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PASSIVE SIGNAL PROPAGATION AND MEMBRANE PROPERTIES IN MEDIAN PHOTORECEPTORS OF THE GIANT BARNACLE

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SUMMARY

1. The light-induced electrical responses of barnacle photoreceptors spread decrementally along the cells' axons. The decay of the depolarizing and hyperpolarizing components of the visual signal was studied by recording intracellularly from single receptor axons of the median ocellus of the giant barnacle.

2. The resistance of the photoreceptor neurone decreases markedly when the cell is depolarized with respect to its dark resting potential of −60 mV. This rectification results in differential attenuation of the depolarizing and hyperpolarizing components of the visual signal as they spread down the axon. Consequently, the visual signal entering the synaptic region is conspicuously distorted.

3. Bathing the photoreceptor axons in sodium-free or calcium-free saline or in isotonic sucrose does not significantly affect the spread of the visual signal to the terminals. Thus the signal is not amplified by an ionic mechanism along the axon.

4. Membrane characteristics of the photoreceptor for hyperpolarizing voltage changes were estimated from (*a*) the ratio of the amplitudes of the visual signals recorded simultaneously in the axon and in the soma, (*b*) the time constant, and (*c*) the input resistance of the cell. All three independent measurements are consistent with a length constant 1 to 2 times the total length of the cell (λ = 10–18 mm) and an unusually high membrane

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resistivity of about 300 kΩ cm². This resistivity enables the receptor potential to spread passively to the terminal region.

5. Electron microscopic examination of receptor axons reveals an investment of glial lamellae, but demonstrates neither unusual structures which would lead to a high apparent membrane resistivity, nor junctions between cells which would seal off the extracellular space. Thus the observed high resistivity appears to be an intrinsic property of the receptor membrane.

INTRODUCTION

Primary receptors transduce sensory stimuli into electrical potential changes which represent the intensity of the stimuli. These receptor potentials are conveyed to the synaptic regions of the receptor either by eliciting a train of action potentials or simply by decremental conduction. Various primary and secondary sensory cells within the nervous system of both vertebrates and invertebrates transmit receptor potentials decrementally over surprisingly long distances (Gwilliam, 1963; Ripley, Bush & Roberts, 1968; Werblin & Dowling, 1969; Shaw, 1972; Millecchia & Gwilliam, 1972; Zettler & Järvilehto, 1973). In the barnacle lateral photoreceptors, for example, a potential change of a fraction of a millivolt recorded in the cell soma can effect synaptic transmission to post-synaptic cells, yet this signal must spread along an axon 10 mm in length (Shaw, 1972).

It has been suggested that the decremental propagation of light-evoked changes in some vertebrate (Werblin, 1975) and invertebrate (Zettler & Järvilehto, 1973) receptors is supported by a regenerative process. But it is possible that the resistivity of certain axonal membranes is high enough to allow signals to spread passively for long distances with little attenuation; cell membranes with unusually high resistivity have been described previously (Waltman, 1966; Bennett & Trinkaus, 1970; Gorman & Mirolli, 1972).

The present experiments examine how the visual signal is propagated from the photoreceptive element to the synaptic terminals in a decrementally conducting photoreceptor neurone. A careful study of the decay of a receptor potential has already been carried out by Shaw (1972) using the lateral photoreceptors of the barnacle, *Balanus eburneus*; however, his analysis was limited by the electrical coupling of the *B. eburneus* receptors and the difficulty in obtaining recordings from the obscured terminals. We have approached the problem of decremental conduction using the photoreceptor of the median ocellus of the giant barnacle, *B. nubilus*. The receptors of this ocellus are visible along their whole lengths to within a short distance from their final synaptic arborizations, and are not electrically coupled to one another (Hudspeth & Stuart, 1977). These features

permit us to record simultaneously from the soma and axon of a single receptor, and thereby to analyse the transmission of the visual signal along the cell. We conclude that the membrane of the barnacle photoreceptor has a high intrinsic resistivity which enables the receptor potential to spread passively with little attenuation along the axon to the synaptic terminals, but that membrane rectification properties conspicuously distort the visual signals during their passage.

METHODS

Physiology

Experiments were performed on photoreceptors of the giant barnacle *B. nubilus* in a preparation consisting of the median ocellus, the ocellar nerve containing the receptor axons, and the supraoesophageal ganglion in which they terminate. Techniques of dissecting, recording from, and stimulating this preparation have been described in detail in the previous paper (Hudspeth & Stuart, 1977). Photic stimuli were delivered from a white light source and had a maximal irradiance of 1·3 mW/cm^2. Experiments were done at 20–22 °C.

The four median photoreceptors of the giant barnacle are large, long cells, visible under the dissecting microscope for almost their entire length of 5–15 mm. Their somata are 40–70 μm in diameter and are encapsulated together in a peripheral ocellus. The four cylindrical axons travel together in a compartment of the median ocellar nerve to terminate in the supraoesophageal ganglion; each is visibly distinct and maintains a diameter of 10–40 μm, depending on nerve length, until it enters the commissure of the ganglion. Each axon then bifurcates and extends into each hemiganglion one or more processes which travel about 100 μm before arborizing in a restricted spray of presynaptic branches. Thus only about 1 % of the entire length of these cells is devoted to the soma at one end and the synaptic arborization at the other.

To record from receptor axons, it was important that the ocellar nerve be unfolded, extended to its full length, and held securely. Straps made of pieces of nerves from elsewhere in the animals were stretched across the ocellar nerve at intervals along its length and pinned with a tension that did not crush the nerve but slightly flattened the axons. Most experiments involved placing two electrodes in a single axon. However, it was not always possible to follow one cell visually along the entire nerve, for the axons twist and entwine. Accordingly the procedure usually adopted was to place one electrode in the receptor soma, and then to impale successively the four receptor axons in the ocellar nerve with the second electrode until current pulses passed through one electrode elicited potential changes recorded by the other. This criterion sufficed to indicate when both electrodes were located in the same axon since the photoreceptors of the median ocellus are not electrically coupled (Hudspeth & Stuart, 1977).

