The relationship between receptor potential amplitude and duration of dark adaptation is illustrated in Fig. 3. Initially, this cell was fully adapted to a saturating light after which it was interrupted by a series of increasing duration light-off stimuli. After the first 500 ms period in the dark, the receptor potential was only 7 mV; after a 7 s dark interval (7th response), the approximate time after which recovery of the 'resting' membrane potential becomes asymptotic, the receptor potential increased to 41 mV. In another experiment the eye was again adapted to a saturating light stimulus and then presented 100 ms light flashes at intervals following a return to the dark. Results from both experiments are plotted in Fig. 4 and illustrate that dark adaptation in NS-receptors is initially rapid followed by a slower period of recovery.

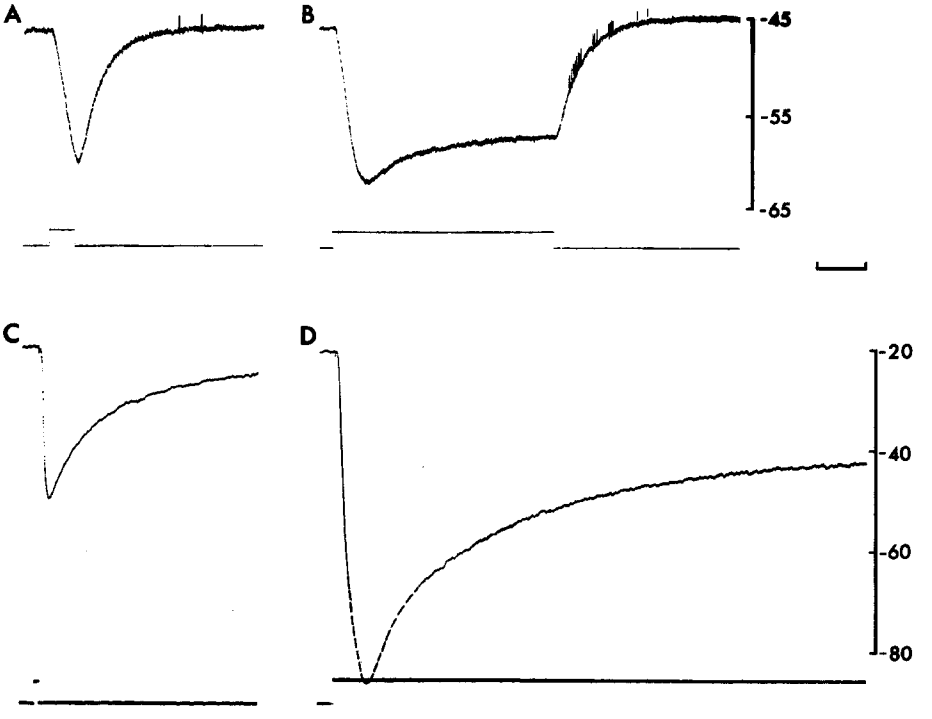

Fig. 1A–D. Comparison of spiking (*S*) and non-spiking (*NS*) receptor potentials in *Tridacna* photoreceptors. The response of S-receptors (**A, B**) and NS-receptors (**C, D**) to brief and prolonged light flashes is illustrated. Trace retouched in **D** (dashed line). S-receptors have membrane potentials of -34 to -58 mV and non-adapting receptor potentials – bursts of spikes are triggered on the repolarizing light-off phase. NS-receptors have membrane potentials of -6 to -27 mV and adapt rapidly to continuous illumination (cf. **B, D**). Light stimuli are monitored by a photocell (lower traces). Onset is indicated by an upward deflection in this and subsequent figures. Membrane potentials (in mV) are indicated by vertical scale bars in all figures. Horizontal bar, 1 s

