Biophys. Struct. Mechanism 4, 263–300 (1978)

Biophysics of Structure and Mechanism

Two Components of Extracellularly-Recorded Photoreceptor Potentials in the Cephalopod Retina: Differential Effects of Na^+, K^+ and Ca^{2+}

R. B. Clark* and G. Duncan**

School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, England

Abstract. The ERG of the isolated, superfused half-eye of the cephalopod *Sepiola atlantica*, evoked by a brief (10 μs) light flash, has been studied by recording intraretinal potentials with glass microelectrodes. The intensity-response characteristics of the potentials recorded at an electrode fixed at the surface (V_s) can be fitted by a simple equation derived from an equivalent circuit model based on a sodium conductance increase mechanism. Raising the external potassium level reduces the maximal response (ΔV_m), but does not alter the half-saturation intensity value (I_0). Reducing external sodium does not affect ΔV_m, but increases I_0. Reducing external calcium also does not affect ΔV_m, but decreases I_0. These effects are adequately described by the model if it is also assumed (a) that changing the external sodium does not significantly alter the transmembrane sodium gradient, and (b) that sodium and calcium ions compete for the sensitivity control mechanism.

Differential-depth recording between the fixed electrode at the surface and another electrode that could be moved into the retina revealed that the two component appearance of the transretinal ERG arose from the superposition of two vitreal-negative waveforms. An initial "fast" component was mainly recorded in the photoreceptive distal segments while a "slow" component was prominent in the more proximal regions of the retina. Perfusion with high K^+ salines resulted in a decrease in the amplitudes of both fast and slow components of the response whereas reducing external Na^+ reduced the amplitude of the fast component at all light intensities but reduced the amplitude of the slow component only at low intensities. The amplitudes of both the fast and slow components increased on reducing external calcium, but the rate of rise and fall of the fast component was independent of external calcium. The rate of rise of the slow component was also independent of the external Ca^{2+} level but a minimum in the recovery time (t_r) was shifted to a lower intensity value at lower calcium concentrations. The shift of the minimum was to a higher intensity value with lowered

* *Present address:* Department of Zoology, University of Nottingham, Nottingham, England

** Author to whom all correspondence should be addressed

0340-1057/78/0004/0263/$ 07.60

sodium perfusing solutions. On the basis of the differential sensitivity of the two components to ion changes, as well as stimulus intensity and intraretinal distribution of the components, it is suggested that they reflect two distinct processes in the light-evoked potential of the photoreceptor cells.

Key words: Cephalopod – Retina – Photoreceptor – Potentials – Cations.

Introduction

The membrane potential of most invertebrate photoreceptors depolarises in response to light. A considerable number of investigations on *Limulus* photoreceptors in which the ionic composition of the saline bathing the receptors has been changed imply that this depolarisation is the result of a light-induced increase in membrane permeability, primarily for sodium ions (Fuortes and O'Bryan, 1972; Wulff, 1973; Stieve, 1974). Localised illumination of photoreceptors of the *Loligo* retina results in an extracellular current flow consistent with a depolarisation of the illuminated cells (Hagins, 1965) and replacement of Na^+ by $choline^+$ ions in the bathing saline reversibly abolishes the photocurrent (Hagins and Adams, 1962). Similarly, photoresponses from the perfused retina of the small cephalopod *Sepiola atlantica* are considerably reduced, but not completely suppressed, by removal of Na^+ (Duncan and Weeks, 1973).

Although it seems very likely that a sodium permebility increase mechanism is largely responsible for generating the light response of most invertebrate photoreceptors, there are indications that in many receptors the photoresponse consists of more than one component. Intracellularly recorded photoresponses, for example from retinular cells of the lateral eye of *Limulus* (Wulff and Mueller, 1973), the compound eye of the honeybee drone *Apis* (Naka and Eguchi, 1962; Fulpius and Baumann, 1969) and the eyes of the gastropod molluscs *Aplysia* (Jacklet, 1969) and *Tritonia* (Chase, 1974) all exhibit more than one component and a variety of experiments suggests that these components are generated by different processes. In the case of *Limulus* and *Apis* the components are differently sensitive to Na^+ removal. Extrinsic current has different effects on the components of the *Limulus* and *Tritonia* photoresponses. More than one component has also been observed in the extracellularly recorded photoresponses (Electroretinogram or ERG) of several invertebrate retinae. The ERGs of the eye of the gastropod *Strombus* (Gillary, 1974), which has a retina very similar in structure to that of the cephalopod, and the compound eye of the crayfish *Procambarus* (Naka and Kuwabara, 1959), both exhibit two corneal-negative peaks with distinctly different properties. In both cases, the two components respond differently to light adaptation, and those in *Strombus* are also affected differently by changes of temperature.

Voltage-clamp experiments with the arthropods *Limulus* (Millecchia and Mauro, 1969b; Lisman and Brown, 1975b) and *Balanus* (Brown et al., 1970) have demonstrated the important role that calcium plays in regulating the flow of photocurrent and thus, in effect, in determining the amplitude and time course of the photoreceptor potentials. However, neither of the above studies dealt with any differential ef-

fects of calcium on the two components of the response and indeed there are technical difficulties involved in following the time course of the fast component using conventional clamping techniques.

The photoresponse of cephalopod photoreceptors also seem to contain more than one component. The photoreceptor generated ERG of the in vitro *Sepiola* retina, when evoked by a short (10 μs) intense flash of light, has an initial rapid phase superimposed on a much slower main response (Weeks and Duncan, 1974). Differential-depth studies of the intraretinal photovoltages in the unperfused retina showed that the ERG waveform arose from a "fast" initial component, apparently generated in the light-sensitive distal segments of the photoreceptors and a "slow" component which was more prominent near the somal region of the receptors (Clark, 1975).

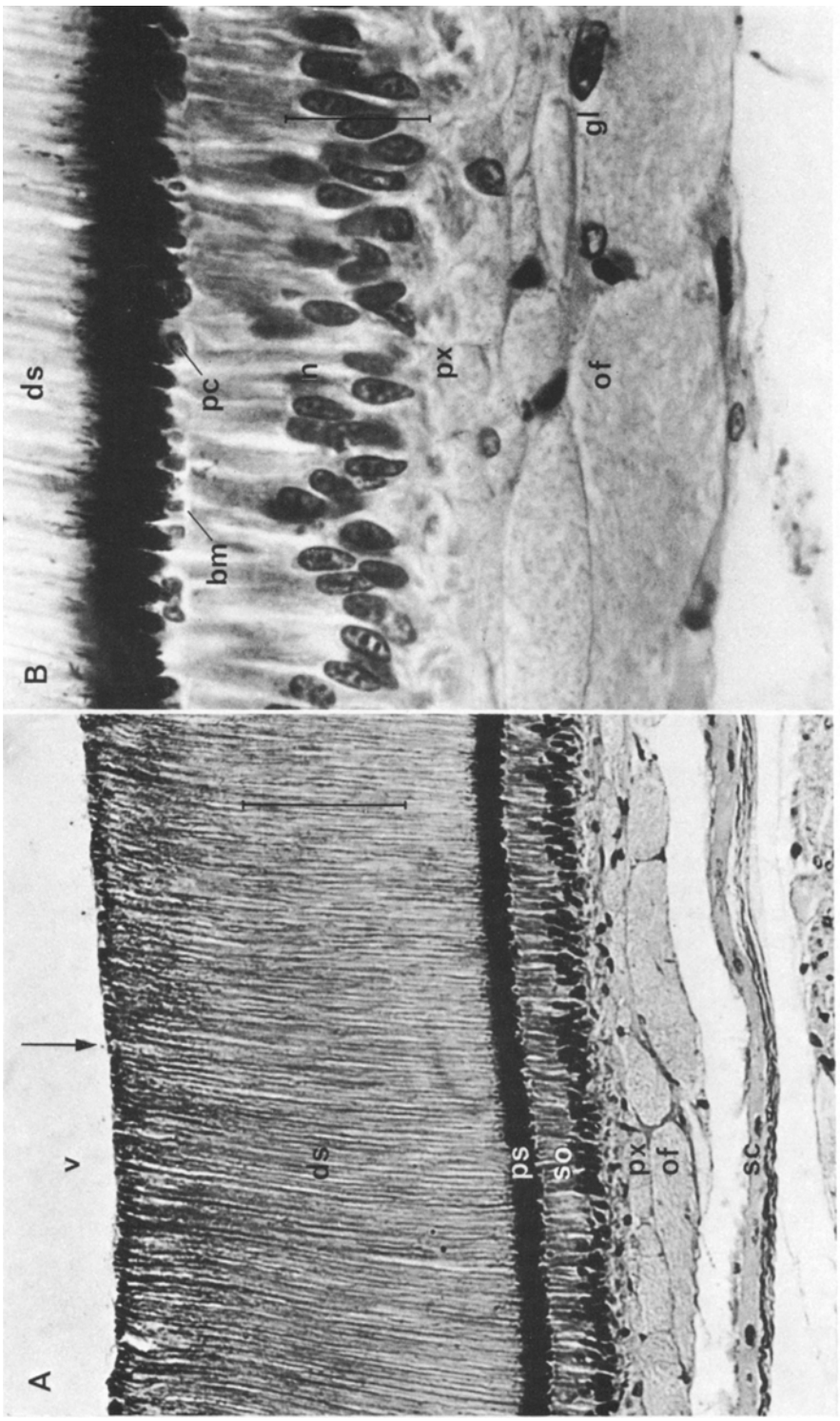
The primary purpose of the present study was to investigate the effects of ionic substitution on the overall ERG and on differential depth recordings as a means of distinguishing the ionic sensitivities of the processes responsible for the two components. We also report the effect of calcium ions on the amplitude and time-course of the two components in the *Sepiola* retina.

Methods

The isolated half-eye of the small cephalopod *Sepiola atlantica* was used in this study. Specimens were obtained locally from the North Sea and maintained in circulating sea water aquaria in the laboratory. Prior to an experiment, animals were dark-adapted for about ½ h, killed and the eyes removed under dim red light. The eye was cut in half perpendicularly to the optic axis and the rear portion, consisting of the sclera and attached retina, was retained. The half-eye was mounted in a Perspex chamber which allowed saline to be circulated over the vitreal surface of the retina (Fig. 1). As the back surface of the *Sepiola* eye has a relatively large radius of curvature and as the retina lies flat when the eye is bisected (in contrast to the vertebrate retina which still retains its hemispherical shape when removed from the eye) the receptor cells were probably not seriously stressed or distorted in the perfusion chamber.

Perfusion salines were based on an analysis of *Sepia* plasma (Robertson, 1953). The standard saline was composed of 460 mM NaCl, 20 mM KCl, 5 mM $CaCl_2$, 5 mM $MgCl_2$, 1 g/l glucose and 5 mM Trizma preset pH crystals which gave a pH of about 7.6–7.8 at 10° C. Changes in ionic composition were made by appropriate replacement of ions in the normal saline. The osmolarity of test salines, measured on a freezing-point depression osmometer (Advanced Instruments, model 31LAS), was never more than 5% different from that of the normal saline, which was about 920 mOsm.

The flow rate of saline was about 0.1–0.2 ml/s and the volume of the chamber was about 1 ml. When saline composition was changed, a by-pass line could be momentarily opened to flush the feedlines and chamber at about 2 ml/s. The temperature of the saline was kept at 10–12° C with a crushed ice-water bath and was monitored with a thermistor inserted in the feedline near the chamber inlet. The


A
v
ds
ps
so
px
of
sc
B
ds
bm
pc
h
px
of
gl

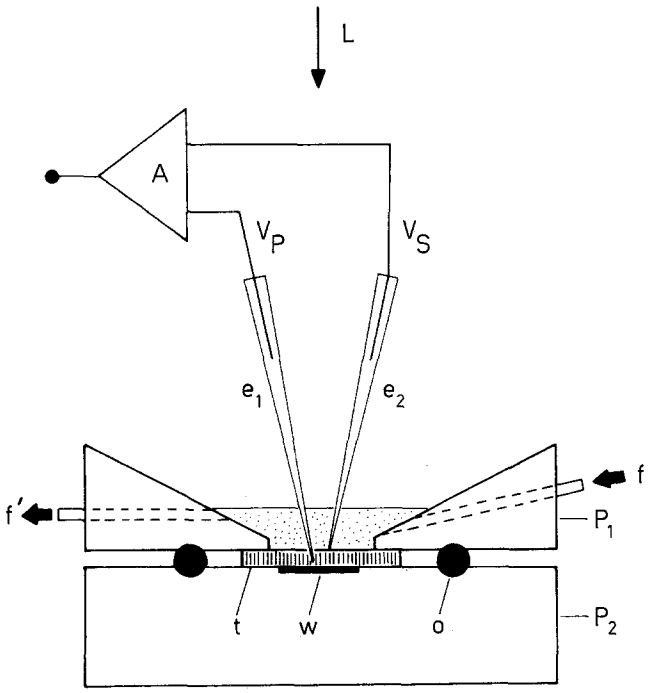


Fig. 1. Schematic diagram of perfusion chamber and recording system. P_1, P_2 circular perfusion chamber plates, shown in cross-section; *o*, rubber *o*-ring; *w*, loop of earthed Ag/AgCl wire, *t*, half-eye preparation; e_1, e_2, glass microelectrodes; *A*, FET-input differential amplifier; *L*, light stimuli. The vitreal side of the half-eye is perfused with saline (*f*, *f'*). One electrode (e_1) is attached to a microdrive which allows the electrode to be penetrated into the retina and the other (e_2) is fixed at the vitreal surface of the retina. Electrode e_1 measures the "proximal" potential V_P, and e_2 measures the "surface" potential V_S relative to *w* (see Methods)

temperature varied slightly from experiment to experiment but was always held to $\pm$ 0.5° C within any single experiment.

Electrical recordings were made with saline-filled glass microelectrodes with tip diameters of about 2 μm and d.c. resistance of 5—10 MΩ, measured in saline. Two separate electrodes, one fixed at the vitreal surface of the retina with a micromanipulator and the other attached to a manually operated hydraulic microdrive, allowed differential-depth recordings to be made. The extracellular potentials were referred to

---

Plate 1. Light micrographs of *Sepiola* retina. **(A)** Longitudinal section of *Sepiola* retina. Levels of the retina indicated are: *ds*, distal segments, *ps*, proximal segments and *so*, cell bodies of the photoreceptor cells; *px*, plexiform layer; *of*, optic fibre region; *sc*, sclera. The tips of the distal segments are separated from the vitreous humour (*v*) by an amorphous membrane about 2 μm thick (not visible in this section). Light is incident on the distal surface of the retina (arrow). Calibration bar indicates 100 μm. **(B)** Longitudinal section of the proximal region of the *Sepiola* retina. *pc*, nucleus of pigmented glial cells; *bm*, basement membrane (indicated by line of fracture); *n*, photoreceptor cell nucleus; *gl*, glial cell nucleus. Other structures are denoted as in (A). Calibration bar indicates 25 μm

an earthed loop of Ag/AgCl wire at the back of the half-eye. Before introducing the saline into the chamber, the electrode tips were initially positioned sufficiently close together on the vitreal surface of the retina (less than 0.5 mm apart) so that a light stimulus elicited an identical response from each electrode. The electrodes were coupled via Ag/AgCl wires to a high impedance FET-input amplifier. Responses were photographed from a storage screen oscilliscope.