For experiments in which test solutions were exchanged around the axon but not around the soma or terminal region of a receptor, the preparation was pinned out in a Plexiglas chamber with three compartments and a resin (Sylgard 184, Dow Corning) floor. The walls separating the central compartment from the end compartments were each 1 mm thick. A centred trough in each of the two walls allowed the ocellar nerve to pass through all three compartments. About 1·5 mm of each end of the receptor extended into the first and third compartments, the next 1 mm of each end was surrounded by petroleum jelly as the nerve passed through the walls,

and the remaining 5–10 mm (depending on the nerve length) were contained within the central compartment. The three compartments were effectively isolated from one another by the petroleum jelly barriers and separate perfusion systems; the soma and terminal compartments were grounded.

For solutions of varying potassium concentration, potassium was substituted for sodium; in sodium-free saline, sucrose was substituted for sodium until the osmolarity of normal barnacle saline was reached. Magnesium replaced calcium in calcium-free saline. Isosmotic sucrose solutions contained no ions. All solutions were isosmotic with normal saline (960 m-osmole/kg) which was prepared by the recipe of Brown, Hagiwara, Koike & Meech (1970).

Anatomy

Preparations were fixed and prepared for examination in the light and electron microscopes as described in the previous paper (Hudspeth & Stuart, 1977). For the estimation of receptor axon diameter from length of ocellar nerve, preparations were removed from ten animals, the extended lengths of the ocellar nerves measured, and the preparations fixed. The length of the cell was taken as the distance from the centre of the ocellus to the entry of the ocellar nerve into the commissure. After embedding and sectioning, the receptor axon diameters were measured and plotted against the lengths of ocellar nerves. The relationship between nerve length and axon diameter was fitted by the power function $d = 5{\cdot}5\ L^{0{\cdot}64}$ (d in μm, L in mm) for the range of dimensions found in experimental animals, and was used to estimate the diameters employed in Table 1. Diameters were corrected for an assumed 10% shrinkage during fixation.

RESULTS

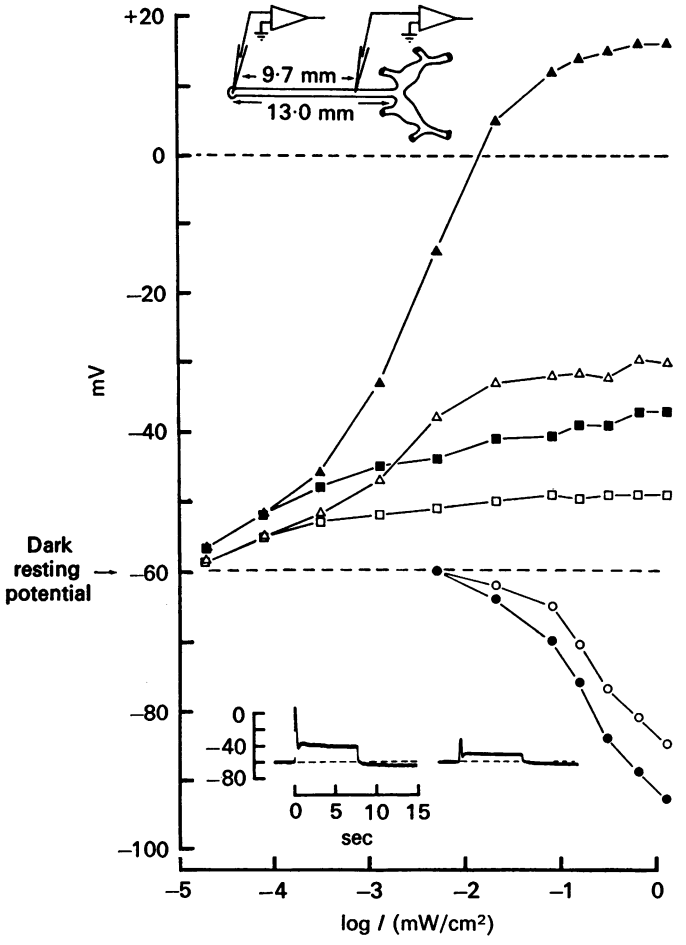
The decay of the light response along the receptor cell

In order to measure the degree of attenuation of the receptor potential as it spreads along the axon, two micro-electrodes were placed in a receptor simultaneously (see Methods). One electrode recorded from the soma or axon-hillock, and the other recorded from the axon at a point 5–15 mm away.