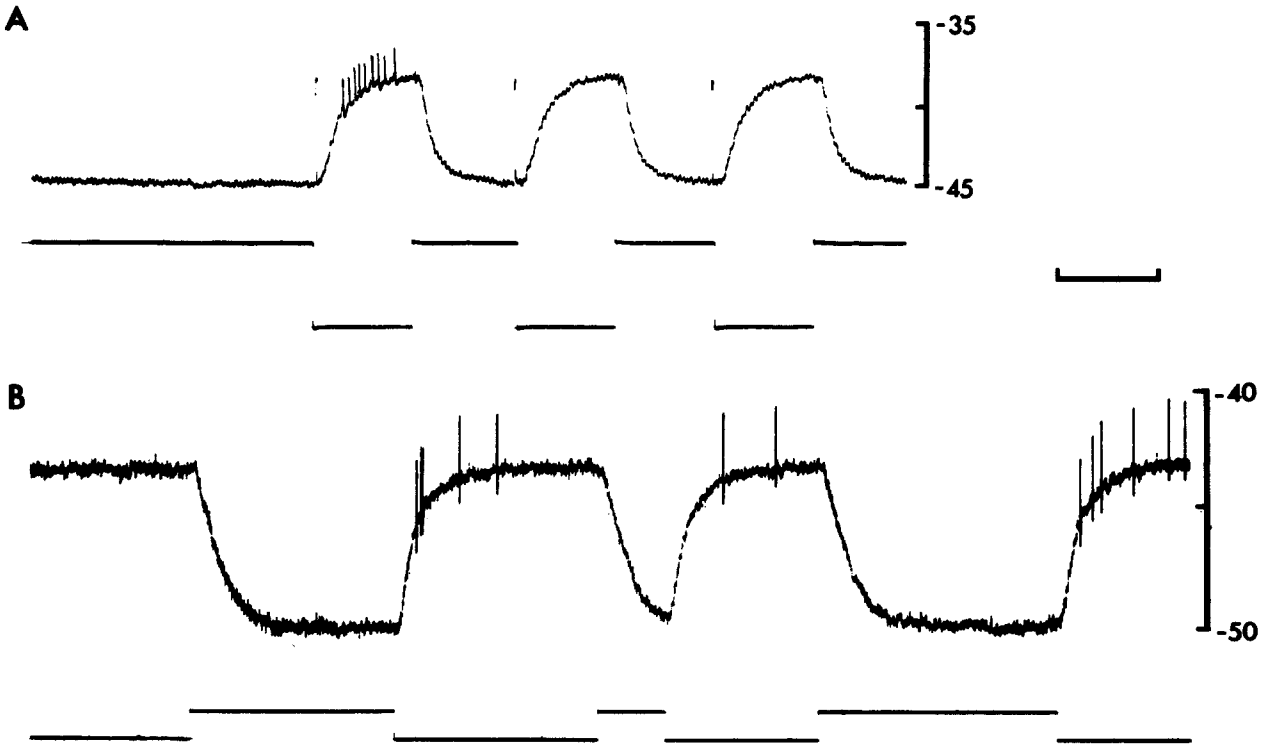

Fig. 2. S-receptor responses to shadow (**A**) and light (**B**) stimuli. Eyes were adapted for several minutes to the light and dark, respectively, at the beginning of each trace. Receptor potential amplitudes are unaffected by light or dark adaptation. Alternatively, spiking increases following light adaptation. Horizontal bar, 30 s (**A**), 5 s (**B**)

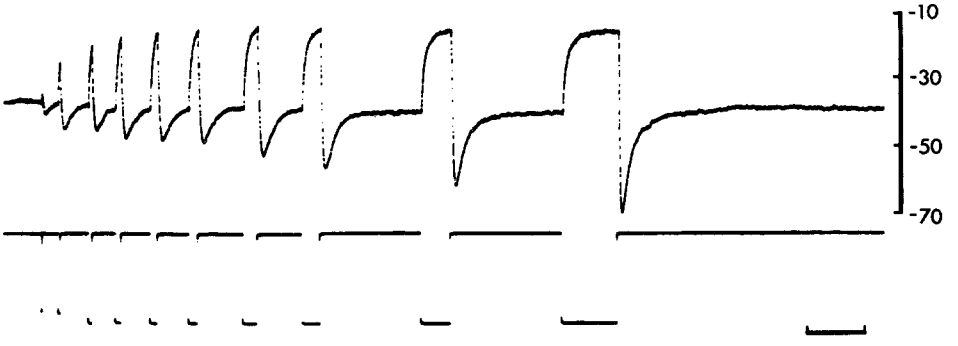

Fig. 3. Response of NS-receptor to shadows of increasing duration (0.5–30 s). The eye was fully light adapted at the onset of the record; repolarization to the resting potential level in the dark occurs after approximately 15 s (9th shadow). Receptor potential amplitudes increase in relation to the duration of the preceding shadow, i.e., with increasing dark adaptation. Horizontal bar, 30 s

The light response in NS-receptors can be facilitated by previous light exposure. This is illustrated in Fig. 5A where trains of constant intensity 100 ms flashes at various frequencies produce a mixture of facilitation and light adaptation. At 0.5 Hz the receptor potential increases in amplitude over the first four stimulus pulses after which the amplitude tapers off due to light adaptation. With shorter intervals, facilitation increases along with an earlier onset of adaptation (cf. trains at 0.5, 0.7, and 1.0 Hz where the response to the second flash is increased by 37%, 74%, and 79%, respectively; the amplitude declines after 10, 4.5, and 4 s, respectively). In another cell (Fig. 5B), light flashes in trains at 1 Hz were varied in duration. With 10 ms flashes, facilitation was gradual, increasing by approximately 6% after 5 s; at 25 and 100 ms, amplitudes were increased by 10 and 6%. No adaptation was apparent in this sequence and the variability in both adaptation and facilitation is evident by comparison of equivalent 1 Hz-100 ms pulse trains in A and B. Repetitive off responses (first record, Fig. 5B) are relatively constant, and under equivalent conditions of duration

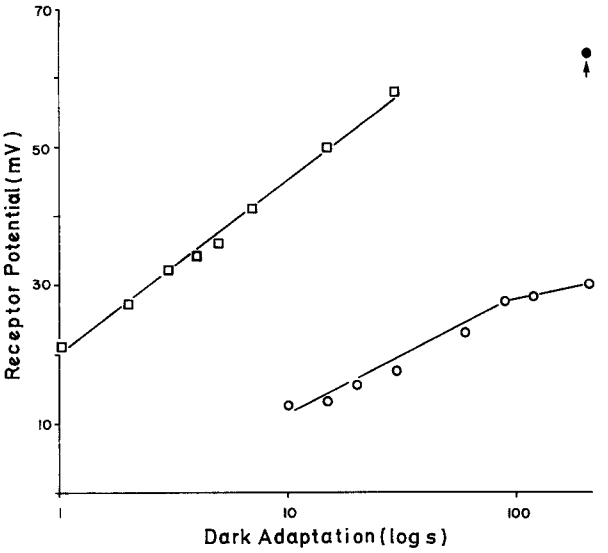

Fig. 4. Relationship between NS-receptor potential amplitude and time of dark adaptation. Peak amplitude increases linearly with log time in the dark for light flashes of long duration (squares), and up to 90 s for dark adaptation of a receptor stimulated with 100 ms light flashes (open circles). Receptor potential amplitude for the cell stimulated by 100 ms light flashes is comparable to the former cell when stimulated by a 2 s light flash (filled circle at arrow)

and frequency, are only one-third the amplitude of on responses (i.e., at 1 Hz and 100 ms, Fig. 5B).

The receptor potentials also differ in terms of latencies and maximum rates of voltage change. In fully dark adapted NS-receptors, the latency to onset is shorter and the rate of change of the hyperpolarizing response is more rapid than in S-receptors (Table 1). The latency and initial rate of change of the response at light offset is similar for both receptors. For complete recovery to the original dark-adapted potential, however, NS-receptors are slower than S-receptors (cf. Fig. 1A, C; 2.7 s and 6.4 s, respectively, following short flashes). After light adaptation, NS-receptors require at least 15 s for recovery to resting levels (Fig. 3, 9th response).