The following convention has been used in describing the recordings. The potential of the electrode at the surface of the retina (relative to the grounded electrode at the back of the sclera) is denoted V_S or "surface" potential, whereas the potential of the electrode attached to the microdrive is denoted V_P or "proximal" potential (Fig. 1). The vertical separation of the two microelectrodes was estimated using the microdrive calibration. The differential amplifier allowed measurement of V_S, V_P and the "distal" potential $V_D = V_S - V_P$.

The preparation was always dark-adapted for approximately ½ h after it was fixed in the chamber and perfusion saline introduced. The photoresponse to a fixed stimulus generally increased during this time and experiments were not begun until it reached a stable value. Preparations in which the photoresponse declined during this period were abandoned.

Light stimuli were delivered by a xenon discharge lamp (Dawes Instruments Ltd., type 1202D stroboscope) which had a flash duration of less than 10 μs. The intensity of the light stimulus is expressed on a logarithmic scale where log intensity = 0 corresponds to energy at the surface of the retina of about 10^{-7} J/cm^2 over the wavelength range 400–600 nm (Duncan and Weeks, 1973). Stimuli of about 20 times this energy could be obtained from a different range setting on the stroboscope, but these were used only occasionally. The intensity of the stimulus was altered by attenuating it with neutral density filters (Kodak Ltd.) which were attached to the lamp housing. Light flashes were spaced sufficiently far apart (at least 1 min) so that the retina was fully dark-adapted before each flash.

Light microscope examination of the *Sepiola* retina was made from 6 μm sections of paraffin-embedded material. The photomicrographs of Plate 1 are of sections from a half-eye fixed in Bouin's fixative and stained with haematoxylin and eosin.

Results

Morphology of the Sepiola Retina

The structure of the *Sepiola* retina is very similar to that of *Octopus* (Young, 1962) and *Loligo* (Zonana, 1961; Cohen, 1973). About 70% of the volume of the retina is occupied by the photoreceptor cells. These consist of a long (200–250 μm) photoreceptive distal segment, the borders of which are covered with stacks of tubular microvilli, a short (15 μm) proximal segment devoid of microvilli and the cell body or soma (40 μm) containing the nucleus (Plate 1A). The proximal segments contain pigment granules, some of which migrate distally through the cytoplasm of the distal segments when the retina is illuminated (Daw and Pearlman, 1974). Interdigitating the proximal segments of the photoreceptors are the glial-like "epithelial" (Zonana,

1961) or "supporting" (Yamamoto et al., 1965) cells, also containing pigment which in contrast to that in the photoreceptors, is not mobile. The glial cells sit on the distal side of a thick lamella or "basement membrane" which is often apparent in the light microscope sections as a line of fracture between the pigmented proximal segment layer and the photoreceptor somae (Plate 1B).

The nuclear region of the photoreceptor somae forms a layer of about 25 μm thick. Immediately proximal to this is the plexiform layer, a complex meshwork composed primarily of axons from the proximal ends of the photoreceptors. In the optic fibre region these axons are gathered into large bundles by glial cells, the nuclei of which form a sparse band between the plexiform layer and optic fibre region (Plate 1B). These axon bundles course over the back of the eye under the sclera and pass into the optic lobes which lie behind the eyes. It is of particular note that, apart from the photoreceptors, the retina contains only cells with a non-nervous function. It seems probable that *Sepiola* photoreceptors, like those in *Octopus* (Young, 1962) are primary neurons whose axons run via the optic fibres directly to the optic lobes.

From the light micrographs (Plate 1B) the average cross-sectional area per photoreceptor in the *Sepiola* retina was about 30 μm^2, corresponding to about 3×10^6 cells/cm^2 of retina. This compares with about 5×10^6 cells/cm^2 in the *Octopus* retina (Young, 1960).

Properties of the Electroretinogram

In response to a brief flash of light the potential of the distal surface of the retina with respect to the back of the sclera ("V_S") becomes transiently negative and both the time course and amplitude of the photoresponse are dependent upon the intensity of the flash (Fig. 2). At relatively low stimulus intensities (i.e. $\log I < -2$ or about 10^{-9} J/cm^2 of retina) the waveform consists of a "latent" period of up to 50 ms (at 10° C) during which there is no detectable electrical response. The potential then rises to a peak value within 50–70 ms ("rise" phase) and finally declines to the baseline.

With increasing stimulus intensities the decay phase is progressively prolonged and, at the highest stimulus intensities, the response persists for as long as several seconds after the stimulus. In contrast, the latent period decreases with increased stimulus and the rising phase speeds up. At a relatively high intensity (−0.6 in Fig. 2), the rising phase dissociates from the main response, i.e., a fast initial wavelet appears superimposed on the slower part of the response. As the intensity increases still further, the time to peak value of the "fast" component becomes shorter whereas that of the "slow" is prolonged.

In order to quantify the effects of various ion substitutions on the waveform, the latency, rate of rise and fall of the response and also the amplitude were chosen to characterise the response. However, the latency was difficult to quantify as the value obtained depends very much on the criterion chosen (Fig. 3A and B).

The relation between stimulus intensity and response amplitude is the simplest to obtain and data from a typical experiment are given in Figure 4. For weak stimuli the response amplitude is approximately proportional to the intensity but gradually

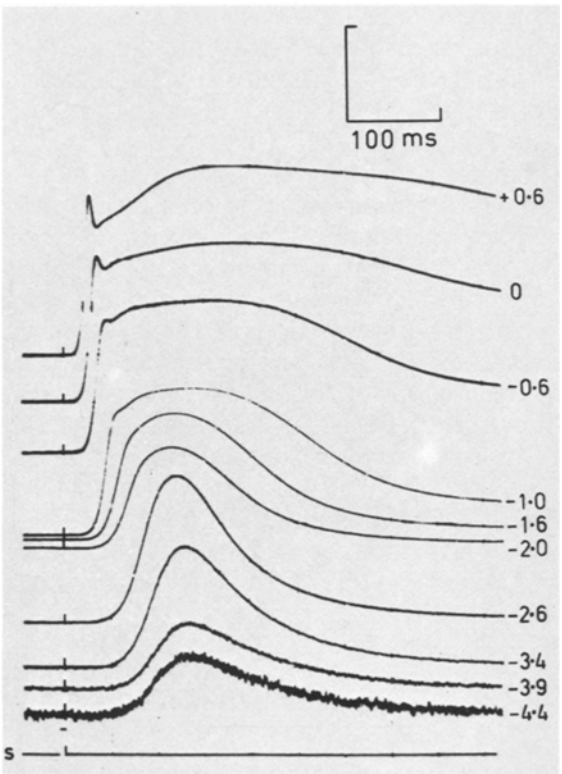


Fig. 2. Effects of stimulus intensity on the ERG. Each of the surface responses V_S (potential of the distal surface of the retina with respect to the back of the sclera) was evoked by a 10 μs light flash of different intensity, shown to the right of each trace in log units relative to a stimulus of about 10^{-7} J/cm^2 incident on the retina. The stimulus occurred at the downward edge of the square pulse on trace *s*. The vertical voltage calibration bar for log $I = -4.4$ is 0.4 mV; −3.9 is 1 mV; −3.4 is 2 mV; −2.6 is 4 mV; −2.0 to +0.6 are 10 mV. The responses in this and all other figures are shown negative upwards

falls below proportionality with increased intensity and apparently tends towards a limiting value. The light source used was not sufficiently intense to saturate the response of most preparations and even at the highest intensity available (log $I = +1.2$) the amplitude continued to increase slightly. Where fast and slow components were present, the amplitude of the slow phase was invariably larger than that of the fast component and was taken as the overall amplitude of the response (V_S).

Although the photoresponses of vertebrate receptors also seem to comprise two components (Werblin, 1975; Arden, 1976), the amplitude-intensity relation for the responses is adequately described by a simple equation of the form (Baylor and Fuortes, 1970; Penn and Hagins, 1972; Dowling and Ripps, 1972)

$$\Delta V = \Delta V_m \left(\frac{I}{I + I_0} \right), \tag{1}$$

where ΔV is the amplitude of the photoresponse evoked by a flash of intensity I, ΔV_m is the maximum or "saturation" amplitude and I_0 is the stimulus required to evoke a response of half-saturation amplitude. Equation (1) adequately describes the amplitude-intensity relation of *Sepiola* photoresponses (Fig. 4) and it was fitted to each set of data by a least-square method which adjusted both ΔV_m and I_0 to give a

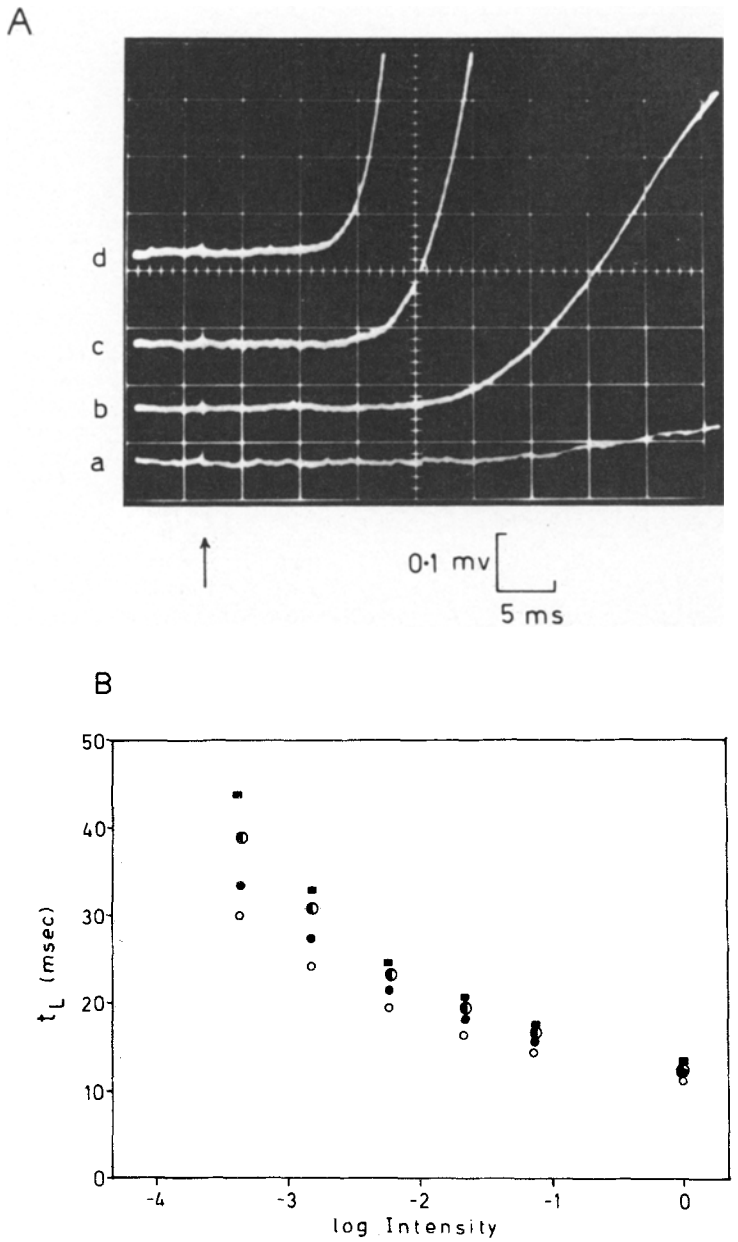


Fig. 3. Effect of stimulus intensity on latency. (**A**) Initial part of photoresponse on expanded time scale and voltage gain. Temperature 11.5° C. (**a**) Stimulus intensity, −3.4; (**b**) −2.2; (**c**) −1.1; (**d**) 0. (**B**) Latency as a function of intensity. The criterion responses from the baseline were: (■) 50 μV; (◐) 35 μV; (●) 20 μV; (○) 10 μV

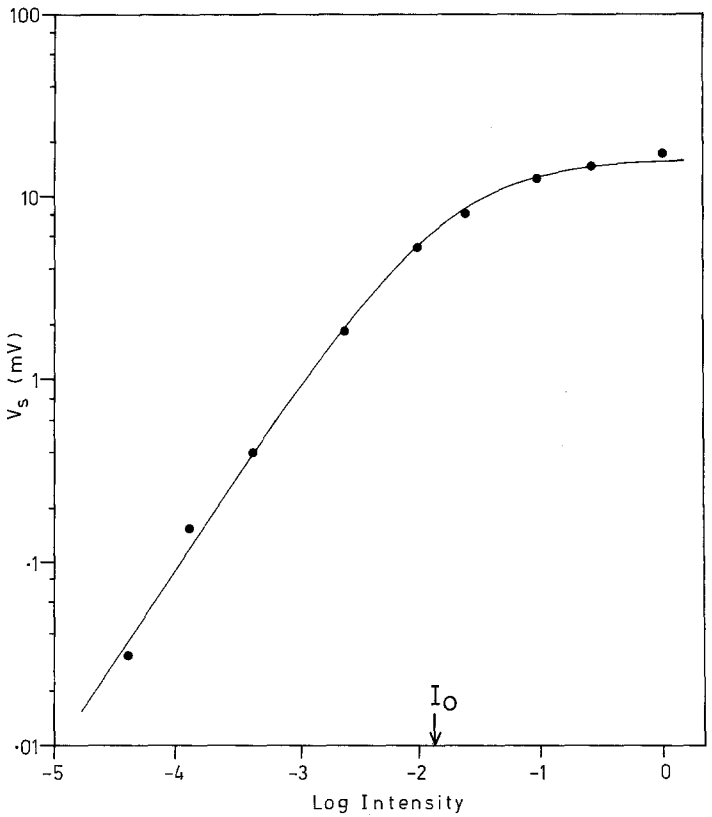


Fig. 4. Amplitude-intensity relation of the ERG. The maximum response height of V_S is plotted, on a logarithmic scale, against log intensity of the stimulus. The data (filled points) are from a single set of responses similar to those in Figure 2. The solid line is a fit of Equation (1) to the data; the values of the fitted parameters for this set of data are $\Delta V_m = 16.0$ mV and $I_0 = 1.7 \times 10^{-2}$

best fit. For eight separate sets of data, the fitted values of ΔV_m and I_0 obtained were

$$\Delta V_m = 14.5 \pm 4.4\ mV$$

$$I_0 = (8.4 \pm 0.6) \times 10^{-3},$$

where the values are the mean ± SD. The value of I_0 corresponds to energy of about 10^{-9} J/cm^2 at the surface of the retina. This value is of the same order as that observed by Dowling and Ripps (1972) for the half-saturation intensity of the isolated photoreceptor potential in the skate ERG.

Weeks and Duncan (1974) have previously shown that the response amplitudes predicted from an equivalent circuit model based on a transient sodium conductance increase mechanism (Duncan and Croghan, 1973) will fit extracellular data from *Sepiola*. The relationship between response amplitude and intensity can also readily

be derived from a simplified equivalent circuit model and the form of the relationship is exactly that given in Equation (1) where

$$\Delta V_m = E_{Na} - E_D \tag{4}$$

and

$$I_0 = \frac{g_K + g_{Na}}{G}. \tag{9}$$

G is the conductance increase per photon and the other terms are defined in the Appendix. Equations (4) and (9) will form the basis for discussion of the effects of varying Na^+, K^+ and Ca^{2+} on the overall photoreceptor response.