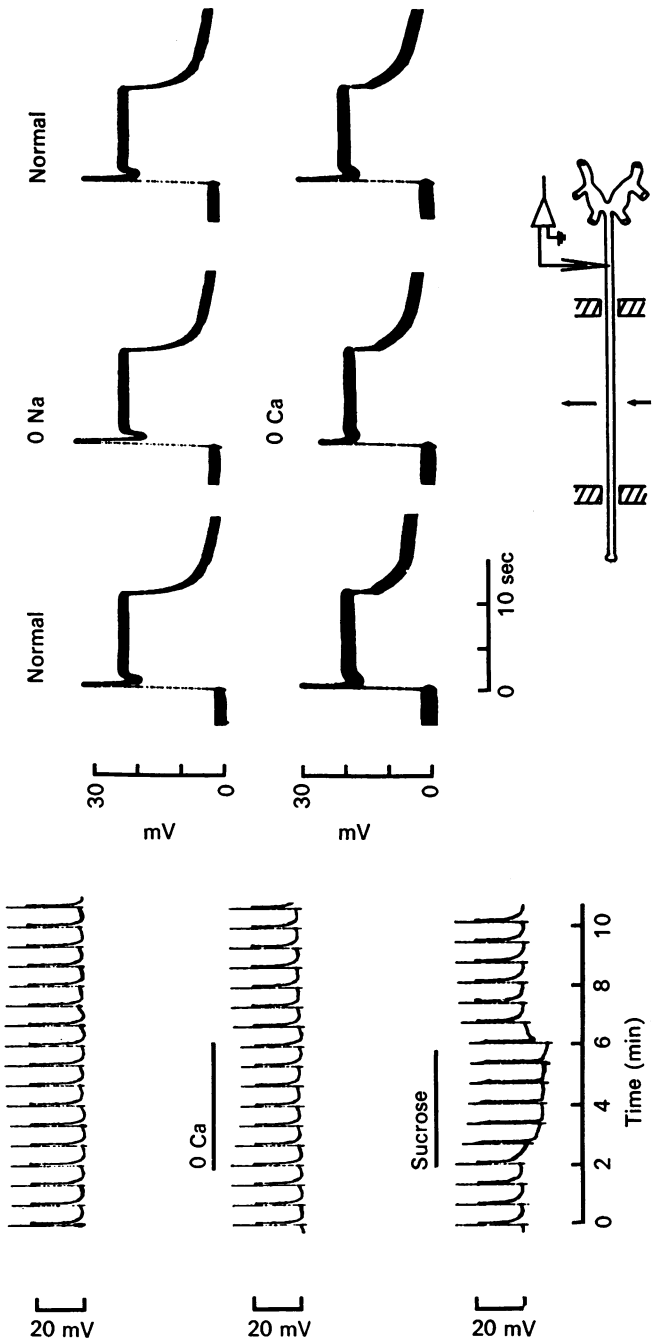
The inset of Text-fig. 1 shows the response to a moderately bright light of a receptor impaled simultaneously in its soma and at a point 9·7 mm down its axon of 13·0 mm total length. The response elicited by light in the soma consists of an initial peak depolarization lasting approximately 300 msec which decays to a maintained plateau, and a post-illumination hyperpolarization when the light is turned off (Hudspeth & Stuart, 1977). Both the depolarizing and hyperpolarizing voltage changes appear at the second point of recording nearly 10 mm away. In order to compare the attenuation of the various phases of the receptor potential, the maximum amplitudes of the peaks, the steady plateaux, and the maximum off-hyperpolarizations at the two recording sites have been plotted as a function of light intensity for eleven different intensities in Text-fig. 1. It is clear that depolarizations are attenuated more than hyperpolarizations.

Superfusion of axons with solutions of altered ionic composition

The small attenuation of the photoreceptor response over long distances (Text-fig. 1), and the differential attenuation of the spike, plateau depolarization, and post-illumination hyperpolarization raised the possibility that the signal might be sustained by one or more voltage-sensitive,



Text-fig. 1. A graph of the peak depolarizations (▲, △), plateaux (■, □), and maximum post-illumination hyperpolarizations (●, ○) recorded simultaneously in a receptor soma (filled symbols) and axon (open symbols) and plotted as a function of light intensity. The dark resting membrane potential was −60 mV (interrupted lines). The duration of the light pulse was 8 sec. The two electrodes were separated by 9·7 mm as shown in the inset diagram. The inset traces show the response to the light of intensity $10^{-1\cdot7}$ mW/cm² recorded in the soma (left) and axon (right).



Text-fig. 2. Receptor potentials recorded from the axon terminal region during immersion of the central segments of the axons in sodium-free, calcium-free or sucrose solutions. Receptor potentials are shown on slow (left-hand traces) and expanded (right-hand traces) time bases. The inset shows the conditions of perfusion and recording. The length of nerve in the central compartment was greater than half that of the entire nerve. Light pulses of 10 sec duration and saturating intensity were delivered every 40 sec. The post-illumination hyperpolarizations following each response sum at this frequency of stimulation. Thus the apparent resting membrane potential is about 15 mV more negative than the dark resting potential of -60 mV.

regenerative processes operative along the axon. The ususual length and isolation of the median photoreceptor axon provide an opportunity for testing whether the visual signal is amplified by an ionic mechanism as it spreads along this cell since significant lengths of axon can be superfused separately from the light-sensitive and synaptic regions.

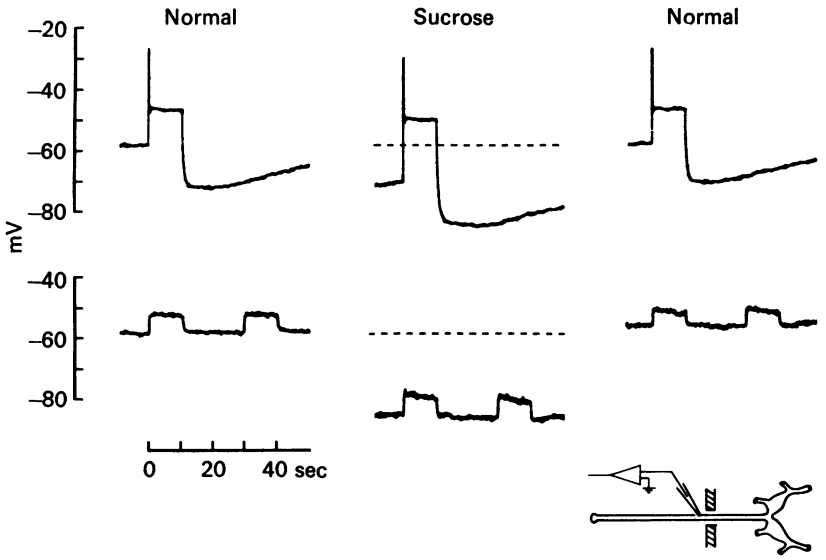
The soma, axon, and terminal region of a receptor were bathed by separate flow systems (see Methods) and the solution bathing the axon was altered to be free of either sodium or calcium, or all ions. The soma was stimulated with light, and a recording was made intracellularly from the axon near its terminal arborization while a long intervening stretch of axon was superfused with the test saline. Thus, if there exists in the axonal membrane an ionic mechanism for amplifying the receptor potential, the response in the axon terminal should have changed with a change in concentration of the relevant ion.