Intensity-response curves of the receptor potential suggest that S- and NS-receptors may have a different sensitivity range as well. V-log I plots for 7 cells, all tested under similar conditions, are presented in Fig. 6. Each of these cells is maximally sensitive to green light (Wilkens 1984). The slope of the S-receptor curve is different from those of NS-receptors and may reflect the unusual non-adapting characteristics of the receptor potential. Unfortunately, comparable data from other S-receptors is not useful here since these test series were conducted at different wavelengths.

Spikes. Only the S-receptors have been observed to produce spikes. Action potentials, as recorded from cell somata, are 3–8 mV in amplitude and occur at a low spontaneous rate (1–3 Hz) in the dark or when the eye is weakly illuminated, or in bursts of up to 60–80 Hz (0.5–2.0 s in duration) at the offset of illumination (Fig. 2). When present,

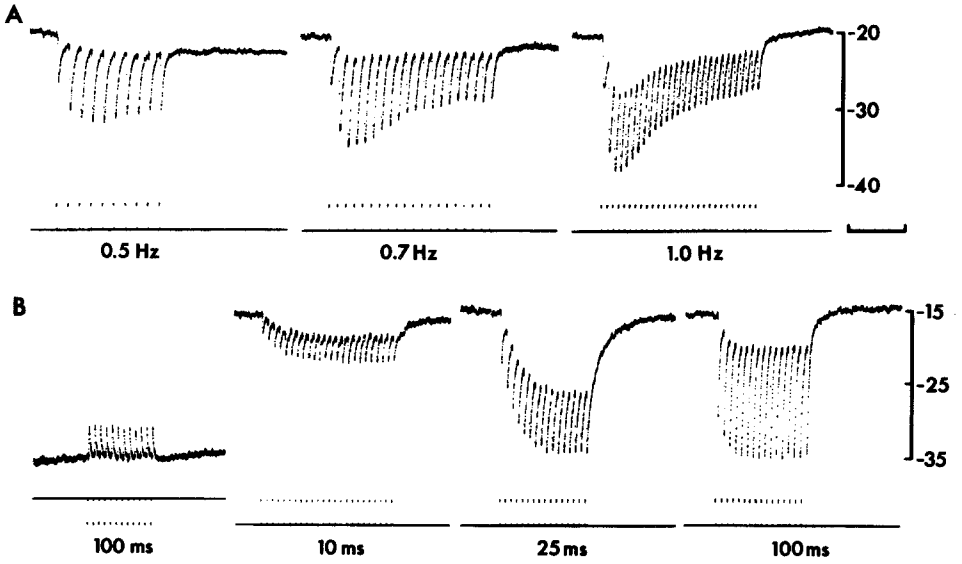

Fig. 5A, B. Facilitation of NS-receptor potentials. **A** Light flashes of 100 ms duration, and at frequencies of 0.5–1.0 Hz, produce facilitated responses following the second and third stimuli. Facilitation is greatest at 1 Hz, but a decrease in amplitude due to light adaptation is also greater. Each sequence follows a 5 min period of dark adaptation. **B** Receptor potentials in another NS-receptor in response to stimulation at 1 Hz and pulse durations of 10 ms, 25 ms, and 100 ms. Depolarizing shadow responses (first record) do not vary over time and are approximately 25% of the amplitude of depolarizing light responses at equivalent stimulus parameters (cf. fourth record). Facilitation and light adaptation are less pronounced in this cell. Horizontal bar, 10 s in all records

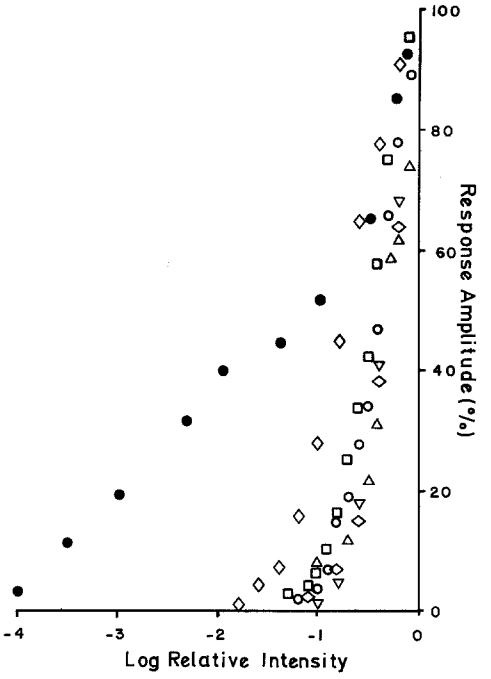

Fig. 6. V-log I plots for 7 photoreceptors; open symbols are for green-sensitive NS-receptors, filled circles represent a green-sensitive S-receptor. Receptor potential amplitudes are plotted as percentage of the response to maximum light intensity (1.2×10^{-6} W). All responses were to 100 ms light flashes at $\lambda = 490$ nm

spikes are rapidly inhibited by any increase in light intensity.

Spikes can also be triggered by passing current through the electrode – spike frequencies increase in proportion to the amount of positive current. The rebound of the membrane potential from negative current injection also triggers short bursts of spikes. Current injection, as with photic stimulation, is incapable of triggering spikes in NS-receptors.

Occasionally, injury discharges were seen following penetration of S-receptors. In cells damaged by penetration, spikes usually disappear in 30–45 s followed by a gradual cell depolarization and attenuation of the receptor potential. Nevertheless, these spikes were always associated with initial resting membrane potentials of approximately -40 to -50 mV and a non-adapting receptor potential. When S-receptors were penetrated without injury, spikes and membrane and receptor potentials were frequently recorded without attenuation for 30–60 min. Injured NS-receptors were characterized by an upward drift of the membrane and receptor potentials.