Effects of K^+ Concentration Changes

The dark resting membrane potential of many invertebrate photoreceptors seems to be controlled to a large extent by the potassium Nernst potential (Wulff, 1973; Stieve, 1974). Enhancement of the extracellular K^+ concentration depolarises the membrane potential and results in a concomitant reduction in the receptor potential. Photoresponses from the *Sepiola* retina exhibit a similar behaviour (Fig. 5A). Replacement of NaCl by an isomolar quantity of KCl resulted in a reduction in the amplitude of the surface photoresponse (V_S), the degree of reduction depending directly on the concentration of K^+ in the saline (Fig. 5B). Reduction of the K^+ concentration from 20 mM to 2 mM resulted in a small (about 10%) increase in the amplitude of the photoresponse, consistent with previous observations in the *Loligo* retina (Hagins et al., 1960) but there was no significant change in I_0. That is, the relative decrease in response amplitude caused by K^+ increase was the same over the entire range of stimulus intensities used (log $I = -4.4$ to -0.6), but the intensity required to evoke a half maximal response (I_0) was substantially unaltered.

In all of the high potassium solutions tested, the potassium was increased at the expense of sodium and so any changes in the waveform brought about by a sodium reduction must be taken into account. The effect of changing the external potassium in the range 2–120 mM K^+ was always simply to change the amplitude without noticeable effect on the time course of the surface (Fig. 5A) or differential-depth responses (Fig. 6A).

Significant changes in the time course only occured when the retina was exposed to K^+ concentrations in the region of 200 mM and moreover, only when high intensity stimuli were employed. Under these conditions, the fast component was eliminated and there was a significant change in the time course of the slow component (Figs. 5A and 6C). Both of these effects can probably be ascribed to a decrease in the sodium concentration alone (see following section).

The K^+ increase effects took several minutes to develop completely after the test saline was initially introduced. Providing the preparation was not exposed to elevated K^+ levels for a too prolonged period, the effects were reversible after return to control saline (Fig. 6). It was found that approximately 10 min was the longest exposure to high K^+ that most preparations would tolerate without irreversible dam-

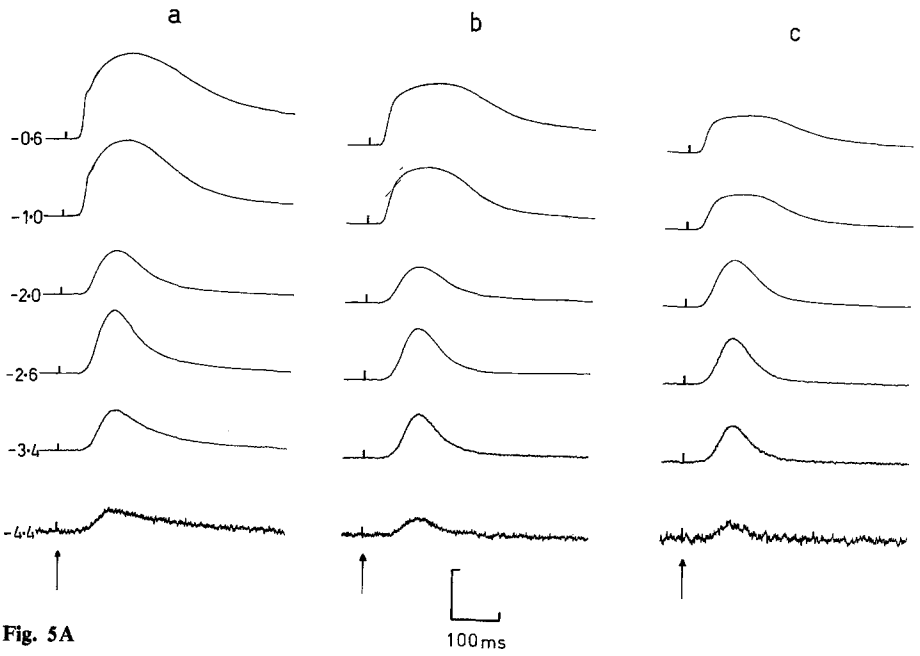

a
b
c
-0·6
-1·0
-2·0
-2·6
-3·4
-4·4
100ms

Fig. 5A

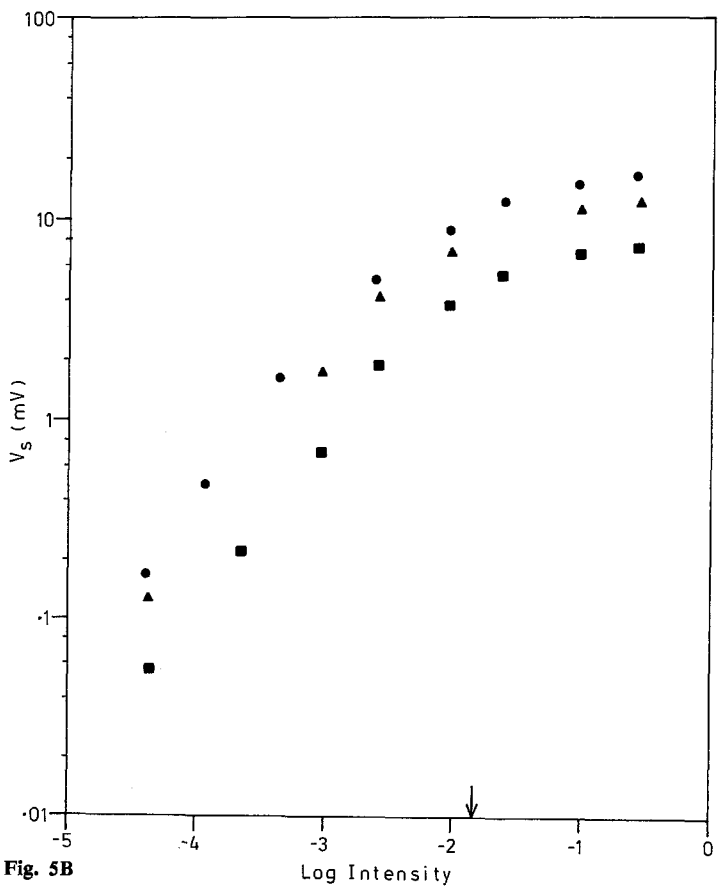

100
10
1
·1
·01
V_S (mV)
-5
-4
-3
-2
-1
0
Log Intensity

Fig. 5B

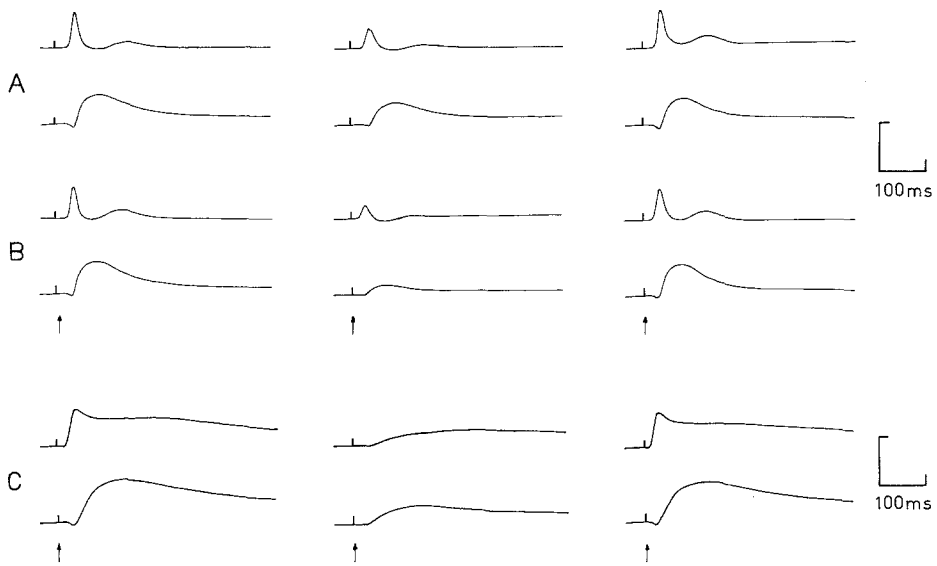


Fig. 6. Effect of increased K^+ concentration on differential-depth responses. **(A)** Left-hand column; the upper of the pair of responses is V_D and the lower is the corresponding V_P, recorded at an apparent electrode depth of 175 μm. At this depth, the V_D records comprise mainly the fast distal component whereas the V_P records contain both the reversal of the fast component and the corneal negative slow component (Clark, 1975). Preparation perfused with normal (20 mM K^+) saline. Centre column; after perfusion for 3 min with 100 mM K^+ (380 mM Na^+). Right-hand column; after perfusion for 3 min in normal K^+ saline. **(B)** Same as (A), except that preparation was perfused with 200 mM K^+ (280 mM Na^+) in centre column. **(C)** Same format as (A) and (B) but from a different preparation. The apparent electrode depth was 200 μm and $\log I = 0$. Left-hand column; Normal K^+ saline. Centre column; after perfusion for 3 min in 250 mM K^+ (230 mM Na^+). Right-hand column; after perfusion for 3 min in normal K^+ saline. The voltage calibration bar is 10 mV for both V_D and V_P

Fig. 5. Effect of K^+ concentration increase on V_S. **(A)** Log stimulus intensity is shown in the extreme left-hand column. The stimulus is marked by vertical arrows. **(a)** Preparation perfused with "normal" saline (20 mM K^+). Voltage calibration bar for $\log I = -4.4$ is 0.4 mV; −3.4 is 2 mV; −2.6 is 4 mV; −2.0 to −0.6 is 10 mV. **(b)** Responses after perfusing for 10 min in 120 mM K^+ saline. Voltage calibration for $\log I = -4.4$ is 0.4 mV; −3.4 is 1 mV; −2.6 is 4 mV; −2.0 to −0.6 is 10 mV. **(c)** Normal saline was reintroduced after (b) for 15 min and the responses were recorded 10 min after introducing 240 mM K^+ saline. Voltage calibration for $\log I = -4.4$ is 0.2 mV; −3.4 is 1 mV; −2.6 is 2 mV; −2.0 is 4 mV; −1.0 and −0.6 is 10 mV. **(B)** Effect of increased K^+ concentration on the amplitude-intensity relation. Data were obtained from the responses in (A). (●) 20 mM K^+; (▲) 120 mM K^+; (■) 240 mM K^+. The I_0 value in each case was approximately 1.7×10^{-2}, while the ΔV_m values were 19 mV, 11 mV and 8 mV respectively

age and in view of the relatively long time taken for the effects of Na^+ reduction to occur, it is possible that the effects of K^+ increase have been underestimated by brief exposure to the test saline. It was often noted that the response shortly after return to control saline following exposure to high K^+ levels was larger that the original control response. This was particularly true for brief exposures to the highest K^+ levels (200 mM K^+). Usually the response gradually decreased after a few minutes to near the original control level. This possibly arose because of a presumed increase in intracellular K^+, which was subsequently reduced to "normal" levels after return to the control saline.

Effects of Na^+ Concentration Changes

Reduction of the Na^+ concentration in the perfusion saline resulted in a corresponding reduction in the amplitude of the photoresponse of the *Sepiola* retina (Fig. 7A). The degree to which the photoresponse was reduced, however, depended inversely on the intensity of the light stimulus. For weak stimuli the response appeared to be nearly abolished but for more intense stimuli there was a substantial photoresponse even in the complete absence of Na^+ from the saline. In terms of Equation (1), reduction of Na^+ resulted in changes in the amplitude-intensity relation which were consistent with an increase in the half-saturation intensity I_0 while the saturation amplitude V_m was substantially unchanged (Fig. 7B) and in some cases actually increased slightly, exactly opposite to the effects of K^+ increase (see previous section).

In addition to the amplitude, the waveform of the photoresponse was considerably altered by Na^+-deficient saline (Fig. 7A). Two features were of special note. The sharp initial rising phase, corresponding to the fast component of the ERG, was considerably reduced by lowering the Na^+ concentration. This resulted in a much slower rising phase of the response, as well as an apparent increase in the latency. By contrast, however, the very prolonged decay phase of the response, particularly at the highest stimulus intensities ($\log I > -1$), became much more rapid in reduced Na^+ saline. Consequently in spite of the reduced rate of rise, the duration of the

---

Fig. 7. Effect of Na^+ concentration decrease on V_s. (**A**) Log stimulus intensity is shown in the left-hand column. The stimulus is marked by vertical arrows. (**a**) Preparation perfused with normal saline (460 mM Na^+). Voltage calibrations bar for $\log I = -4.4$ is 0.4 mV; -3.4 is 2 mV; -2.0 to 0 is 10 mV. (**b**) Same preparation perfused with 100 mM Na^+ (360 mM choline chloride). Responses were recorded 15 min after introducing 100 mM Na^+ saline. Voltage calibration for $\log I = -4.4$ is 0.2 mV; -3.4 is 0.4 mV; -2.0 is 4 mV; -1.0 and 0 is 10 mV. (**c**) Same preparation perfused with Na^+-free saline (460 mM choline chloride). Normal Na^+ saline was reintroduced after (b) and the preparation perfused for 15 min. Responses recorded 15 min after introducing Na^+-free saline. Voltage calibration bar for $\log I = -4.4$ is 0.2 mV; -3.4 is 0.4 mV; -2.0 is 2 mV; -1.0 and 0 is 10 mV. (**B**) Effect of decreased Na^+ concentration on the amplidude-intensity relation. Data were obtained from the responses in (A). (●) 460 mM Na^+; (△) 100 mM Na^+, after 15 min; (■) Na^+-free saline, after 15 min; (□) Na^+-free saline after 30 min. The V_m value for all four sets of data was approximately 20 mV, while the I_0 values were (●) 10^{-2}; (△) 3×10^{-2}, (■) 10^{-1} and (□) 0.9 respectively