To test that the salines had access to the axonal membrane, the axon was superfused with solutions containing high potassium (100 mM). This concentration depolarized the cell in less than 1 min to a potential which depended on the length of axon being perfused. These experiments, and those in which slight, stable changes were observed when calcium or sodium was altered, made it unlikely that a physical barrier prevented access of the test salines to the axons.

Text-fig. 2 shows that removing sodium or calcium from the medium bathing the axon has little effect on the spread of the receptor potential and the post-illumination hyperpolarization. Saturating light pulses of 10 sec duration were presented to the cell every 40 sec, and sodium-free or calcium-free saline was introduced into the central compartment until any changes were stable (about 4 min). At this rate of stimulation with light, the post-illumination hyperpolarizations sum and hold the membrane potential between stimuli at a value more negative than the dark resting potential. In sodium-free saline, there is a slight increase in the amplitude of the peak, a slight decrease in the level of the summed post-illumination hyperpolarizations, and no change in the value of the plateau. Changes in calcium concentration also had little effect on the plateau or on the summed hyperpolarizations, although calcium-free saline did decrease the amplitude of maximum peak responses by up to 15%. Since smaller peak depolarizations and all plateau values were not substantially affected by changes in calcium concentration, it is unlikely that calcium is used to amplify the visual signal along the axon.

In three experiments we bathed over half the length of an ocellar nerve with a solution of isosmotic sucrose which contained no ions. Superfusion with sucrose in the dark caused a hyperpolarization of the cell to about -100 mV which was reversible as long as superfusion times were kept

brief. When the cell was briefly superfused with sucrose while being stimulated with light, the receptor potential still spread well down the axon (Text-fig. 2); although the values attained by the peak and plateau became slightly more negative because the response was superimposed on the hyperpolarization, the receptor potential itself actually became larger in absolute magnitude. Thus, removal of all ions from the medium surrounding the axon does not decrease the amplitude of the receptor potential in the terminal region of the cell, and it can be concluded that an ionic mechanism of amplification is not necessary for the effective propagation of this graded signal.



Text-fig. 3. Receptor potentials recorded from the axon during superfusion of the presynaptic terminal region of the cell with isosmotic sucrose solution containing 8 mM potassium and 20 mM magnesium (upper traces) or 8 mM potassium only (lower traces). The diagram shows the conditions of perfusion and recording. The upper traces show receptor potentials 10 sec in duration evoked by bright ($10^{-2 \cdot 3}$ mW/cm^2) lights, and the lower traces show a pair of receptor potentials each 10 sec in duration evoked by dim ($10^{-5 \cdot 3}$ mW/cm^2) lights. During sucrose perfusion the membrane hyperpolarized to about −85 mV. The receptor was 9·3 mm in total length, and the electrode was located 4·8 mm from the soma. The dark resting potential indicated during sucrose perfusion by dotted lines, was −58 mV.

It could be argued that the light response spreads passively to the presynaptic terminal region of the receptor cell and there is amplified by an ionic mechanism localized to the terminal membrane. If this were the case, the signal recorded in the axon might be a combination of the light-

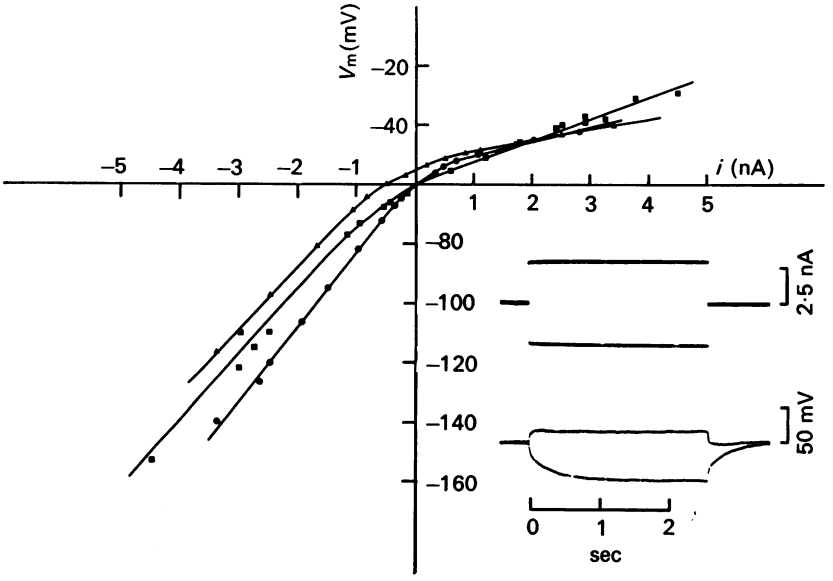
induced voltage change and the amplified terminal potential. To test this possibility, we removed ions from around the terminals while recording the receptor potential in the axon to see if the light response diminished. A preparation was placed in a two-compartment chamber, an electrode was inserted into a receptor axon in the soma compartment just next to the wall separating the compartments (slightly more than half the distance from the soma to the terminals), and the terminal compartment was perfused with sucrose. 8 mM potassium and 20 mM magnesium were added to the sucrose solution to lessen the large hyperpolarization caused by perfusing with sucrose alone; the soma compartment was perfused with normal saline. Text-fig. 3 shows that the receptor potentials set up by either bright or dim lights are not diminished during the sucrose superfusion of the terminal. Rather, the depolarizing light response becomes slightly larger, while the post-illumination hyperpolarization remains about the same in magnitude. One possible explanation for the increase in the receptor potential is that the sucrose-induced hyperpolarization of the terminals spreads to the soma and there displaces the resting membrane potential further from the equilibrium potential of the light-induced conductance change. Essentially the same result is seen when the magnesium is left out of the perfusant, although the sucrose-induced hyperpolarization is substantially larger in the absence of a divalent cation. Thus, there is no significant contribution to the receptor potential recorded in the axon of any ionic amplification mechanism that might exist in the terminal region.