Unlike the receptor potential, the spikes of S-receptors are affected by the relative state of dark or light adaptation. As seen in Fig. 2A, a vigorous

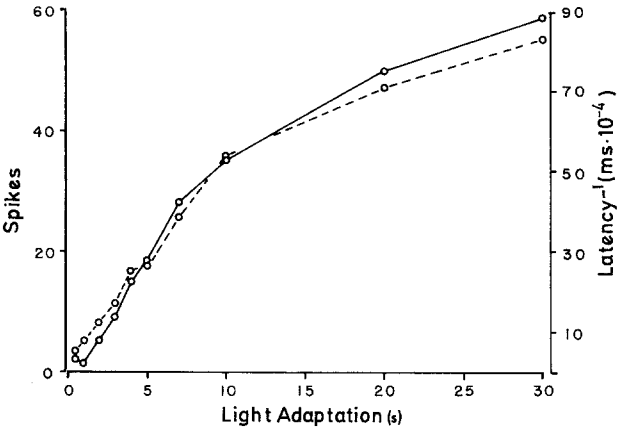

Fig. 7. S-receptor spikes (solid line) arising during the first 5 s of a shadow response and plotted in relation to the preceding period of light adaptation. Latency from light offset to the first spike (broken line) decreases with light adaptation and in proportion to the increase in number of spikes. Data from cell represented in Fig. 1 (A, B)

burst of spikes is triggered by the first shadow whereas a similar receptor potential response to the second shadow is not accompanied by spikes. In this instance, the photoreceptor has been partially light adaptated by the preceding 30 s shadow. In a similar test sequence from another cell, 500 ms shadows triggered 0, 4.4 ± 0.4, and 9.0 ± 1.0 spikes (SE; $n = 6$) in response to intervals of 0.5, 2, and 5 s of light adaptation. To further quantify the effect of light adaptation, eyes were exposed to a saturating light for various periods of time, in each case following 5 min of dark adaptation. Spikes were then counted over a 5 s interval following a return to the dark. These results are illustrated in Fig. 7 along with the latency to the first spike. Spike output rises rapidly with increasing light adaptation up to 10 s and this is accompanied by a corresponding decrease in the latency. The maximum rate of rise of the receptor potential remained constant.

Ionic conductances. The receptor potentials in both cell types are due to increases in membrane conductance. This is illustrated in Fig. 8 where constant current injections through the recording micropipette before, during, and after light stimulation produce potential drops that decrease in amplitude in association with the hyperpolarizing responses. At the current levels used here, conductance changes probably include both light- and voltage-dependent ionic currents. The increase in relative membrane conductance in S-receptors varies between 10% and 150% and, like the receptor potential itself, remains nearly constant for the duration of the stimulus (Fig. 8A). For NS-receptors,

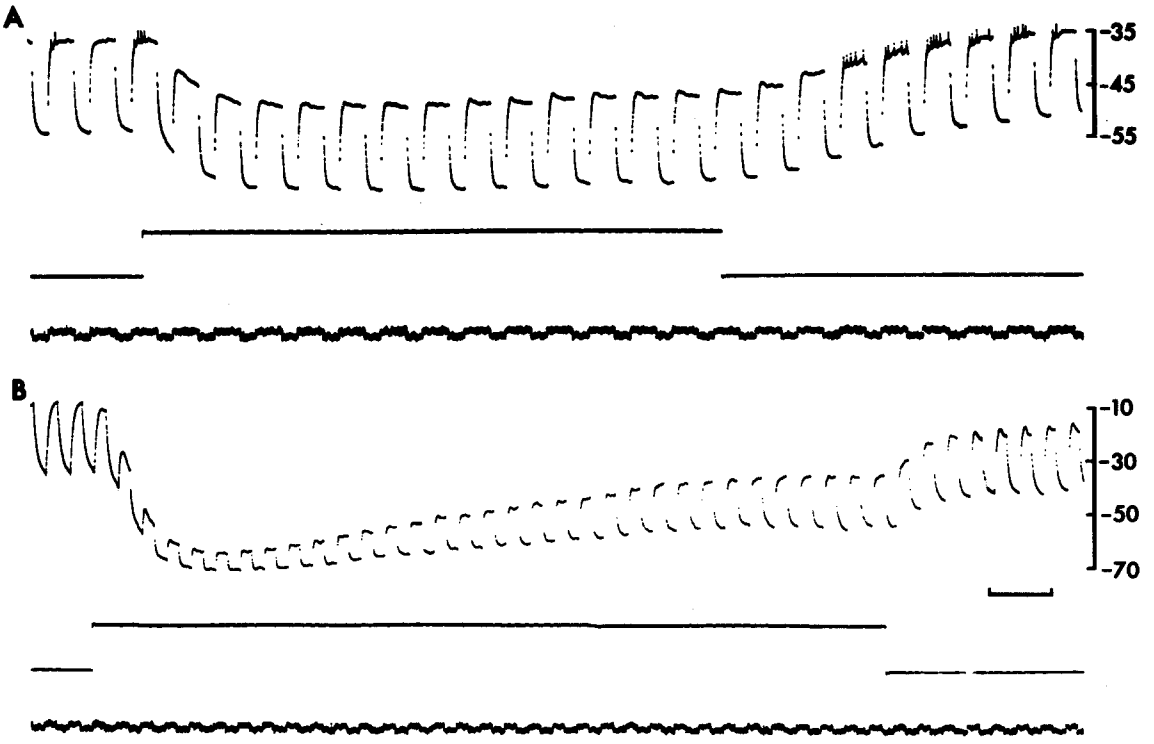

Fig. 8. Conductance increases underlie receptor potentials in both S-receptors (**A**) and NS-receptors (**B**). S-receptor spikes triggered on the rebound repolarization are also inhibited by light (**A**). Negative current pulses (0.5 nA; 250 ms) injected through the recording pipette are monitored in the lower traces of each record. Horizontal bar, 1 s (**A**), 2.5 s (**B**)