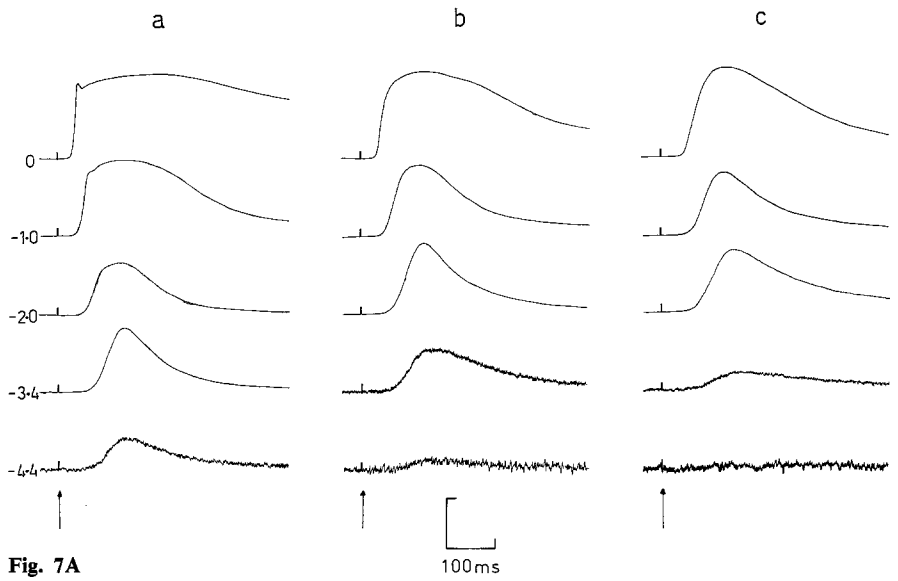


Fig. 7A

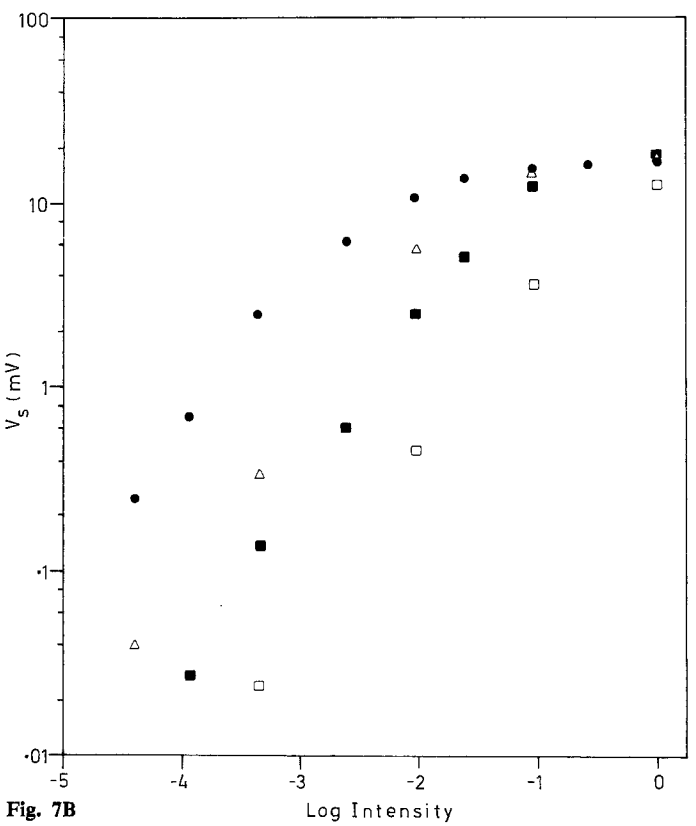


Fig. 7B

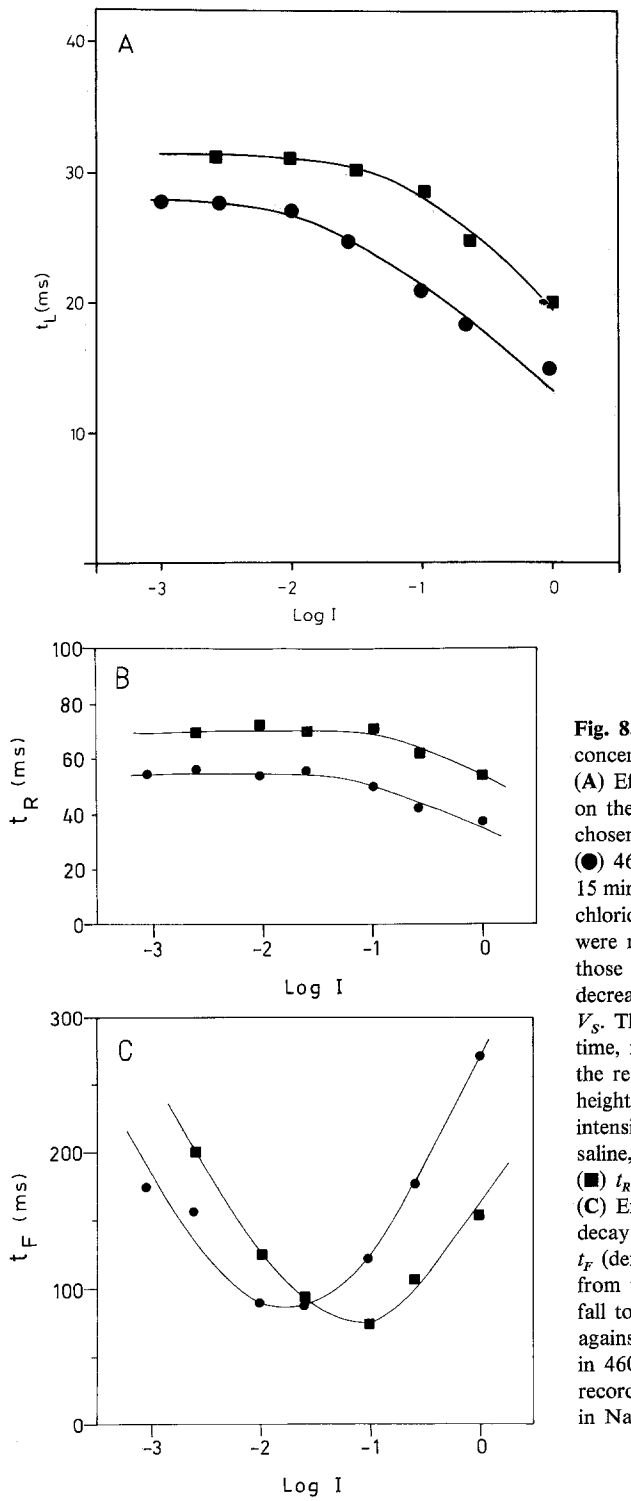


Fig. 8. Effects of decreased Na^+ concentration on the waveform of V_S. **(A)** Effects of decreased external Na^+ on the latency. A 20 μV criterion was chosen for the latency (see Fig. 3). (●) 460 mM Na^+ saline; (■) after 15 min in Na^+-free (460 mM choline chloride) saline. The measurements were made from records similar to those in Figure 7A. **(B)** Effect of decreased Na^+ on the rise phase of V_S. The "rise time" t_R (defined as the time, measured from the stimulus, for the response to reach 50% of peak height) is plotted against log stimulus intensity. (●) t_R in 460 mM Na^+ saline, from same records as in **(B)**; (■) t_R after 15 min in Na^+-free saline. **(C)** Effect of decreased Na^+ on the decay phase of V_S. The "decay time" t_F (defined as the time, measured from the peak, for the response to fall to 50% of peak height) is plotted against log stimulus intensity. (●) t_F in 460 mM Na^+ saline, from same records as in (B); (■) t_F after 15 min in Na^+-free saline

photoresponse at high stimulus intensities became progressively shorter as the Na^+ concentration was reduced. For weak stimuli the opposite effect occurred and the response became more prolonged in the Na^+-deficient salines.

These changes in the waveform of V_S are described quantitatively in Figure 8. The latency was estimated by taking a 20 μV criterion (Fig. 3) and the data clearly show (Fig. 8A) that removing the external sodium prolongs the latent period, as would be expected from the fact that removing external sodium abolishes the fast component of the response (Figs. 7A and 9). Because of the difficulty involved in estimating latency, the rise-time of the response (t_R) defined as the time, measured from the stimulus, for the response to reach 50% of peak amplitude was routinely used to give a more reliable measure of the effect of removing sodium on the initial part of the response. In normal Na^+ (460 mM) the rise time (Fig. 8) was relatively insensitive to low stimulus intensities, remaining nearly constant up to about log $I = -2$, then progressively decreasing with increased intensity. In the absence of Na^+, t_R had a very similar dependence on intensity but was consistently greater than the corresponding value in normal Na^+. The decay phase of V_S was measured by the "decay time" t_F which was defined as the time, measured from the time of the peak of the slow component, for the response to fall to 50% of peak amplitude. Unlike the rise time, the decay time t_F exhibited a striking dependence on stimulus intensity (Fig. 8C). The response to weak flashes had a very prolonged decay phase but with increasing intensity t_F decreased to a minimum and at still higher intensities became progressively prolonged. In the absence of Na^+, t_F exhibited a similar dependence on intensity but the minimum was shifted towards higher stimulus intensities. The amount by which the minimum was shifted varied from preparation to preparation but was typically about one log unit.

The changes in waveform of the surface photoresponse V_S were examined in relation to the components of the ERG using differential-depth recordings. Responses at a fixed electrode depth indicated that removal of Na^+ from the bathing saline had a significantly greater effect on the amplitude of the fast component of the ERG than it did on the slow component. This is clearly evident in Figure 9. The apparent electrode depth was about 175 μm and both the initial fast component and the slow second component were present in part in both V_D and V_P. After a brief (5 min) exposure to Na^+-free saline, both V_D and V_P were considerably reduced in amplitude but not abolished at the high stimulus intensity. The waveform of both V_D and V_P changed markedly and in particular the fast component evident in V_D appeared to be completely suppressed whereas the slow component was diminished but clearly not abolished. At lower stimulus intensities the responses in Na^+-free saline were very small and for the weakest stimuli used (log $I = -3.0$), both of the ERG components appeared to be abolished (Fig. 9). The effects of Na^+ removal on the components were generally reversible and after re-introducing the normal Na^+ saline (460 mM) the prominent fast component reappeared. Differential-depth recordings made with the movable electrode in the region of the retina where V_P inverted polarity showed that the changes in the amplitude and waveform of V_S caused by Na^+ removal were mirrored by very similar changes in the "inverted" response (see also Fig. 6 and Fig. 14 later).

The results of the differential-depth studies clarified the changes in the waveform of V_S caused by Na^+ reduction (Fig. 8). The slow component of the ERG is appar-

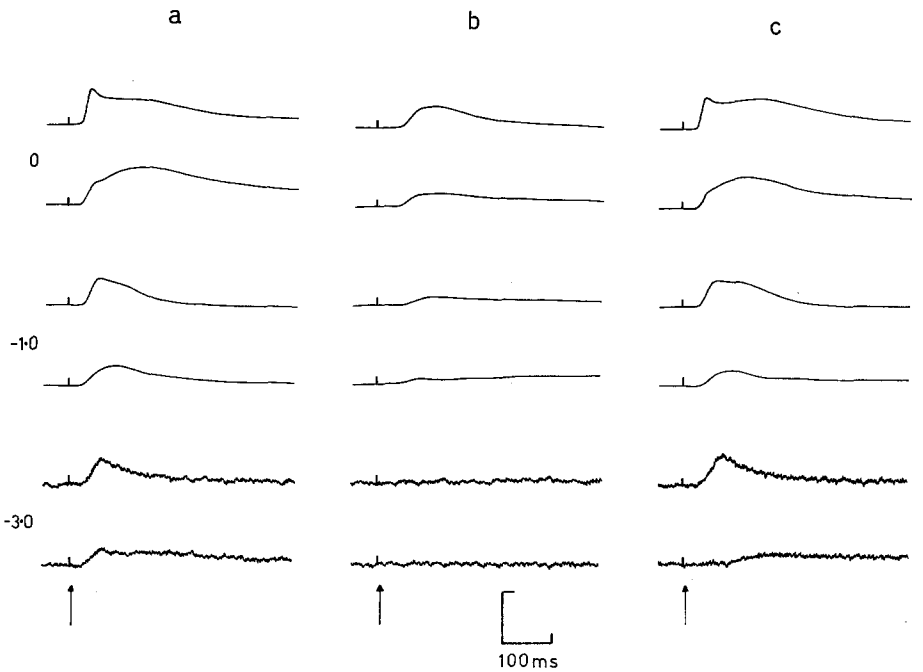


Fig. 9. Effect of Na^+ concentration decrease on differential-depth responses. The log stimulus intensity for each pair of records (upper record is V_D; lower record is V_P) is shown at the extreme left. The apparent electrode separation is 200 μm. In this particular preparation the reversal potential was small indicating there was probably little leakage of current through short-circuit pathways. (**a**) Preparation perfused in normal Na^+ (460 mM Na^+) saline. (**b**) Same preparation after 5 min in Na^+-free (460 mM $Tris^+$) saline. (**c**) Same preparation 5 min after return to normal Na^+ saline. The voltage calibration bar for log $I = -3.0$ is 0.4 mV for both V_D and V_P; -1.0 and 0 is 10 mV for both V_D and V_P

ently less sensitive to Na^+ removal than is the fast and the latter's disappearance in Na^+-free saline resulted in the increased rise time (Fig. 8B). The changes in decay time at low stimulus intensities were probably also a result of the abolition of the fast component, because at low stimulus intensities the time course of the fast component was similar to that of the slow and consequently the decay time of the resulting surface response V_S was influenced by both components. Suppression of the fast component in Na^+-free saline resulted in a time course which was that of the slow and hence t_F was increased. At high stimulus intensities, however, the time course of the fast component does not significantly influence the decay phase of V_S and t_F reflects the properties of the slow component.

The effects of Na^+ reduction developed gradually and it generally took several minutes after initially introducing the test saline into the chamber for the photoresponse to reach a relatively stable value. The photoresponse of preparations exposed to test saline for about 10—15 min recovered their original amplitude when replaced in normal saline, although the waveform was often slightly altered. In particular the sharp rising phase, corresponding to the fast component, was often absent if the preparation was exposed to reduced Na^+ levels for more than about 15 min. The

amplitude of the slow phase of the response, on the other hand, was not significantly changed. It is of note that there were substantial photoresponses from preparations which had been perfused with Na^+-free salines for as long as 30–40 min (Fig. 7B).

Choline chloride, Tris chloride and sucrose were used as replacements for NaCl. The effects of replacing Na^+ by either choline$^+$ or Tris$^+$ were very similar and substantially reversible. Sucrose, however, proved to be a poor replacement, as the photoresponse was greatly reduced when the retina was replaced in normal Na^+ saline after a few minutes exposure to sucrose saline. This was similar to previous observations (Stieve et al., 1972) that isosmotic substitution of glucose for NaCl irreversibly reduced photoresponses of the *Astacus* retina.

Effects of Ca Concentration Changes

It was generally found that when the preparation was exposed to reduced calcium concentrations (< 1 mM) for more than 10–15 min, the light sensitivity on return to the control solution (5 or 10 mM Ca) was often irreversibly reduced. It was also found that exposure to solutions outside the range 0.1–10 mM Ca^{2+} even for much shorter times invariably resulted in a permanent decline of sensitivity on return to the control solution. In this communication, we only report the results of those experiments where a complete recovery of sensitivity was achieved and so exposure of the tissue to various test solutions (0.1 mM < Ca^{2+} < 10 mM) was for a maximum of 10 min.