Current–voltage characteristic of the receptor

If the electrical signals of the photoreceptors are propagated passively, the differential attenuation of hyperpolarizing and depolarizing response components implies that the cell membrane exhibits rectification. Current–voltage relationships of three dark-adapted receptors are shown in Text-fig. 4. The current–voltage relation of the photoreceptor was derived by impaling the axon with two closely juxtaposed electrodes, one for passing current and the other for recording the resulting voltage change. The shape of the current–voltage characteristic is typical of that seen in photoreceptors of other invertebrates (Baumann, 1968; Millecchia & Mauro, 1969; Lisman & Brown, 1971; Brown, Hagiwara, Koike & Meech, 1971) in that it displays marked rectification close to the resting potential. For hyperpolarizations greater than about 10 mV, the membrane behaves linearly with a slope resistance whose mean value is 25 MΩ (range 15–45 MΩ, $n = 11$; see Table 3). For depolarizations greater than 10 mV, where the membrane again behaves linearly, the mean value of the slope resistance is 4·6 MΩ (range 2·3–7·9, $n = 5$).

Estimation of membrane resistivity of the receptor

It has been suggested (Shaw, 1972) that the effective transmission of the receptor potential along the axon of a photoreceptor may be due solely to high membrane resistivity (R_m). To test this hypothesis, we have estimated the membrane resistivity of these cells in three ways, using the equations of cable theory: (1) from the length constant λ, calculated from the spatial decay of the light response; (2) from the time constant, τ; (3) from the input resistance, R_{in}, measured with two electrodes at one point. Because



Text-fig. 4. Current–voltage relationships for three receptors whose hyperpolarizing slope resistances lie near the mean value. The inset shows sample recordings used in construction of the curve and taken from the cell symbolized by circles (●). Note that the voltages are plotted on an absolute scale, the resting potentials ($i = 0$) ranging from -56 to -60 mV. Cells correspond to numbers 2 (▲), 3 (●), and 11 (■) of Table 3.

cable theory applies only to the linear part of a current–voltage relation, we have analysed only moderate or large hyperpolarizations for which the membrane appears to behave like an ohmic resistance (Text-fig. 4). The separate calculations from the three methods agree and provide a reasonable estimate of the resistivity of the hyperpolarized membrane.

Estimation of R_m from the spatial decay of the receptor potential. In theory one could measure the decay of voltage along the cell by inserting three electrodes into the receptor, one for passing current and the other two for

recording the voltage at the point of current injection (V_0) and at a point along the axon (V_x). In practice, however, this is a technically difficult experiment. We therefore chose to insert two recording electrodes into the cell, one into the soma and one into the axon, and to inject current into the soma by means of the photo-response. Various degrees of depolarization or hyperpolarization could be achieved by presenting light stimuli of varying intensity to the cell.

The attenuations (V_x/V_0) of the post-illumination hyperpolarizations were measured in five experiments similar to that presented in Text-fig. 1. The values of V_x/V_0 and the calculations made from them are presented in Table 1. In all five receptors, the length constant of the receptor is longer than the cell itself. The average value of R_m calculated from these length constants was 296 kΩ cm², the individual values varying from the mean by less than twofold.

TABLE 1. Membrane properties of median ocellar axons from measurements of spatial decay of the receptor potential

Expt.	L (mm total length)	x (mm from soma)	Est. d (μm)	V_x/V_0 average for hyperpol. (and n)	Calculated λ average and range (mm)	Calc. R_m (kΩ cm²)
1	6·0	5·5	17·3	0·88 (4)	12 (11–14)	298
2*a*	7·0	2·4	19·1	0·88 (3)	10 (9–13)	188
2*b*	7·0	4·4	19·1	0·85 (2)	14 (8–19)	368
3	8·2	4·5	21·1	0·78 (5)	10 (9–11)	170
4	10·8	10·5	25·2	0·83 (4)	18 (16–24)	463
5	13·0	9·7	28·4	0·72 (3)	15 (15–15)	286

Axonal diameters were estimated from the lengths of median ocellar nerves (see Methods). The number of receptor potentials at different light intensities used to calculate V_x/V_0 for hyperpolarizations is shown in parentheses after each value. The average R_m for the six measurements was 296 kΩ cm². In expt. 2 the axonal electrode was moved from a first recording position near the soma to a second position nearer the terminals and the measurements repeated.

We used the equations of cable theory that describe the case in which current is applied at one end of a short cable terminated at either end by an infinite resistance (Weidmann, 1952). In this circumstance

$$V_x = V_0 \frac{\cosh[(L-x)/\lambda]}{\cosh(L/\lambda)},$$

where L is the length of the receptor (measured from the soma to its entry into the commissure) and x is the distance between the electrodes. The equation describing a short cable with a terminated, as opposed to an open, end was selected because the amplitude of the voltage change did not change significantly over the final several mm of the axon before the nerve entered the ganglion; this result is consistent with the cosh function. From measurements of L and x for each receptor, and the above

equation, a theoretical curve was constructed for V_x/V_0 as a function of selected values of λ. From this curve, a value of λ was found for each experimentally observed V_x/V_0 (measured from graphs similar to that of Text-fig. 1). The values of λ were then averaged for each cell and are presented in Table 1.