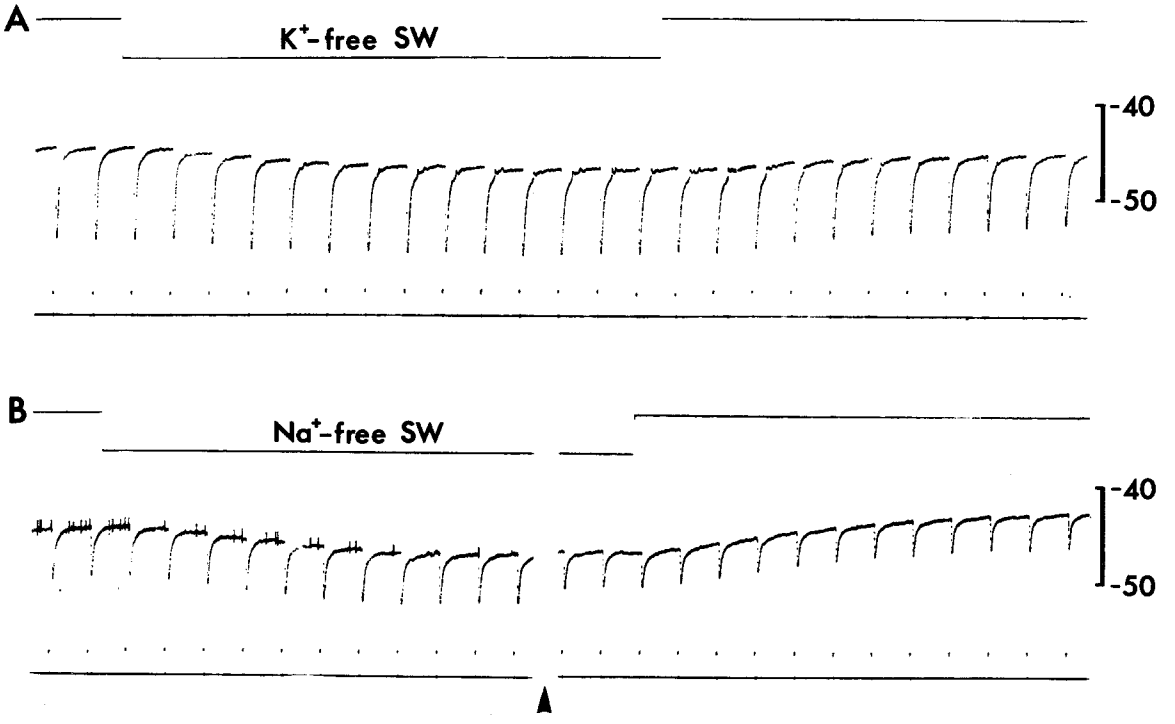

Fig. 9A, B. Effects of ion substitutions on S-receptors. Removal of both potassium (**A**) and sodium (**B**) increases the membrane potential, the effect being greater for sodium, but does not directly alter receptor potentials (elicited by light flashes at 5 s intervals). **B** Spikes are blocked in the absence of sodium ions after 40 s; return of spiking occurs following end of record. Time scale: 5 s stimulus marks. Arrow indicates 85 s break in the record

Table 2. Effects of ion substitutions on mean membrane and receptor potential values for *Tridacna* photoreceptors

	Change in membrane potential	Change in receptor potential	Spikes
S-Receptors			
K^+-free SW ($N=7$)	−1.6 mV	NC	NC
Na^+-free SW ($N=3$)	−10.0 mV	NC	Blocked
54 mM K^+ SW ($N=2$)	+12.0 mV	Abolished	
108 mM K^+ SW ($N=1$)	+32.0 mV	Abolished	
NS-Receptors			
K^+-free SW ($N=5$)	−3.3 mV	NC	
Na^+-free SW ($N=2$)	−17.0 mV	−6.2 mV	
54 mM K^+ SW ($N=1$)	+29.0 mV	Abolished	

A negative sign indicates hyperpolarization of the membrane and a decrease in the receptor potential. A positive sign indicates depolarization of the membrane. (NC no change)

increases in relative conductance of between 100% and 600% accompany peak hyperpolarization (e.g., Fig. 8B). A subsequent rapid decline in conductance coincides with light adaptation of the receptor potential and reaches a steady-state value only 25% greater than that in the dark.

Ion substitution experiments were conducted to determine the ionic currents associated with the physiological responses of *Tridacna* photoreceptors. In these experiments, brief light flashes of 100–200 ms were presented at 5 s intervals throughout sequences of control, test, and control solutions superfused over the eyes. Muscle contractions in the tissues surrounding the eyes associated with the changes in ionic composition made it especially difficult to maintain stable recordings in these experiments. The results of these experiments are summarized in Table 2 and illustrated in Figs. 9 and 10.

The removal of potassium ions increases the membrane potential in both receptors, but does not affect the amplitude of the receptor potential in either type of cell. This is illustrated for a representative S-receptor in Fig. 9A. The substitution for sodium ions in the bathing medium increased the membrane potential to a greater extent in both cells, with the increase in potential being greatest for NS-receptors with lower initial potentials. Na^+-free conditions had little effect on receptor potential amplitude in either cell – a 6.2 mV decrease in NS-receptor amplitude (Table 2) is attributable largely to hyperpolarization of the underlying membrane potential. The effect of sodium removal on an S-receptor is illustrated in Fig. 9B. High-potassium solutions rapidly depolarize both types of receptor and reduce or abolish receptor potentials and spikes (in S-receptors). In one instance, small 3 mV S-receptor potentials, blocked initially in 54 mM K^+ SW, reappeared after several minutes while in this test solution.

Ion substitution effects on S-receptors were also noted with respect to spiking. K^+-free SW apparently had no effect on spike generation as evidenced from the fact that spikes could be triggered by positive current injections in potassium-free solutions. On the other hand, spikes were reversibly blocked by sodium-free solutions (Fig. 9B), even when positive current was injected (not shown). The return of spiking in control situations was generally much slower than the recovery of the membrane potential.