The effect on the amplitude of the surface response of reducing external calcium is, as observed in the case of sodium substitution, dependent on stimulus intensity and the data from a typical experiment covering more than 3 log units of intensity are given in Figure 10. At the lowest intensity there is an almost 10-fold increase in sensitivity for a 10-fold decrease in external calcium, whereas at the highest intensities employed there is almost no change in amplitude. The changes in the time course of V_S brought about by changes in the external calcium levels are also dependent upon intensity (Fig. 11). At nearly saturating light intensities (log $I = 0$), where the dual nature of the light response is evident, the rising phase of the response is identical in both solutions whereas the falling, or recovery, phase is greatly prolonged in low Ca^{2+}. With intensities in the range -2 to -1 log units there is an increase in amplitude in low calcium, but the time course of the response is not very different from the control. At lower light levels, however, the falling phases are again different and, somewhat surprisingly, the position is now reversed: with the recovery phase faster in the low calcium solution. Throughout the entire intensity range the rising phase of the response seems relatively insensitive to changes in the calcium concentration. These observations can be quantified by measuring the time to reach 50% of the peak height from the stimulus onset (t_R), and also the time to decay to 50% from the maximum response (t_F). The rise time of the response (t_R), is indeed, relatively insensitive to changes in external calcium and does not in fact show a very marked dependence on stimulus intensity, except at the highest levels (Fig. 12A). The decay time, however, is both dependent upon intensity and on the external calcium concentration. The data from one experiment are given in Figure 12B, but

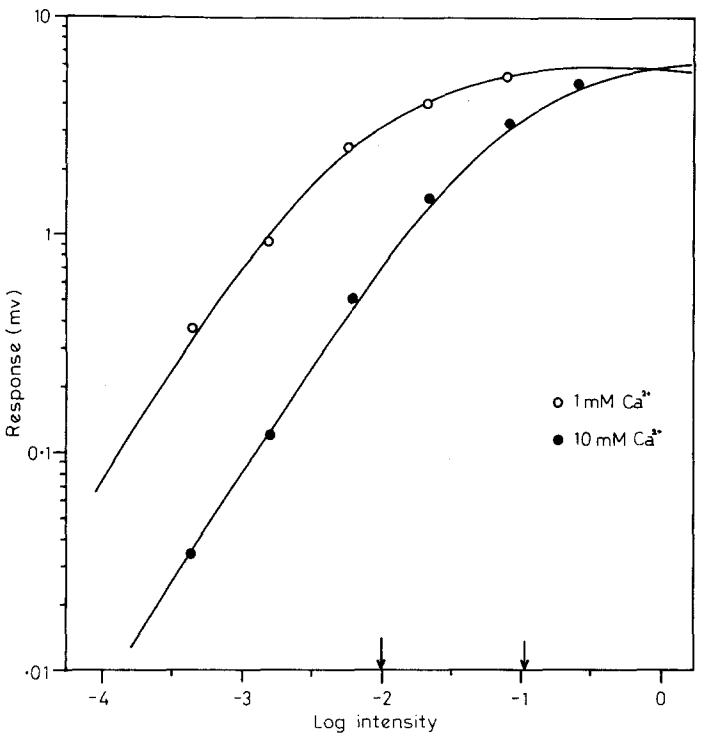


Fig. 10. Effect of reducing calcium ion concentration on response-intensity relation for short flashes (V_S). Reducing calcium from 10 mM to 1 mM reduces the half-saturation intensity (I_0) from 1.1×10^{-1} to 1×10^{-2} indicated by arrows (c.f. Fig. 8C). The ΔV_m value in both cases was approximately 15 mV

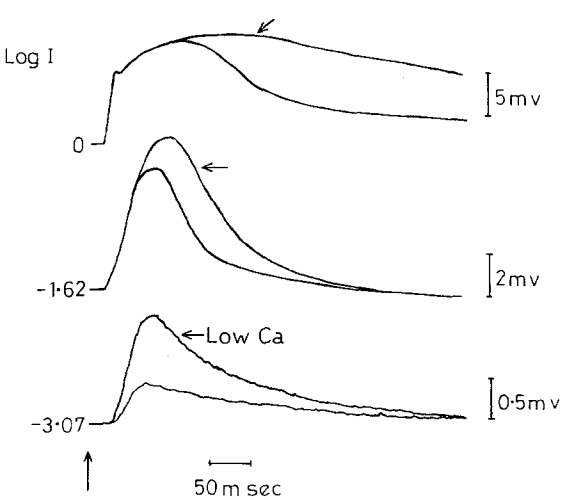


Fig. 11. Effect of reduced calcium on response waveform (V_S). Log stimulus intensity is shown in the extreme left near each pair of records. The upper trace in each pair arrowed is the response in reduced calcium (1 mM) approximately 5 min after introduction of the test solution. The control solution (lower traces) in this case contained 10 mM Ca^{2+}

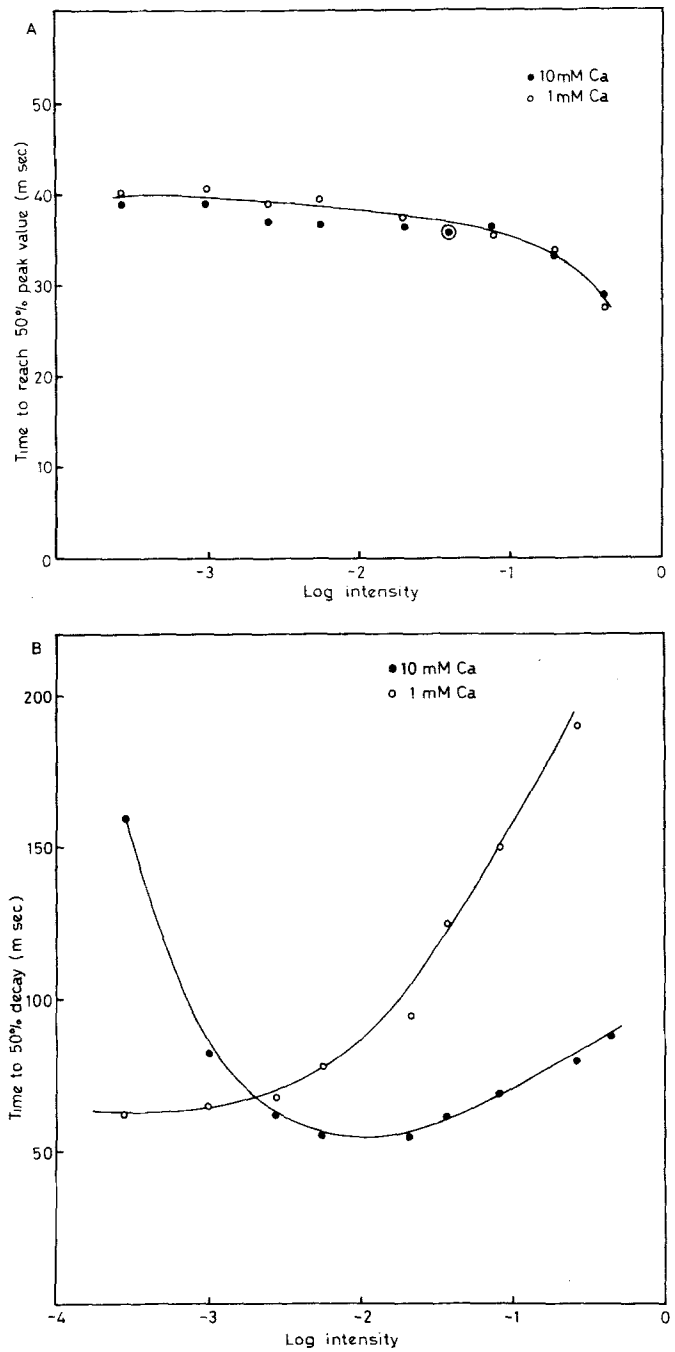


Fig. 12. Effect of reduced calcium on the waveform of V_S. (**A**) The rising phase. The "rise-time" t_R (defined as the time, measured from the stimulus, for the response to reach 50% of peak height) is plotted against log stimulus intensity. The measurements were made from records similar to those in Figure 11. (**B**) The "decay time" t_F (defined as the time, measured from the peak, for the response to fall to 50% of peak height) as a function of log I

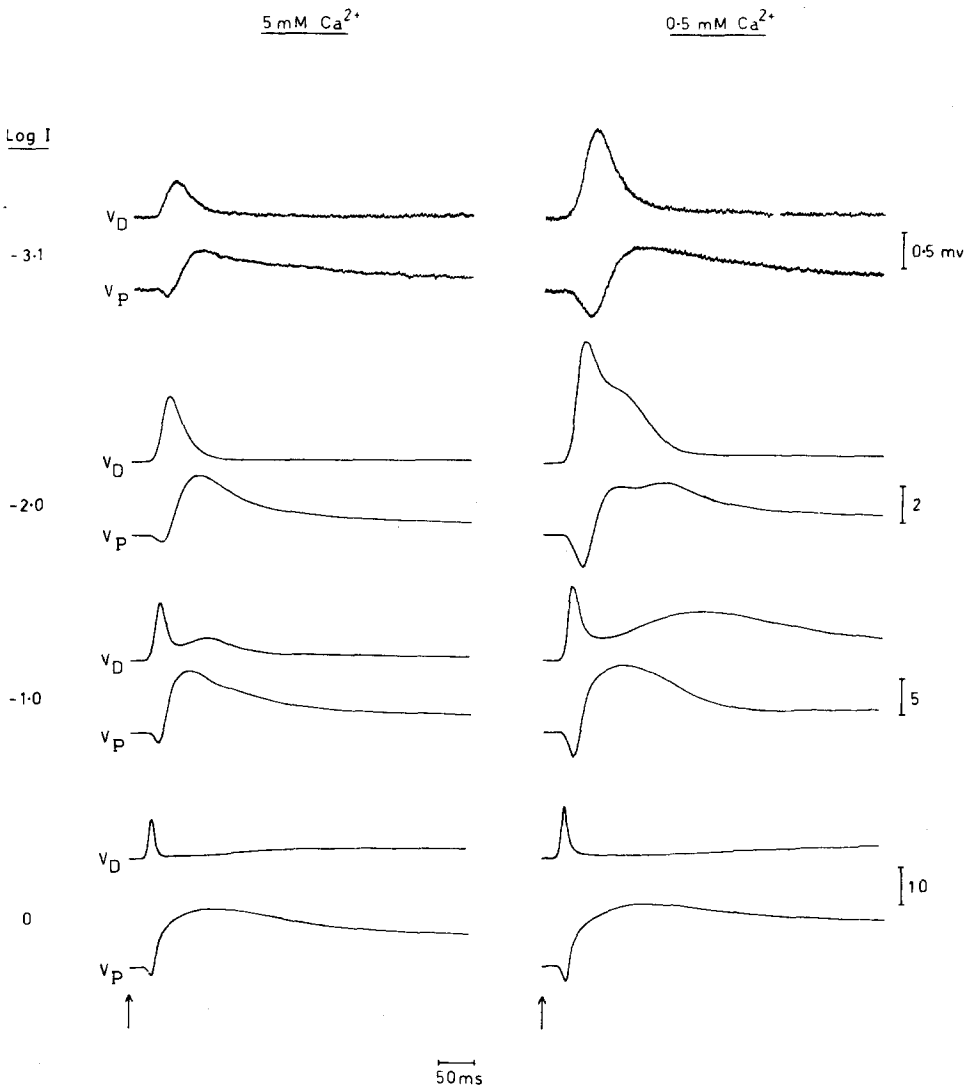


Fig. 13. Effect of Ca^{2+} reduction on differential-depth waveforms. Apparent tip separation between surface and inner electrodes was 150 μm. The control solution contained 5 mM Ca and the records in the test solution were obtained after 3 min exposure (V_D = distal p.d.; V_P = proximal p.d.). Note the clear reversal of the fast component (V_P records) at this depth

we found that all retinae behaved in the same way. As the flash intensity increases, the recovery time t_F decreases, until a minimum is reached and increasing the intensity still further leads to a lengthening of the recovery time. Although the actual position of the minimum varied with intensity from retina to retina, the effect of reducing the external calcium was always to shift the minimum towards a lower intensity value (see also Fig. 17 later).

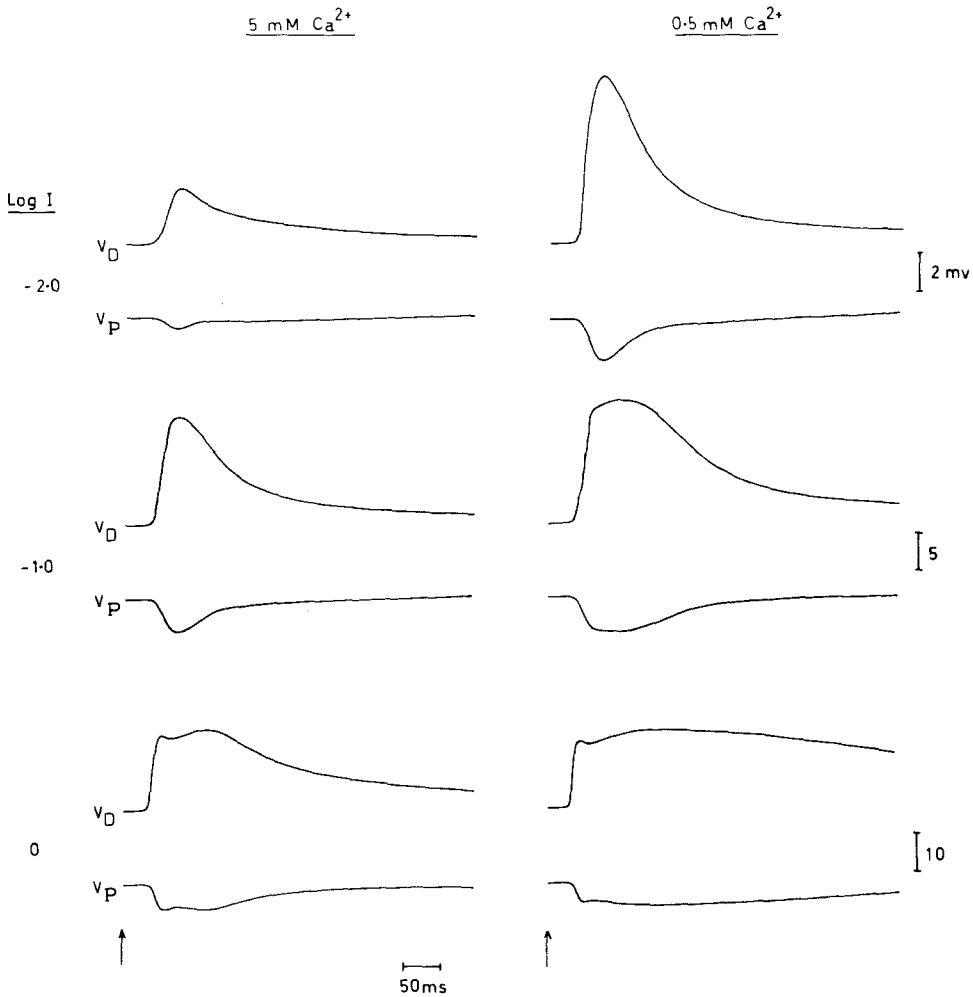


Fig. 14. Effect of Ca^{2+} reduction on differential-depth waveform. Same format as in Figures 13, except that the apparent tip separation was 300 μm. Response from same preparation as in Figures 13. At this depth both the fast and slow components have reversed

Figure 13 shows a series of differential depth recordings from the same retina over a wide range of flash intensities and for two different calcium concentrations. The tip separation between the surface and inner glass micropipettes was about 150 μm. At relatively low intensities (log $I = -3.1$ and -2.0) there is a very marked increase in the amplitude of the fast component on lowering the external calcium (V_D records) and there also appears to be an increase in the amplitude of the slow component (V_P). While there appears to be little change in the kinetics of the fast component, there are clearly changes in the decay rate of the slow component. The records at higher light intensities (log $I = -1.0$ and 0) show that while there is little change in the amplitude or time course of the fast component on reducing calcium,