From these values of λ it is possible to calculate the membrane resistivity R_m if one also knows the diameter of the axon and assumes an internal resistivity, R_i. Thus

$$R_m = \frac{4\lambda^2 R_i}{d},$$

where d is the diameter of the fibre. R_i was assumed to be 90 Ω cm (Shaw, 1972); the axonal diameter was estimated from the length of the median ocellar nerve (see Methods). This calculation ignores the additional membrane in the dendrites and synaptic terminals at either end of the cell.

Estimation of R_m from the time constant. The membrane resistivity is equal to the ratio of the membrane time constant, τ, to the membrane capacitance, C_m. Measurements of the time constant for the photoreceptor were made from traces such as those shown in the inset of Text-fig. 4. The τ for a membrane is the time of rise to a specified fraction of the final membrane voltage change in response to an applied current pulse. This fraction depends on the L/λ ratio of the cell (Jack, Noble & Tsien, 1975). The ratios of the lengths of our cells to their length constants ranged from 0·50 to 0·87, with an average value of 0·68. The time constant for a cable with a ratio of 0·68 has been calculated (Jack, Noble & Tsein, 1975, Fig. 4.10*b*) to be that time required for the membrane voltage to change to 67 % of its final value. Time constants measured on this basis from the potential changes produced in the receptor by hyperpolarizing current pulses ranged from 244 to 475 msec (Table 2). Taking the average value of 342 msec, and assuming a membrane capacitance of 1 μF/cm^2, the usual

TABLE 2. Time-constant measurements for hyperpolarizing current pulses

Expt.	Electrode location	Average τ at current onset (msec)*	Average τ at current offset (msec)*
1	Soma	450	—
2	Soma	475	325
3	Mid-axon	375	346
4	Axon hillock	280	275
5	Terminal†	325	280
6	Axon hillock	387	244

* Overall average τ = 342 msec.

† The portion of axon just before its entry into the ganglion.

τ was measured from pulses whose final steady voltage was within the linear portion of the current–voltage relationship. In all cases the voltage displacement was from the dark resting potential.

value for biological membranes, we find for R_m a value of 342 kΩ cm² ($n = 6$).

Estimation of R_m from input resistance. The membrane resistivity may be determined from measured input resistances of photoreceptor cells on the assumption that they behave as short cables terminated at each end by infinite resistances (Weidmann, 1952). If current-passing and potential-measuring electrodes are situated within such a cell near its end, the input resistance, R_{in}, is given by

$$R_{in} = \frac{2}{\pi}\sqrt{\frac{R_i R_m}{d^3}} \quad \coth 2L\sqrt{\frac{R_i}{dR_m}}.$$

We performed measurements of input resistance on eleven receptors with both electrodes placed in various parts of the axon (Table 3). Assuming a value for R_i of 90 Ω cm, we then solved for R_m for each receptor using the above equation and arrived at a mean value of 140 kΩ cm². A more detailed analysis, which takes into account the fact that the electrodes were often not at the end of an axon, provides a better estimate of the mean R_m of 157 kΩ cm².

TABLE 3. Estimation of membrane resistivity from input resistances to hyperpolarizing current pulses

Expt.	L (mm total length)	x (mm from soma)	Est. d (μm)	R_{in} (MΩ)	Calc. R_m (kΩ cm²)
1	6·6	2·9	18·4	20	51
2	8·6	7·4	21·8	21	86
3	8·7	7·9	22·0	24	106
4	9·0	5·8	22·4	32	161
5	9·2	1·0	22·8	15	60
6	9·2	1·0	22·8	16	66
7	9·5	8·5	23·2	45	266
8	9·5	8·6	23·2	19	89
9	10·5	1·0	24·8	22	131
10	11·2	10·1	25·8	43	334
11	13·0	9·7	28·4	22	189

The variability in the values of slope resistance shown in Table 3 is probably due to some extent to damage caused by the micro-electrodes (see Discussion). The two highest values were obtained from the terminal end of the axon where we might have inserted the electrode into one of the first branches of the receptor beyond the bifurcation instead of into the main axon (Hudspeth & Stuart, 1977). The lower values tend to be associated with the axon hillock region, but our results are too variable to permit the conclusion that the hyperpolarizing slope resistance depends on electrode position in a systematic manner.

Comparison of the three independent estimates of R_m. Our values of R_m calculated from λ, τ, and R_{in} are in reasonable agreement. Thus, we

calculate an average value of R_m of 296 kΩ cm² from λ, 342 kΩ cm² from τ, and 140 kΩ cm² from R_{in}. In one experiment, illustrated in Text-fig. 1, we estimated R_m independently from all three measurements made on the same cell. After the spatial decay of the receptor potential was recorded, the soma electrode was withdrawn, inserted close to the axonal electrode, and a current–voltage relation was obtained. From the decay of the receptor potential R_m was calculated to be 286 kΩ cm², from the time constant measurements 140 kΩ cm², and from input resistance 189 kΩ cm².

The agreement of the R_m calculated from τ with that calculated from λ and R_{in} implies that our assumption of a value for C_m of 1 μF/cm² for the receptor membrane is valid, and that the receptor axon has a single membrane of the usual capacitance. This suggests that the observed high value of R_m is an intrinsic property of the membrane and is not due to myelin-like wrapping of the axon.

Electron microscopy of photoreceptor axons

The results presented above show that the effective resistivity of the hyperpolarized receptor membrane is unusually high. This property might be intrinsic to the receptor membrane, or a consequence of a high extracellular series resistance. Although the large values of time constant make the possibility unlikely, a resistance to extracellular current flow might be caused by tight wrapping of the receptor axons by other cells, as in certain invertebrates (Hama, 1966; Heuser & Doggenweiler, 1966) or in the myelinated nerves of vertebrates. Alternatively, occluding junctions between glial cells or fibrocytes might seal off the extracellular space. Shaw (1972) showed with light microscopy that the dye Procion yellow could penetrate the sheath surrounding the photoreceptors of the barnacle lateral eye, apparently filling extracellular space quite close if not next to the axonal membrane. We examined cross-sections of receptor axons in the electron microscope to determine the nature of, and the junctions between, the structures wrapping the axons.