These photoreceptors appear to be sensitive to ionic changes with respect to complete reversibility. For example, receptor potential amplitude often continued to decline following a 2–3 min exposure to the test solution, even after recovery of the membrane potential to control levels in normal

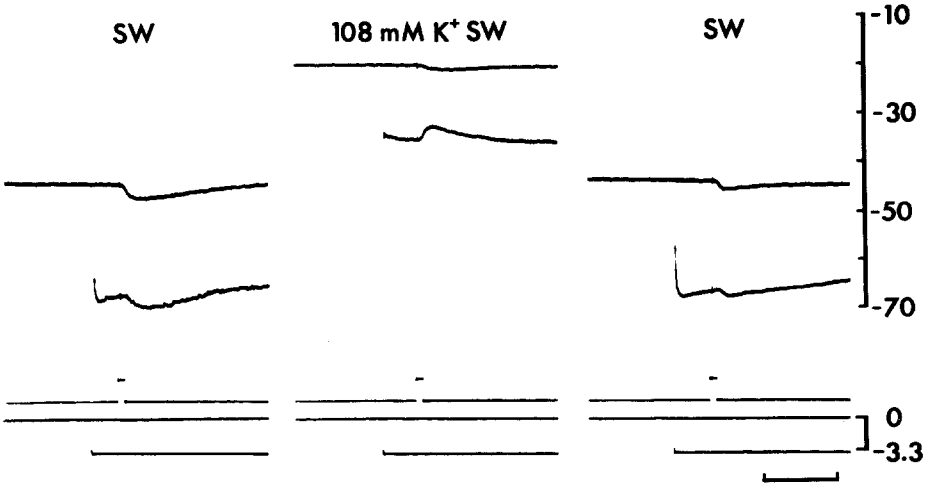

Fig. 10. Reversal of receptor potential in high potassium. Hyperpolarizing current injections were not sufficient to reverse the polarity of the receptor potential in normal seawater (SW). Superimposed traces show receptor potential at resting and hyperpolarized levels; current (in nA) is monitored in lower traces. In high-potassium SW, the receptor potential is reduced in amplitude (top trace), and reversed with the addition of hyperpolarizing current (second trace). Polarity returns to normal in control SW solution. Light monitor, third trace; horizontal bar, 4 ms

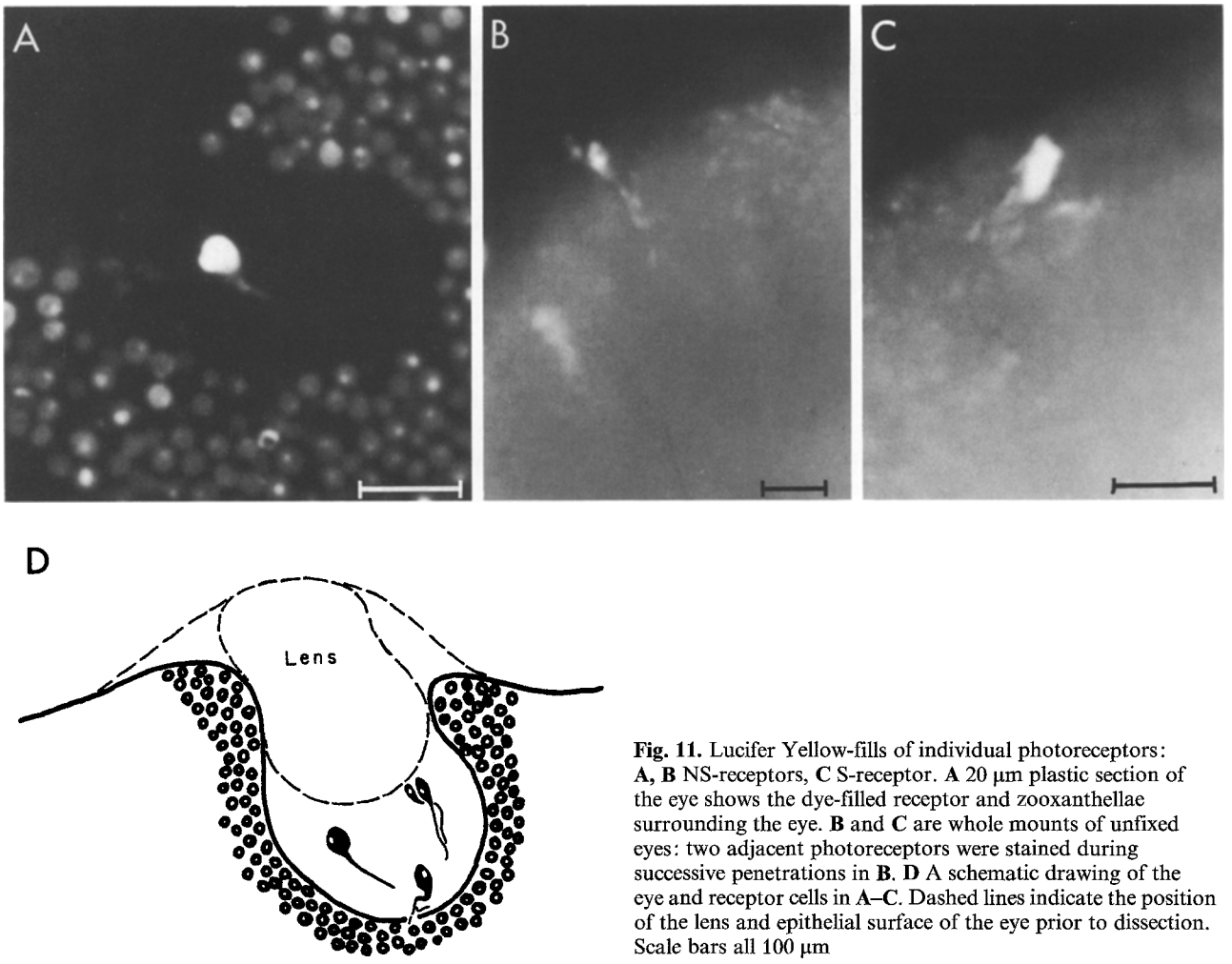

Fig. 11. Lucifer Yellow-fills of individual photoreceptors: **A, B** NS-receptors, **C** S-receptor. **A** 20 μm plastic section of the eye shows the dye-filled receptor and zooxanthellae surrounding the eye. **B** and **C** are whole mounts of unfixed eyes: two adjacent photoreceptors were stained during successive penetrations in **B**. **D** A schematic drawing of the eye and receptor cells in **A–C**. Dashed lines indicate the position of the lens and epithelial surface of the eye prior to dissection. Scale bars all 100 μm

seawater. This is illustrated in Fig. 9 where the receptor potential amplitude decreases following the removal of either sodium or potassium ions.