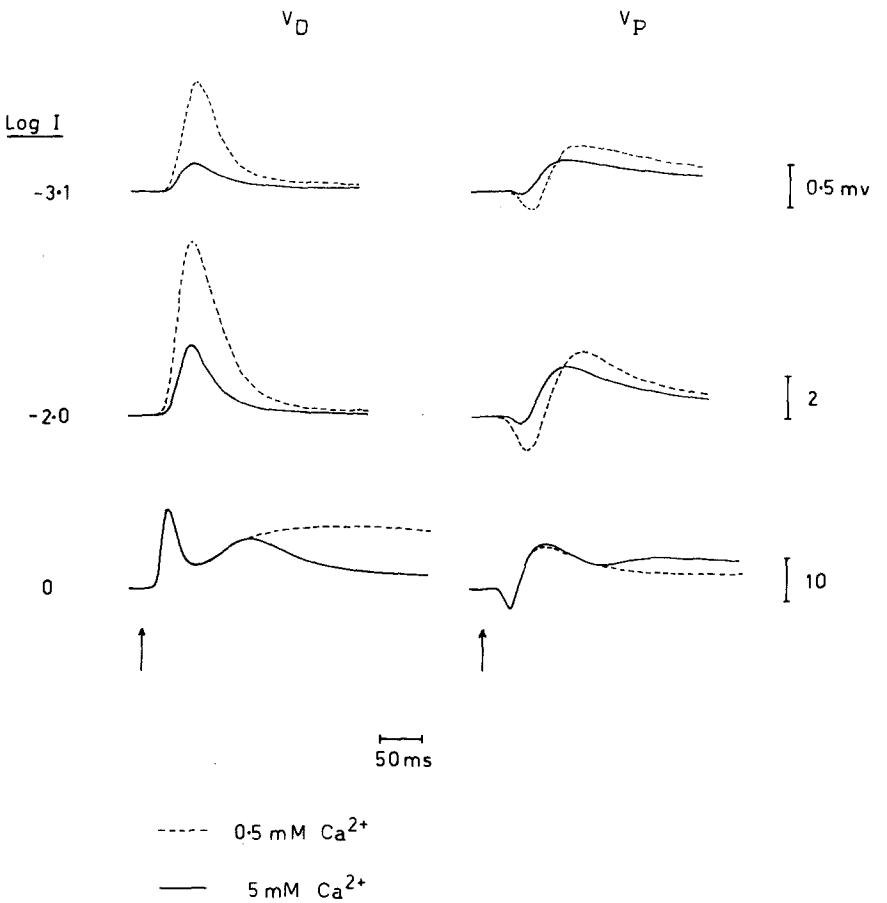


Fig. 15. Effect of Ca^{2+} reduction on differential-depth waveforms. Comparison of V_D and V_P in 5 mM and 0.5 mM Ca^{2+} salines. Apparent tip separation was 150 μm

the decay phase of the slow component is prolonged. The effects on the decay of the slow component can be seen more clearly by moving the proximal electrode further into the retina and a tip separation of about 300 μm gives a maximal distal response (V_D records in Fig. 14). The proximal response at this depth consists solely of a potential that is of reverse sign to that of V_s (and V_D at this depth). The fact that the time course of V_P closely mirrors that of V_D, irrespective of the calcium concentration, indicates that this reversal potential is in fact probably set up by the flow of current through a short-circuit shunt pathway traversing the retina (see Tomita, 1972; Rodieck, 1973 for good accounts of reversal potentials).

The superposition of proximal records at different calcium concentrations (Fig. 15) show the difficulty in obtaining any information concerning the rise time of the slow component as any changes are accompanied by a change in the reversal potential of the fast component. However, if the distal records at $\log I = 0$ give an accurate picture, then the rising phase of the slow component is insensitive to calcium

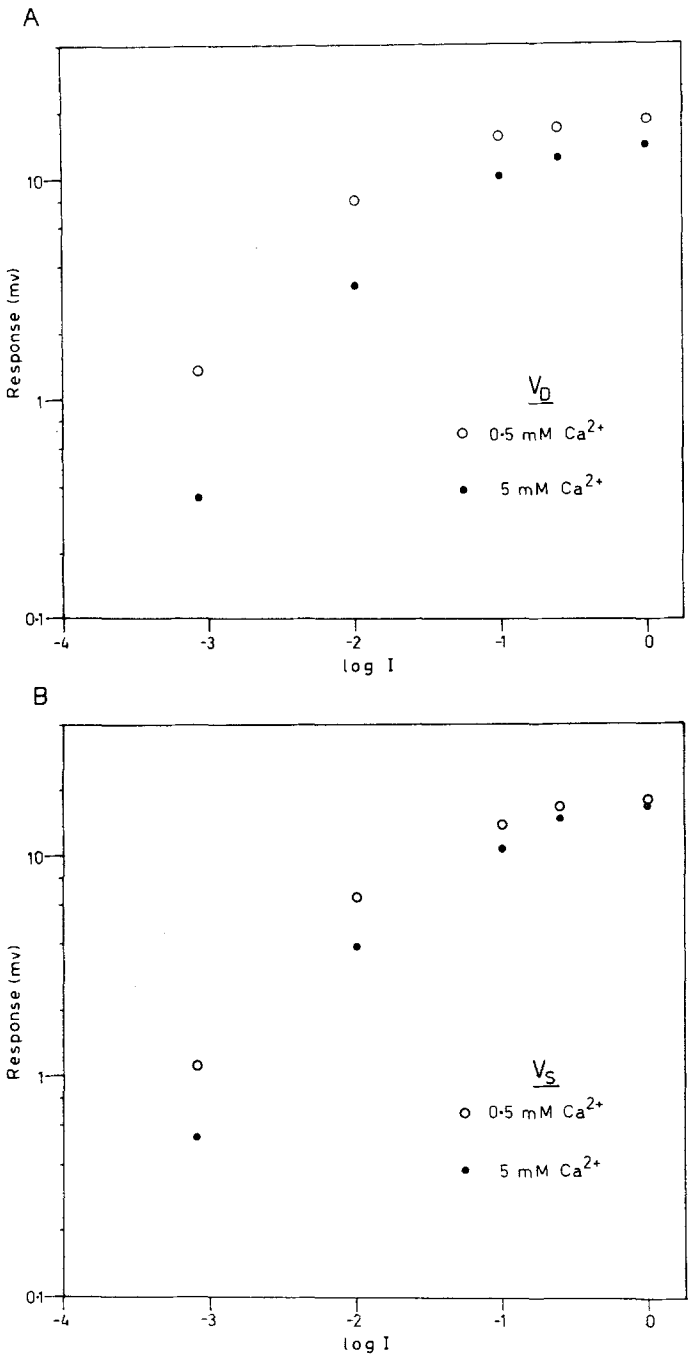


Fig. 16. Effect of Ca^{2+} concentration on response intensity of fast and slow components. **(A)** Response-intensity relation for V_D (fast component) in 5 and 0.5 mM Ca^{2+} salines. The peak values of V_D were measured from the records of the experiment illustrated in Figure 13. **(B)** Response-intensity relation for V_S in 5 and 0.5 mM Ca^{2+} salines. The peak value was obtained from a reconstruction of V_S ($V_S = V_D + V_P$) from the records of Figure 13

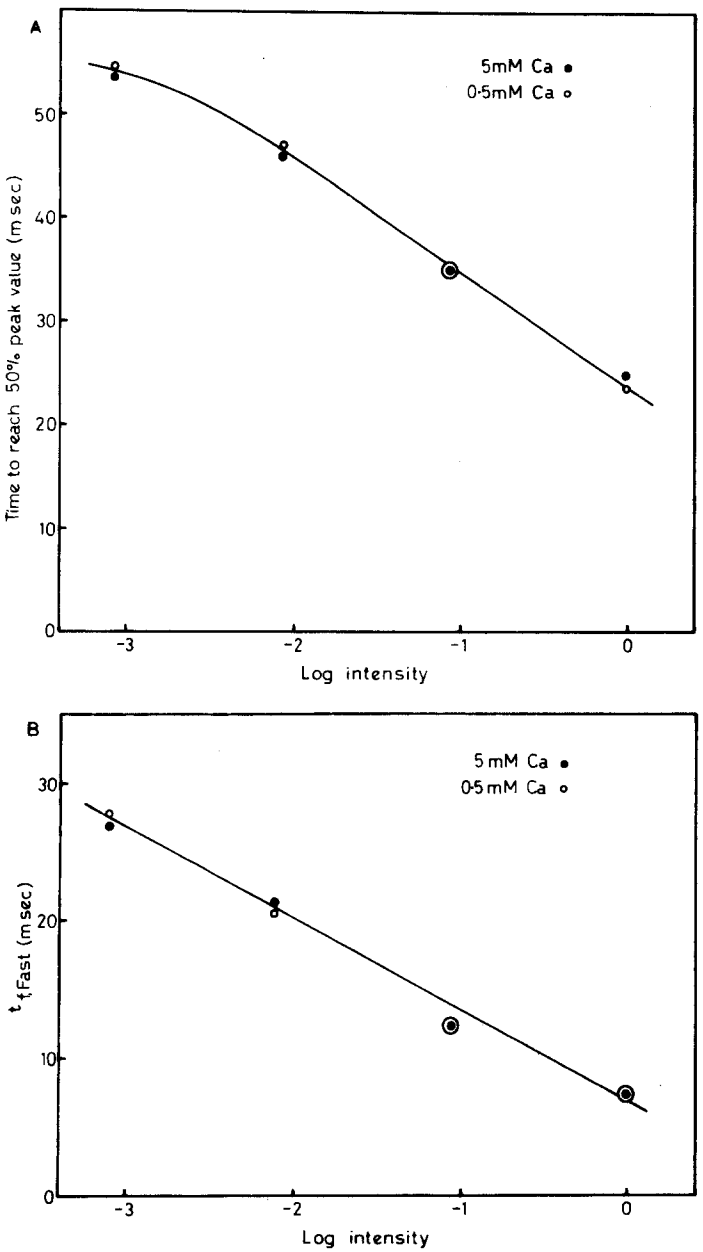


Fig. 17A and B

changes, just as the kinetics of the fast components are. A quantitative estimate of the effects of calcium on the kinetics and amplitude of the fast component was obtained by measuring the rise time ($t\hat{R}$), the fall time (t_F) and V_{max} from the distal records at a tip separation of 150 μm. The amplitude recovery kinetics of the slow

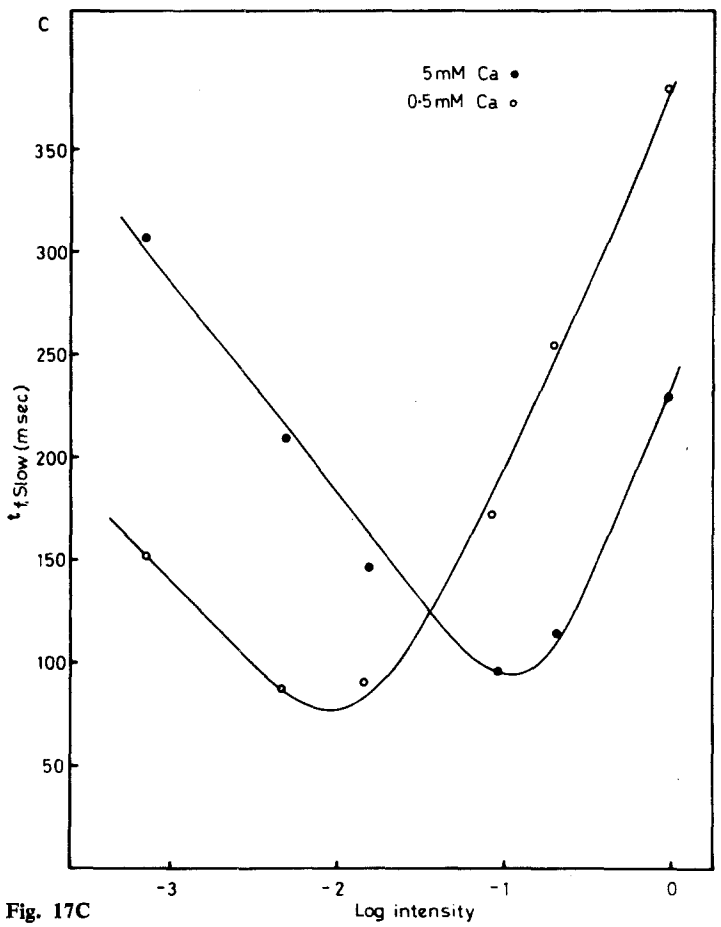


Fig. 17C

Fig. 17. Effect of Ca^{2+} concentration on waveform characteristics of fast and slow responses. (**A**) Effect of reduced Ca^{2+} on rise-time (t_R) of the fast component. Data obtained from V_D records for a tip separation of 175 μm. (**B**) Effect of reduced Ca^{2+} on decay-time (t_F) of the fast component. (**C**) Effect of reduced Ca^{2+} on decay-phase of the slow component measured from the corresponding records of V_S

component were estimated by reconstituting the surface response (as $V_s = V_D + V_P$, this was accomplished by simply adding the potentials recorded on V_D and V_P at convenient times after the stimulus onset). The amplitude data are summarised in Figure 16 and the kinetic data in Figure 17. The effect of calcium on the amplitudes of the fast component and the overall surface response are similar in that reducing the external calcium shifts the intensity required for half-maximal saturation to a lower value, i.e. the system is sensitized. The kinetics of the fast component (t_R and t_F) are independent of the external calcium concentration throughout the intensity range (Fig. 17A and B), whereas it is only at the very highest intensities that the amplitude does not vary with external calcium (Figs. 11 and 16). As observed above, the rising phase of the slow component is probably also independent of the external

calcium concentration. The fact that the kinetics of the fast component are also calcium independent means that the recovery phase of the surface response, an easily measured parameter, can be taken as a reliable indicator of changes in the recovery kinetics of the slow component. Once more it was observed that reducing the calcium concentration 10-fold is equivalent to increasing the stimulus intensity by about one log unit (Fig. 17C).

Discussion

Although the responses reported here were measured extracellularly and so need not bear a close resemblance to the photoreceptor transmembrane potential, there is a growing body of data indicating that extracellular and intracellular potentials in some invertebrate photoreceptors are quite closely related and that any difference that does exist is one of magnitude rather than degree. Fein and Charlton (1975) have reported simultaneous measurements of intracellular and extracellular potentials in *Limulus* ventral photoreceptors that show the two potentials have virtually the same time course in response to both short and long light flashes.

Stieve et al. (1978) go so far as to maintain that "simultaneous intracellular and extracellular recording of receptor potentials of the isolated crayfish retina reveal a correspondence sufficient to justify the use of only extracellular recording". Recently in this laboratory it has been shown (Duncan and Pynsent, 1977) that the time course of intracellular and extracellular potentials in *Sepiola* are also very similar and only begin to diverge at very high stimulus intensities. It is justifiable therefore to compare extracellular and intracellular measurements from other invertebrate photoreceptor systems. Before such a comparison is made, however, the stimulus conditions used in the various studies should be noted. Weeks and Duncan (1974) have already pointed out that, although the rate of rise of the *Sepiola* surface response is relatively insensitive to changes in external calcium when short intense flashes are used, the rising phase sharpens up in low calcium solutions when long flashes are employed. Similarly, the intensity of the flashes (whether long or short) is important because Millecchia and Mauro (1969a) have reported that reducing external calcium greatly increases signal height, while Stieve (1965, 1974) and Wulff (1973) have reported little or no significant effect of calcium on amplitude. Low intensity illumination was used in the first study and near-saturating flashes in the latter two.