At low magnification it is apparent that the glial and capsular wrapping of the axon is similar to that surrounding other, normally conducting invertebrate axons (Villegas & Villegas, 1960) and the inexcitable axon of the *Limulus* ventral photoreceptor (Clark, Millecchia & Mauro, 1969); layered glial processes loosely wrap the axon and are themselves surrounded by a thick collagenous sheath (Pl. 1*A*). One somewhat unusual feature of the relationship between the glial cells and these axons is the lack of trophospongium, or finger-like glial projections into the axonal cytoplasm (Shaw, 1972).

Higher magnification of the axonal perimeter (Pl. 1*B*) shows that typical extracellular spaces of 10–30 nm exist between the membrane of the

receptor axon and that of the innermost glial cell and between successive glial cell membranes. While desmosomes and gap junctions (Type B, Gilula, 1974) exist between glial cells (Pl. 1*C*,*D*), they are plaques rather than zonulae and would not be expected to impede the movements of ions in the extracellular spaces. Of further note are the large amount of smooth endoplasmic reticulum and variety of multi-vesicular bodies found in the receptor cytoplasm, and the regular array of microtubules (Pl. 1; Hama, 1966) seen in cross-section in the cytoplasm of the glial cells.

We conclude, as did Shaw (1972), that there is no obvious anatomical barrier to the flow of ions in the extracellular spaces surrounding the axons of barnacle photoreceptors.

DISCUSSION

Passive conduction of the visual signal

It has previously been observed that certain neurones of both vertebrates and invertebrates conduct graded signals along processes whose length would be expected to prohibit successful passive conduction if the cell had the membrane properties commonly found for nerve (Ripley *et al.* 1968; Ioannides & Walcott, 1971; Paul, 1972; Shaw, 1972; Zettler & Järvilehto, 1971, 1973). In the present experiments, simultaneous recordings of light-induced voltage changes at two positions along the receptor permitted a more thorough description of the spread of the receptor potential than has been possible in other preparations. We establish that the high intrinsic membrane resistivity of the barnacle median photoreceptor can adequately account for the spread of visual signals to the receptor terminals.

Since the receptors are located in a peripheral nerve which can be more than 10 mm long, significant lengths of their axons can be superfused with solutions free of ions. Bathing axons in sucrose leads to a distortion of the signal reaching the axon terminal, but not to the diminution that one would expect if amplification along the axon were significantly involved in conduction of the signal. The level of membrane potential achieved by plateau depolarizations and post-illumination hyperpolarizations does not change significantly when half or more of the axon is superfused with sodium-free or calcium-free solutions. These results constitute strong evidence that amplification is not a factor in the conduction of at least the plateau depolarization and post-illumination hyperpolarization of the receptor potential.

Calculation of membrane resistivity

In calculating the membrane properties of the hyperpolarized receptors we made several assumptions. One is that the cell is a cylinder stopped

by an infinite resistance at either end. This must be only an approximation to the real case; the membrane area at the ends, in the light-sensitive dendrites and in the synaptic arborization, is extensive. While the amount of convoluted membrane is difficult to estimate, it can only lower the assumed infinite resistance of the ends; the actual values of λ and R_m must be accordingly larger than we calculate.

It is also an assumption that the cell membrane is ohmic during the post-illumination hyperpolarization as it is during the injection of hyperpolarizing current pulses (Text-fig. 4). The post-illumination hyperpolarization in the barnacle median photoreceptor is probably generated both by an electrogenic Na pump, as in other invertebrate photoreceptors (Koike, Brown & Hagiwara, 1971; Brown & Lisman, 1972), and by a long-lasting increase in potassium conductance (D. Edgington, personal communication). Conductance changes or an electrogenic pump operating during the post-illumination hyperpolarization could lead to error in the calculation of λ, but only if they occurred in membrane between the two recording electrodes. By removing potassium from around the ocellus or from around the axon, we can demonstrate that the Na pump component is associated with the somatic but not the axonal membrane (D. Edgington and A. E. Stuart, unpublished observations). We cannot rule out as yet an increase in potassium conductance in the axon, but if an increase in membrane conductance interferes at all with the measurements it should lead to an underestimate of R_m.

Another source of error might arise from the insertion of the electrode through this high-resistance membrane. We have noticed that the insertion of a second electrode often causes the amplitude of the post-illumination hyperpolarization to decrease, which indicates that the input resistance has decreased. Again, errors caused by insertion of the electrodes could only lead to an underestimate of the membrane resistivity. Insertion of a second electrode would affect the input resistance and time constant measurements more than the measurements of attenuation of the visual signal since, in the attenuation experiments, the shunt caused by the electrode closer to the cell body should affect the amplitude of the response to a given light pulse but not its decrement.

Since all of our sources of error would lead to an underestimate of R_m, our value of 100–300 kΩ cm^2 is a lower limit. Estimates of membrane resistivity have been derived for photoreceptors of two other barnacle species using terminated-cable equations (Millecchia & Gwilliam, 1972; Shaw, 1972). The higher value was obtained by Shaw, who calculated an R_m of 57 kΩ cm^2 from his data and an assumed R_i of 90 kΩ cm, an R_i value which we also employed. His value for R_m was lower than ours, perhaps because he estimated the degree of attenuation of the light res-

ponse from the peak depolarization of the receptor, at which voltage the resistance of the cell is low due to rectification. Unusually high membrane resistivities have been found in other cell types: 10 MΩ cm² in the ampullary canals of Lorenzini (Waltman, 1966); 1 MΩ cm² in a giant neurone of the mollusc *Anisodoris* (Gorman & Mirolli, 1972); and 50–100 kΩ cm² in membranes of the developing *Fundulus* egg (Bennett & Trinkaus, 1970).