The ionic currents in *Tridacna* photoreceptors were further examined in relation to the reversal potential of the light response. Even though the electrometer could not be balanced when using currents sufficient to reverse the receptor potential, evidence for an increase in potassium conductance was obtained by current injection in the presence of high-potassium concentrations in the bathing medium, as illustrated in Fig. 10 for an S-receptor cell. In this experiment, the receptor potential is still negative-going when elicited during hyperpolarization with a current of −3.3 nA (left sequence of traces). With equivalent light and current stimulus intensities, but in the presence of 108 m*M* K^+ SW, the receptor potential becomes depolarizing (middle sequence, Fig. 10). A control sequence in seawater again shows a hyperpolarizing receptor potential at both normal and hyperpolarized levels of membrane potential.

Photoreceptor morphology

Intracellular staining with Lucifer Yellow has been used to examine the gross morphology of *Tridacna* photoreceptors. Two NS-receptors (A, B) and one S-receptor (C) are illustrated in Fig. 11. Although no absolute morphological distinction between receptor types has emerged from these experiments, several features of the receptors are apparent. All are monopolar cells with a nucleus evident in the original color transparencies. The NS-receptors have axons which taper rapidly as they emerge from the cell soma and extend downward toward the base of the eye. Due to the limitations of the intracellular staining technique in these eyes, it has not been possible to determine whether the axons of NS-cells extend out of the eye or eventually branch. The lone S-receptor successfully stained also gives rise to an axon. After a short distance it appears as if several short branches may have arisen but this assertion needs further supporting evidence.

Discussion

The unique adaptations of giant clams, other than their large size, include hypertrophy of the siphon (Stasek 1962) in conjunction with endosymbiotic zooxanthellae (Yonge 1980; Trench et al. 1981), behavioral adaptations corresponding with the siphon complex (Stasek 1965; Wilkens 1986), and sensitivity to ultraviolet light (Wilkens 1984). In this report, the electrophysiological properties of the visual receptor cells are also shown to be unique.

Two receptor cell types

Two features of the retinal constituency in *Tridacna* contrast with the photoreceptors present in other bivalve eyes – the apparent absence of depolarizing cells, which comprise one of the two retinal layers in the scallop, and the presence of hyperpolarizing photoreceptors which do not generate spikes. The absence of depolarizing receptors seems clear since a depolarizing receptor potential was never observed in recordings from well over 100 light-sensitive cells. The dichotomy of hyperpolarizing photoreceptor cell types also seems clear due to the variety of non-overlapping physiological characteristics of each cell, e.g., differences in resting membrane potentials, relative light and adaptation sensitivity, response latency, and the presence of spikes (Table 1). Both S- and NS-receptors have a monopolar soma, the presumed site of phototransduction. However, due to the limitations of the dye injection studies, we unfortunately cannot correlate the length and/or branching characteristics of either cell with its physiological properties.

Alternate interpretations might be that S-receptors are interneurons instead of primary photoreceptors and receive synaptic input from NS-receptors, or that NS-receptors may be some type of retinal glial cell electrically-coupled to the photoreceptor. The former is analogous to the vertebrate retina where all photoreceptors are hyperpolarizing and postsynaptic cells respond to a decrease in transmitter output during light stimulation. That NS-receptors are presynaptic to the spiking cells is theoretically possible since the onset latency of the receptor potential is shorter. At offset, however, the S-receptor latency is shorter. The wave form of the two receptors in *Tridacna* during all but short light flashes is also hard to reconcile according to a synaptic mechanism in that the sustained hyperpolarizations of S-receptors do not reflect the adaptation characteristics of NS-receptors. A glial role for NS-cells is also untenable due to their shorter response latency and low resting (dark) membrane potential. The emergence of an axon from the cell body and the fact that dye coupling was never observed also argue against a glial hypothesis.

A final consideration is whether the appearance of two cell types results from injury during penetration or recordings from different regions of the same cell. Injury during penetration was always detectable, resulting in attenuated receptor potentials and an upward drifting of the membrane potential in both types of cells. A loss of spikes in S-receptors was generally accompanied by a decrease in receptor potential amplitude although in a few experiments spikes disappeared after several minutes with no other changes. Unlike the scallop, however, where spikes were recorded infrequently or always disappeared in a short period of time (McReynolds and Gorman 1970), spikes in *Tridacna* were invariably associated with the receptor potential characteristics of S-cells and their persistence suggests that the impulse generating region is in the axonal region and less easily damaged. Even with the loss of spikes the receptor potentials of the two cells remain distinct. Finally, the simple monopolar structure of the photoreceptors does not favor the idea that response characteristics will be greatly dissimilar from different recording sites in the cell body.

Structure and function of Tridacna photoreceptors

Further evidence in support of two physiologically distinct photoreceptors is available from anatomical studies. In light micrographs from plastic-embedded tissue, clusters of cells characterized by greater cytoplasmic density can be seen at the outer margin of the retina (unpublished results). Kawaguti and Mabuchi (1969) present ultrastructural evidence for two photoreceptor cells despite difficulties with tissue fixation, i.e., A-cells with numerous basal cilia protruding into a distal region of microvilli and B-cells with fewer basal cilia and which form rhabdomeric matrices of microvilli. The smaller B-cells are found scattered along the outer edge of the retina. This structure and arrangement of cells has been compared with the scallop eye which has distal ciliary and proximal rhabdomeric photoreceptors organized in distinct layers (Miller 1958; Barber et al. 1967).