The effects of Na^+ and K^+ concentration changes on the *Sepiola* ERG are qualitatively similar to those reported in previous studies on the cephalopod retina (Hagins et al., 1960; Hagins and Adams, 1962; Duncan and Weeks, 1973) and these effects will be interpreted in terms of the equivalent circuit given in Figure 18. Although the overall response consists of two major components, the slow response in fact dominates the response, especially at high intensities (Fig. 2) and so the model described relates primarily to the slow component. As chloride ions appear to play an insignificant role either in the maintenance of the dark resting potential or the photoresponse in all invertebrate photoreceptor systems studied so far (Millecchia and Mauro, 1969a; Brown et al., 1970; Hagins and Adams, 1962), probably be-

cause chloride ions distribute passively across photoreceptor membranes (Brown, 1976), the membrane potential was assumed to arise from a combination of the Nernst potentials for sodium and potassium alone. Analyses of the intracellular ion concentrations of the cephalopod retina (Adams and Hagins, 1960; Duncan et al., 1971; Hagins, 1965; Duncan and Weeks, 1973) have shown that like most cells the intracellular concentration of K^+ is large (240 mM in *Sepiola*) and that of Na^+ (250 mM) small compared with the respective extracellular values (20 mM K^+ and 460 mM Na^+). The sodium and potassium Nernst potentials calculated by Duncan and Weeks (1973) for *Sepiola* are +17 and −63 mV respectively and the intracellular resting potential is in the region of −50 mV (Hagins, 1965; Pynsent and Duncan, 1977). The ratio of sodium to potassium conductance obtained by applying these data to Equation (2) is 0.19 indicating that, as expected, the resting potassium conductance is higher than that of sodium. However, the ratio is much higher than that of squid nerve for example (Hodgkin, 1965), and as P_{Na}/P_K for barnacle photoreceptors can be as much as 0.5 it appears that generally photoreceptor membranes are more permeable to sodium than nerve membranes (Brown, 1976).

The maximum response that can be elicited from the photoreceptors (ΔV_m) is given by

$$\Delta V_m = E_{Na} - E_D\,, \tag{4}$$

and transmembrane light-induced responses of the order of 50 mV have recently been observed in *Sepiola* (Pynsent and Duncan, 1977). The results of this study indicate that the overall response amplitude is related to stimulus intensity by an expression of the form

$$\frac{\Delta V}{\Delta V_m} = \frac{I}{I + I_0}\,. \tag{1}$$

As shown in the Appendix, an expression formally resembling Equation (1) may be derived from the model if it is assumed that the change in Na^+ conductance induced by illumination is linearly related to stimulus intensity.

Changes in the intensity response characteristics elicited by changes in Na^+ and K^+ concentrations may be related to changes in ΔV_m, I_0 or both. The dramatic decrease in response amplitude observed in high K^+ throughout the entire intensity-response range (Fig. 5) is consistent with a decrease in ΔV_m and I_0 is apparently unchanged. Increasing K^+ decreases E_D (Equation 2) and hence ΔV_m decreases, whereas increasing K^+ would be expected to have little effect on g_K, g_{Na} or G and so, from Equation (9), I_0 is not affected. As the fast and slow components are similarly reduced on increasing K^+ (Fig. 6) this indicates that the two processes originate in the same cell or in two populations of cells with similar potassium permeability properties.

Reduction of Na^+ in the bathing solution, on the other hand, results in a dramatic increase in I_0 while ΔV_m is apparently little changed (Fig. 7B). At first sight this appears to be inconsistent with Equation (4) as a large change in external sodium would be expected to reduce E_{Na} and hence ΔV_m. However, Duncan and Weeks (1973) have produced indirect evidence suggesting that the intracellular concentra-

tion of sodium decreases when the external level is reduced. It is possible, therefore that the *transmembrane gradient* for sodium (and hence E_{Na}) remains relatively constant under these conditions, leaving ΔV_m relatively unchanged. It is now accepted that the sensitivity of invertebrate photoreceptors (i.e. I_0 in the present model) is controlled by the internal calcium level (Lisman and Brown, 1975; Bader et al., 1976) and I_0 increases with increasing internal Ca^{2+}. As sodium and calcium ions seem to compete for the sensitivity control mechanism (Brown et al., 1970) a reduction in the internal level of sodium might well account for the observed increase in I_0. However, as the fluxes of sodium and calcium are known to be linked in several membrane systems (Baker, 1972) it is also possible that a reduction in the external sodium level leads directly to an increase in internal calcium perhaps through an exchange diffusion system linking sodium influx to calcium efflux.

The persistence of photoresponses in the complete absence of Na^+ in the external bathing medium has been observed in numerous photoreceptive systems and several explanations have been proposed to account for this phenomenon. In the case of *Limulus* lateral eye photoreceptors, Kikuchi et al. (1962) suggested that Na^+ remained trapped in extracellular regions which were not easily accessible to changes in the external solution. Fulpius and Baumann (1969) observed large responses from retinular cells of *Apis* compound eyes which had been exposed to Na^+-free salines for as long as 12 h and postulated that both the retinular cells and the surrounding pigmented cells actively pump sodium into the small extracellular spaces near the light-sensitive rhabdomeres, thus maintaining a transmembrane Na^+ gradient adequate to produce photoresponses. A similar explanation recently been advanced to account for the differences between the photoresponse of dark and partially light-adapted *Limulus* lateral eye photoreceptors exposed to Na^+-deficient salines (Wulff et al., 1975).

In a number of photoreceptor systems, the response in the absence of Na^+ depends on the replacement used for Na^+ and this may be of relevance to the present observations. In both *Limulus* ventral (Brown and Mote, 1974) and *Astacus* (Stieve and Wirth, 1971) photoreceptors, choline$^+$ appeared to partially substitute for Na^+ during a light response. On the other hand, both sucrose and Tris$^+$ were reported to be impermeant in *Limulus* ventral photoreceptors (Brown and Mote, 1974). The photoresponses of the *Sepiola* retina in Na^+-free salines may be partly due to a light-induced permeability increase for the substitute ions.

The differential effect of Na^+ removal on the fast and slow components of the *Sepiola* photoresponse suggests either that different processes are responsible for their generation or that similar processes are located in different regions of the cell that would not be equally accessible to changes in the external ion concentrations. The photoresponse in *Limulus* lateral eye receptor also appears to consist of two components that are differentially sensitive to changes in external sodium concentrations (Wulff, 1973; Wulff and Mueller, 1973). However; in *Limulus,* the slow component (C_2) is more sensitive to changes in external sodium levels than the fast (C_1) component and it was suggested that the site of generation of C_1, assumed to be the rhabdomere, was much less accessible to the bathing solution than the superficial non-rhabdomeric membrane where C_2 was presumed to be produced (Wulff and Mueller, 1973). Although a similar effect may be responsible for the differential sensitivity to Na^+ of the fast and slow components of the *Sepiola* ERG, the fact that

Ca^{2+} depletion rapidly affects the time course of the slow component without notable changes in the fast (Figs. 11 and 17) argues against it, although it is always possible that Na and Ca have different penetration velocities and pathways.

As reducing external calcium does not seem significantly to change either the resting potential (E_D) or the reversal voltage for the light-stimulated voltage clamp currents (Millecchia and Mauro, 1969a and b; Brown and Mote, 1974) the model [Appendix Equations (4) and (9)] predicts that ΔV_m should be independent of external calcium concentration, which was indeed the case (Figs. 10 and 11). Reducing external calcium has, however, a dramatic effect on I_0 and a 10-fold reduction in external calcium is equivalent to a shift in I_0 of approximately one log unit in intensity. As the impedance of invertebrate photoreceptors is not significantly affected by changing external calcium (i.e., $g_K + g_{Na}$ is constant), then the change in G produced by a 10-fold reduction in external calcium may be found directly from Equation (9). G is calculated to increase 10-fold when the calcium concentration is reduced from 10 mM to 1 mM. Reducing external calcium therefore sensitizes the cephalopod photoreceptor cell, just as it does in *Limulus* photoreceptors (Millecchia and Mauro, 1969b; Brown et al., 1970). This increase in sensitivity may be a secondary consequence of in fact reducing the *internal* calcium concentration as Lisman and Brown (1975b) have shown that the conductance increase per photon (G) is greatly increased on injecting EGTA into *Limulus* ventral photoreceptors.

Reducing calcium also affected the time course of the response in *Sepiola* (Figs. 10 and 11). The effect on the recovery kinetics of the response (t_F) was to shift the minimum value to a lower intensity, and again a 10-fold reduction in external calcium was equivalent to a shift of a log unit of intensity (Figs. 12B and 17C). The data are remarkably similar to those obtained on reducing external sodium (Fig. 8C), only there the minimum was shifted to a higher intensity value which corresponded with a shift in I_0 towards a higher value in low sodium solutions. It thus appears that the time-course of the response and sensitivity are intimately connected in *Sepiola,* just as Fuortes and Hodgkin (1964) found they were in *Limulus.* However, in *Sepiola,* the kinetics of the rising phase of the response do not change with increased sensitivity in low calcium solutions (Figs. 11 and 12A). Brown and Lisman (1975) have recently shown that following iontophoretic injection of calcium into *Limulus* ventral photoreceptors (which desensitizes the cell), the rising phase of the response was significantly sharper than in the control. They were, however, using long flashes (> 100 ms) in their experiments and so the data are not directly comparable. In *Sepiola,* Weeks and Duncan (1974) found that reducing external calcium (sensitizing the system) sharpened the rate of rise of the response to a long flash and so it seems in this respect there is a basic difference in the behaviour of *Limulus* and *Sepiola* photoreceptors.

Most of the *Limulus* data on the effect of reducing external calcium on the response waveform has been carried out with very high stimulus intensities and so a prolonging of the decay phase of the response has generally been reported (Stieve, 1965, 1974; Millecchia and Mauro, 1969a and b). There are few, if any, reports of a sharpening of the decay phase that occurs at low intensities (Figs. 11 and 12). Reducing external calcium prolongs the decay phase of the squid action potential and the half-time for the sodium conductance shut down under anodal stimulation is increased from 90 μs at 112 mM Ca to 260 μs at 22 mM Ca (Frankenhauser and

Hodgkin, 1957). This change in kinetics is equivalent to a depolarisation of 10—20 mV, at a constant calcium level and so in the axon there is a relation between sensitivity and kinetics which is analogous to that observed in photoreceptors.

There seems little doubt that a light-stimulated increase in sodium permeability is involved in the generation of the fast component in *Sepiola* as this component appears to be abolished at all light levels in sodium-free saline (Figs. 8B and 9). It is unlikely that the slow component is a regeneratively driven amplification of the fast component as seems to be the case in vertebrate rods (Werblin, 1975; Arden, 1976), since it is still present after the fast component has been abolished. The amplitudes of both components are increased on reducing external calcium when non-saturating light intensities are used (Fig. 16), whereas the time course of the slow component alone is affected. Although the kinetics of the fast component are insensitive to changes in external calcium, they are sharpened by increasing the stimulus intensity. This can be most simply explained in terms of the RC characteristics of the photoreceptor membranes and any simple equivalent-circuit model that ultimately depends upon a primary conductance increase mechanism to set up the potentials (Fuortes and Hodgkin, 1964; Duncan and Croghan, 1973; Kramer, 1975) will predict such a behaviour. It is also likely, however, that the dynamics of the underlying conductance changes also contribute to the sharpening of the response kinetics, because the time-course of the light-stimulated currents in the voltage-clamped photoreceptors of *Limulus* are to some extent dependent on intensity (Lisman and Brown, 1975a).

The rate of rise of the slow component is probably not greatly influenced by the external calcium level (see for example log $I = 0$ responses in Figs. 11 and 15) whereas the decay rate is affected. As the recovery rate of the fast component is also independent of calcium (Fig. 17B), this implies that the changes in the recovery rate of the surface response (V_S) are under these conditions entirely due to changes in the decay of the slow component (Figs. 12B and 17C). This view is strengthened by the fact that recovery kinetics have the same U-shaped form in the absence of sodium and hence of the fast component (Fig. 8C). The decay rate increases at first with increasing light intensity, as might be expected from a simple RC-limited response (Fuortes and Hodgkin, 1964; Duncan and Croghan, 1973). However, at a certain intensity value a minimum is reached and the decay (t_F) is prolonged with increasing intensity, suggesting that a substance (or mechanism) involved in the recovery phase becomes depleted at high intensities.

Light stimulated voltage clamp currents in *Limulus* (Millecchia and Mauro, 1969b) and barnacle (Brown et al., 1970) are much greater in low calcium salines than in the control solution and so it has been suggested that calcium ions control the magnitude of the primary sodium conductance increase. Brown et al. (1970) and Stieve (1965) have further suggested that calcium ions can compete with sodium for the light-induced pores or carriers. Competition between sodium and calcium is a feature of many membrane-based phenomena and such a competition could explain much of the data presented here (Figs. 7B and C, 10, and 17C). Although there is no direct evidence that a sodium permeability change alone is responsible for generating the slow component, it seems unlikely that calcium ions carry a significant fraction of the current in the normal medium (460 mM Na) as reducing external calcium (and so presumably reducing the calcium gradient across the photoreceptor membrane) actually increases the response rather than decreasing it (Figs. 10 and 16). However,

it is possible that calcium ions do contribute significantly in the absence of sodium as appears the case in *Balanus* (Brown et al., 1970) and in *Eupagarus* (Stieve, 1974).

The fast and slow components seen here may bear some relation to the two channels for sodium and calcium ions that have been observed in invertebrate nerve (Baker, 1974), giant synapse (Llinas et al., 1972), and molluscan neurones (Stinnacre and Tauc, 1973). The system with two channels that has probably been investigated in most detail is the vertebrate cardiac muscle membrane (Noble, 1974) and there the fast channel is sodium specific and is blocked by tetrodotoxin (TTX). There is some evidence that TTX blocks the initial component of the *Limulus* lateral eye photoresponse (Benolken and Russell, 1967), although it should be noted others have failed to find such an effect (Dowling, 1968; Wulff and Mendez, 1973). The second channel in cardiac muscle membranes allows both calcium and sodium through and the current is determined by the ratio $(Ca_0)/(Na_0)^2$ (Rougier and Vassort, 1971) just as the late (> 50 ms) voltage clamp currents are in barnacle photoreceptors (Brown et al., 1970). The second channel in heart is blocked by manganese ions (Rougier et al., 1969) and Wulff and Mendez (1973) have shown that the C_2 component in *Limulus* lateral photoreceptors is much more sensitive to manganese than is C_1. As the second channel in heart muscle inactivates much more slowly than the first, this channel determines the falling phase of the action potential (Noble, 1974). It also appears that the slow phase dominates the overall recovery kinetics in *Sepiola* (Figs. 8, 12B, and 17C).