The rectification shown in Text-fig. 4 considerably complicates calculations of the resistivity of the depolarized membrane. It is not known whether the rectification is a feature of the membrane of the entire cell, or is a localized property of the somatic, dendritic, axonal or synaptic membranes. At least one component resides in the terminal region where there is an increase in calcium permeability during depolarizations (Ross & Stuart, 1976). There are also potential-dependent conductance changes in somata of lateral ocelli of other barnacles (Brown *et al.* 1970). The relative contributions of various portions of the cell to depolarizing responses will probably best be understood from recordings from isolated somata, axons, and terminals. Until this information is available, it is not feasible to construct a cable model for depolarizing responses, and we have therefore not presented estimates of resistivity for the depolarized membrane.

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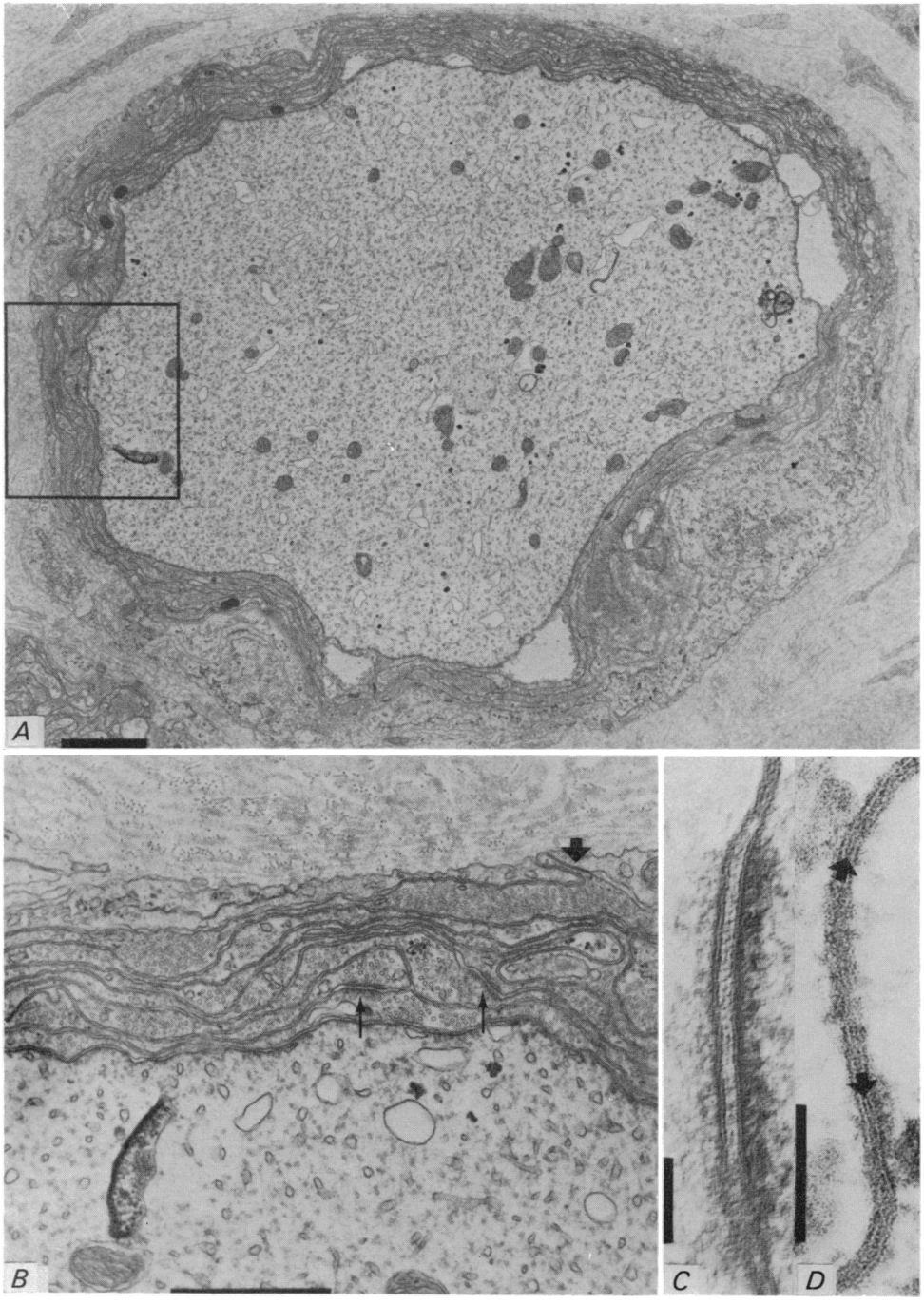
A
B
C
D

EXPLANATION OF PLATE

A, low-power electron micrograph of a single axon in the median ocellar nerve of the giant barnacle. The fibre contains smooth endoplasmic reticulum, mitochondria, glycogen particles, and several multivesicular bodies. The axonal sheath consists of several wrappings of glial cells with varying amounts of cytoplasm; outside it lie fibrocytes in a collagenous matrix. Scale, 2 μm.

B, an enlarged view of the portion of the axonal periphery delineated in Pl. 1*A* above. The glial processes which form the sheath are filled with microtubules and joined together by desmosomes (fine arrows) and gap junctions (coarse arrow). The intercellular spaces are otherwise unremarkable; there is no myelin-like membrane fusion. Scale, 1 μm.

C, high magnification electron micrograph of a desmosome between two glial cells of the median ocellar nerve. The junction is characterized by a dense intercellular line and by associated cytoplasmic densities. Scale, 0·1 μm.

D, a gap junction between glial cells of the median ocellar nerve sheath. The junction, which appears in our freeze fracture preparations as a plaque of the B type, is characterized in thin sections by the 3 nm intercellular space (arrows). Scale, 0·1 μm.