Due to functional similarities between *Tridacna* S-receptors and the scallop ciliary (distal) receptors, i.e., both are off receptors, it is reasonable to expect that S-receptors in *Tridacna* correspond to the ciliary A-cells described by Kawaguti and

Mabuchi (1969). Another bivalve photoreceptor with a ciliary structure, and which responds physiologically as an off receptor, is found in the cockle *Cardium* (Barber and Land 1967; Barber and Wright 1969). One might also expect that *Cardium* photoreceptors, as well as other bivalve off receptors (e.g., *Mactra*, Kennedy 1958; *Spisula*, Kennedy 1960; *Mercenaria*, Wiederhold et al. 1973) will have hyperpolarizing receptor potentials, although no intracellular studies have been performed on these or other bivalve species.

On the contrary, if NS-receptors in *Tridacna* correspond to the B-cells described by Kawaguti and Mabuchi (1969), there would be no correlation between structure and function of rhabdomeric photoreceptor types. The proximal cells in the scallop have depolarizing receptor potentials and produce on spikes (McReynolds and Gorman 1970) whereas NS-receptors have quite different physiological properties.

Membrane properties of hyperpolarizing receptor potentials

The receptor potentials in both types of photoreceptors in *Tridacna* are due to an increase in membrane conductance, as evidenced by sharp decreases in the membrane potential response to injected current during light stimulation. These light-dependent conductances are different in the two cells inasmuch as the conductance increase peaks early in the response of NS-receptors and subsequently decreases in concert with light adaptation, whereas the conductance increase remains constant along with the hyperpolarizing light response in S-receptors. The relative increase in membrane conductance is also higher in NS-receptors. Ionic substitution experiments, which are of a preliminary nature in this report, suggest that outward potassium currents are a major component of the receptor potential. For example, the receptor potential is decreased in the presence of elevated potassium concentrations and similar treatments are instrumental in reversing the polarity of the receptor potential (in S-receptors). Sodium appears not to contribute significantly to the receptor potential in either cell, although removal of sodium from the bathing medium increases the membrane potential, presumably due to a reduction in sodium leakage current. Action potentials, however, are sodium-dependent.

Although the hyperpolarizing (distal) photoreceptor of the scallop is also dependent on a light-activated potassium conductance (Gorman and McReynolds 1978; Cornwall and Gorman 1983), several aspects of receptor physiology in *Tridacna* photoreceptors are different. For example, the scallop distal photoreceptor exhibits a peak hyperpolarization to a prolonged light flash followed by a partial repolarization, a response which corresponds to a peak and subsequent plateau in the outward potassium current (Cornwall and Gorman 1983). The response to the onset of light is therefore similar to receptor potentials in NS-cells, although spikes (as in scallop distal receptors) have not been observed in these cells. The off response in the scallop also contains a voltage-dependent spike or overshoot (Cornwall and Gorman 1979) which is seen in neither of the *Tridacna* photoreceptors. In both S- and NS-cells, the off response appears more as a slow decay of the underlying light-activated current. Despite these differences, the small axonal spikes in both *Tridacna* S-receptors and scallop distal photoreceptors appear to share similar properties. With equivalent delays after the onset of repolarization, both cells give rise to spikes which are sensitive to the removal of sodium from the bathing medium (this report, and Cornwall and Gorman 1979), appear to be the result of currents spreading into the soma from spike generation regions in the axon, and increased spike output following light adaptation.

Evolution of hyperpolarizing photoreceptors in molluscs

In all bivalve species tested, off responses of spikes can be recorded in the pallial nerves supplying the mantle and/or siphon. These fibers originate in functional eyes or from undifferentiated light-sensitive regions of the nerves or nerve endings in the siphon. In several instances, off responses have been shown to correspond with receptors which are hyperpolarized by light, i.e., *Tridacna*, the scallop, and the file clam *Lima* which is closely related to the scallop and has apparently equivalent photoreceptor properties (Mpitsos 1973). Interestingly, however, *Tridacna* appears to have evolved somewhat different physiological characteristics than those previously described and furthermore appears to lack depolarizing on receptors. It is difficult to determine at this point whether the properties of *Tridacna* photoreceptors exhibit properties which reflect their early association with infaunal ancestors in the superfamily Cardiacea (Yonge 1980), relative to those in the more advanced eyes of the Pectinacea (e.g., scallops), or whether *Tridacna* photoreceptors exhibit specializations linked to the other unique adaptations found in these obligate, epifaunal coral reef inhabitants. Intracellu-

lar studies on additional bivalve species will be needed to resolve this question.

The existence of a non-spiking photoreceptor in *Tridacna* also raises interesting questions in regard to the functional organization of siphonal eyes. Whereas S-receptors appear to send axons to the visceral ganglion (I have recorded off spikes extracellularly in the pallial nerves, unpublished results) and are candidates for mediating the shadow and/or sight reactions in *Tridacna* (Wilkens 1986), the function of NS-receptors is unknown. Non-spiking vertebrate photoreceptors rely on synaptic contacts made with cells in adjacent layers of the retina. From available ultrastructural evidence, however, no synaptic connections have been seen in *Tridacna* eyes (Kawaguti and Mabuchi 1969; Fankboner 1981). Non-spiking photoreceptors are also found in the barnacle where specialized cable properties of the axons are such that signals can be conducted decrementally to the supraoesophageal ganglion over distances of 1–2 cm (Shaw 1972; Oland et al. 1983). In *Tridacna*, the distances between the siphon margin and visceral ganglion would seemingly preclude the possibility of effective decremental conduction. NS-receptors must therefore synapse peripherally within some type of local circuit in the mantle. Shadow-induced movements in isolated pieces of the mantle (Wilkens 1986) are evidence for some form of local circuit and may rely on NS-receptor activity. This is also a departure from the pattern in scallops where, although passing through a peripheral ring ganglion, photoreceptor axons do not synapse until reaching the parietovisceral ganglion (Spagnolia and Wilkens 1983).

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