Acknowledgements. We wish to thank Messrs. P. Trett, F. Symons, and F. Moore for supplying the *Sepiola* specimens, Mr. A. R. Bushell for preparing the retina sections and Mrs. G. M. Rowell for typing the manuscript. We also wish to thank Dr. Peter C. Croghan for many stimulating discussions and Professors Eduardo Rojas and Hennig Stieve, Dr. John Noble-Nesbitt and Mr. Paul B. Pynsent for their critical comments on the manuscript. R. C. acknowledges the receipt of a scholarship from the Commonwealth Scholarship Commission.

The data in this communication were presented in a preliminary form at the EMBO Workshop on Transduction Mechanism of Photoreceptors held in Julich, West Germany (October, 1976).

Appendix

Equivalent circuit models have long been used to interpret electrophysiological data from invertebrate photoreceptors (Fuortes, 1959; Rushton, 1959; Fuortes and Hodgkin, 1964; Duncan and Croghan, 1973) and the model shown in Figure 18 is one of the simplest possible. Assuming that the membranes are permeable only to sodium and potassium then the membrane potential is given by

$$E = \frac{1}{g_K + g_{Na}} (g_K E_K + g_{Na} E_{Na}) , \tag{2}$$

where g_K and g_{Na} are the conductances of potassium and sodium respectively and E_K and E_{Na} are the corresponding Nernst potentials. In the dark, the resting potential is E_D. If it is also assumed that in the light g_{Na} increases to g'_{Na} (Hagins, 1964; Duncan and Weeks, 1973), then the membrane potential will be displaced from its resting level by an amount ΔV, where

$$\Delta V = \frac{1}{g_K + g'_{Na}} (g_K E_K + g'_{Na} E_{Na}) - E_D . \tag{3}$$

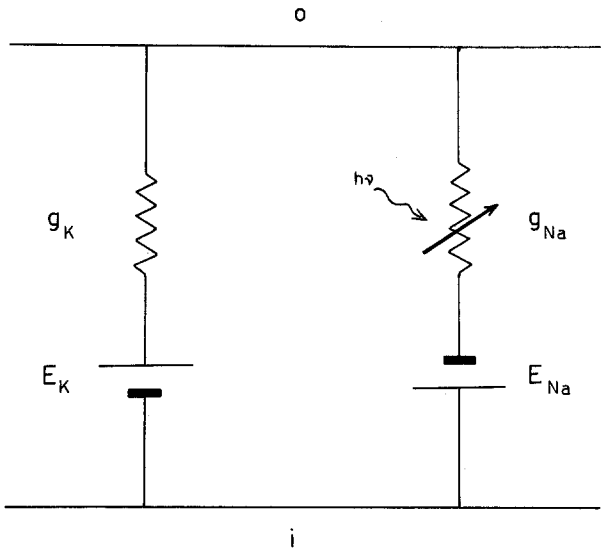


Fig. 18. Equivalent circuit for the photoreceptor membrane. g_K and g_{Na} are the conductances for potassium and sodium respectively and E_K and E_{Na} are the corresponding Nernst potentials. The sodium conductance is assumed to increase linearly with light intensity and on stimulation, current will flow from the outside phase (*o*) to the inside (*i*)

The maximum or "saturation" value of ΔV, ΔV_m is obtained in the limit of very intense lights where $g'_{Na} \gg g_K$. Under these conditions Equation (3) reduces to

$$\Delta V_m = E_{Na} - E_D \,. \tag{4}$$

Because E_{Na} and E_D have different polarities, ΔV_m is larger than the resting potential.

Assuming there is a linear relation between g'_{Na} and light intensity I, then

$$g'_{Na} = g_{Na} + GI \,, \tag{5}$$

where G is the coefficient connecting light intensity and the light-induced change. G effectively measures the Na^+ conductance change per photon absorbed and in fact Lisman and Brown (1975a) have recently shown from voltage-clamp measurements that G is linearly related to I in *Limulus* over a very wide intensity range. This is in contrast to the situation in vertebrate cones for example (Baylor et al., 1974), where a hyperbolic relation between conductance and intensity seems to apply. Equation (5) can only be valid providing I is not so large that it saturates the number of sodium channels available. This again seems likely from the *Limulus* data (Lisman and Brown, 1975a) which show that the voltage response saturates many log units of intensity before the voltage clamp currents.

Substituting Equation (5) into Equation (3) and rearranging gives:

$$\Delta V = \frac{GI}{g_K + g_{Na} + GI}\left[\frac{g_K}{g_{Na} + g_K}(E_{Na} - E_K)\right] . \tag{6}$$

It can readily be shown that Equation (4) may be written in the form

$$\Delta V_m = \frac{g_K}{g_K + g_{Na}}(E_{Na} - E_K) \tag{7}$$

and therefore Equation (6) becomes

$$\frac{\Delta V}{\Delta V_m} = \frac{I}{I + I_0}, \tag{8}$$

where

$$I_0 = \frac{g_K + g_{Na}}{G} \tag{9}$$

is the "half-saturation" intensity.

Equation (8) is formally identical to Equation (1) which was fitted to the short flash intensity-response relations (Fig. 4). The light-induced potentials measured in the present experiments are not transmembrane potentials but extracellular potentials between the photosensitive distal segments and the cell bodies of the photoreceptor cells. Although the time course of the extracellular potential is not identical to that of the intracellular at all intensities, the amplitudes of the two responses are in proportion and indeed are almost comparable throughout most of the intensity response range (Pynsent and Duncan, 1977).

List of the More Important Symbols

V_s Potential of fixed electrode at the surface of retina relative to grounded electrode
V_p Potential of moveable electrode relative to ground
V_D $V_s - V_p$
ΔV_m Maximum value of V_s produced by saturating light intensities
I_0 Stimulus required to evoke a response of half-maximal amplitude
g_K Membrane potassium conductance
g_{Na} Membrane sodium conductance
E_K Nernst potential for potassium
E_{Na} Nernst potential for sodium
G Coefficient connecting light intensity and light-induced change of conductance
t_R Time, measured from the stimulus onset, for the response to reach 50% of peak amplitude
t_F Time, measured from the time to peak, for the response to fall to 50% of its maximal value

References

Adams, R. G., Hagins, W. A.: The ionic composition of squid photoreceptors. Biol. Bull **119,** 300–301 (1960)

Arden, G. B.: Voltage gradients across the receptor layer of the isolated rat retina. J. Physiol. (Lond.) **256,** 333–360 (1976)

Bader, C. R., Baumann, F., Bertrand, D.: Role of intracellular calcium and sodium in light adaptation in the retina of the honey bee drone (*Apis mellifera,* L.). J. gen. Physiol. **67,** 415–491 (1976)

Baker, P. F.: Transport and metabolism of calcium ions in nerve. Progr. Biophys. molec. Biol. **24,** 177–223 (1972)

Baker, P. F.: Excitation-secretion coupling. In: Recent Advances in Physiology (ed. R. J. Linden), p. 51–86. London: Churchill Livingstone 1974

Baylor, D. A., Fuortes, M. G. F.: Electrical responses of single cones in the retina of the turtle. J. Physiol. (Lond.) **207,** 77–92 (1970)

Baylor, D. A., Hodgkin, A. L., Lamb, T. D.: Reconstruction of the electrical responses of turtle cones to flashes and steps of light. J. Physiol. (Lond.) **242,** 759–791 (1974)

Benolken, R. M., Russell, C. J.: Tetrodotoxin blocks a graded response of the eye of *Limulus.* Science **155,** 1576–1577 (1967)

Brown, J. E., Lisman, J. E.: Intracellular calcium modulates sensitivity and time scale in Limulus ventral photoreceptors. Nature (Lond.) **258,** 252–254 (1975)

Brown, J. E., Mote, M. I.: Ionic dependence of reversal voltage of the light response in *Limulus* ventral photoreceptors. J. gen. Physiol. **63,** 337–350 (1974)

Brown, H. M.: Intracellular Na, K and Cl activities in *Balanus* photoreceptors. J. gen. Physiol. **68,** 281–296 (1976)

Brown, H. M., Hagiwara, S., Koike, H., Meech, R. M.: Membrane properties of a barnacle photoreceptor examined by the voltage clamp technique. J. Physiol. (Lond.) **208,** 385–413 (1970)

Chase, R.: The electrophysiology of photoreceptors in the nudibranch mollusc, *Tritonia diomeda.* J. exp. Biol. **60,** 707–719 (1974)

Clark, R. B.: Components of the cephalopod electroretinogram. Exp. Eye Res. **20,** 499–504 (1975)

Cohen, A. I.: An ultrastructural analysis of the photoreceptors of the squid and their synaptic connections. I. Photoreceptive and non-synaptic regions of the retina. J. comp. Neurol. **147,** 351–378 (1973)

Daw, N. W., Pearlman, A. L.: Pigment migration and adaptation in the eye of the squid *Loligo pealei.* J. gen. Physiol. **63,** 22–36 (1974)

Dowling, J. E.: Discrete potentials in the dark-adapted eye of the crab *Limulus.* Nature (Lond.) **217,** 28–31 (1968)

Dowling, J. E., Ripps, H.: Adaptation in skate photoreceptors. J. gen. Physiol. **60,** 698–719 (1972)

Duncan, G., Croghan, P. C.: Electrical activity of the isolated cephalopod retina: An equivalent circuit model. Exp. Eye Res. **15,** 401–408 (1973)

Duncan, G., De Pont, J. J. H. H. M., Bonting, S. L.: Biochemical aspects of the visual process. X. Distribution and movements of ions in the dark-adapted retina of the cuttlefish (*Sepia officinalis*). Pflügers Arch. **322,** 264–277 (1971)

Duncan, G., Pynsent, P. B.: Intracellular and extracellular photoreceptor potentials in the retina of the cephalopod *Sepiola atlantica.* J. Physiol. (Lond.) **267,** 36–37P (1977)

Duncan, G., Weeks, F. I.: Photoreception by a cephalopod retina in vitro. Exp. Eye Res. **17,** 183–192 (1973)

Fein, A., Charlton, J. S.: Local membrane current in *Limulus* photoreceptors. Nature (Lond.) **258,** 250–252 (1975)

Frankenhauser, B., Hodgkin, A. L.: The action of calcium on the electrical properties of squid axons. J. Physiol. (Lond.) **137,** 218–244 (1957)

Fulpius, B., Baumann, F.: Effects of sodium, potassium and calcium ions on slow and spike potentials in single photoreceptor cells. J. gen. Physiol. **53,** 541–561 (1969)

Fuortes, M. G. F.: Initiation of impulses in visual cells of *Limulus.* J. Physiol. (Lond.) **148,** 14–28 (1959)

Fuortes, M. G. F., Hodgkin, A. L.: Changes in time scale and sensitivity in the ommatidia of *Limulus.* J. Physiol. (Lond.) **148,** 14–28 (1964)

Fuortes, M. F. G., O'Bryan, P. M.: Generator potentials in invertebrate photoreceptors. In: Handbook of Sensory Physiology, vol. 7 (2) (ed. M. G. F. Fuortes), pp. 279–319. Berlin-Heidelberg-New York: Springer 1972

Gillary, H. L.: Light-evoked electrical potentials from the eye and optic nerve of *Strombus:* response waveform and spectral sensitivity. J. exp. Biol. **60,** 383–396 (1974)

Hagins, W. A.: Electrical signs of information flow in photoreceptors. Cold Spr. Harb. Symp. quant. Biol. **30,** 403–415 (1965)

Hagins, W. A., Adams, R. G.: The ionic basis of the receptor current of squid photoreceptors. Abstr. 22nd Int. Congr. Physiol. Sci., Leiden, Abstr. 970. Amsterdam: Excerpta Medica 1962

Hagins, W. A., Adams, R. G., Wagner, H. G.: Light-induced current from the receptors of the squid retina. Biol. Bull. (Woods Hole). **119,** 317 (1960)

Hodgkin, A. L.: Conduction of the nervous impulse. Liverpool University Press 1964

Jacklet, J. W.: Electrophysiological organization of the eye of *Aplysia.* J. gen. Physiol. **53,** 21–42 (1969)

Kikuchi, R., Naito, K., Tanaka, I.: Effect of sodium and potassium ions on the elctrical activity of single cells in the lateral eye of the horseshoe crab. J. Physiol. (Lond.) **161,** 319–343 (1962)

Kramer, L.: Interpretation of invertebrate photoreceptor potentials in terms of a quantitative model. Biophys. Struct. Mechanism **1,** 239–257 (1975)

Lisman, J. E., Brown, J. E.: Light-induced changes of sensitivity in *Limulus* ventral photoreceptors. J. gen. Physiol. **66,** 473–488 (1975a)

Lisman, J. E., Brown, J. E.: Effects of intracellular injection of calcium buffers on light adaptation in *Limulus* ventral photoreceptors. J. gen. Physiol. **66,** 489–506 (1975b)

Llinas, R., Blinks, J. R., Nicholson, C.: Calcium transient in presynaptic terminal of squid giant synapse: detection with aequorin. Science **176,** 1127–1129 (1972)

Millecchia, R., Mauro, A.: The ventral photoreceptor cells of *Limulus* II. The basic photoresponse. J. gen. Physiol. **54,** 310–330 (1969a)

Millecchia, R., Mauro, A.: The ventral photoreceptor cells of *Limulus* III. A voltage-clamp study. J. gen. Physiol. **54,** 331–351 (1969b)

Naka, K., Eguchi, E.: Spike potentials recorded from the insect photoreceptor. J. gen. Physiol. **45,** 663–680 (1962)

Naka, K., Kuwabara, M.: Two components from the compound eye of the crayfish. J. exp. Biol. **36,** 51–61 (1959)

Noble, D.: Cardiac action potentials and pacemaker activity. In: Recent Advances in Physiology (ed. R. J. Linden), p. 1–50. London: Churchill Livingstone 1974

Penn, R. D., Hagins, W. A.: Kinetics of the photocurrent of retinal rods. Biophys. J. **12,** 1073–1094 (1972)

Pynsent, P. B., Duncan, G.: Reconstruction of photoreceptor membrane potentials from simultaneous intracellular and extracellular recordings. Nature (Lond.) **269,** 257–259 (1977)

Robertson, J. D.: Further studies on ionic regulation in marine invertebrates. J. exp. Biol. **30,** 277–296 (1953)

Rodieck, R. W.: The vertebrate retina: principles of structures and function. San Fransisco: Freeman 1973

Rougier, O., Vassort, G., Garnier, D., Gargouie, Y. M., Coraboeuf, E.: Existence and role of slow inward current during the frog atrial action potential. Pflügers Arch. **308,** 91–110 (1969)

Rougier, O., Vassort, G.: Interaction des ions sodium et calcium dans le courant entrant lent et la contraction des fibres auriculaires; cardiaques de grenouille. J. Physiol. (Paris) **63,** 92A (1971)

Rushton, W. A. H.: A theoretical treatment of Fuortes's observations upon eccentric cell activity in *Limulus*. J. Physiol. (Lond.) **148,** 29–38 (1959)

Stieve, H.: Das Belichtungspotential der isolierten Retina des Einsiedlerkrebses (*Eupagarus bernhardus* L.) in Abhängigkeit von den extrazellulären Ionenkonzentrationen. Z. vergl. Physiol. **47,** 457–492 (1964)

Stieve, H.: Interpretations of the generator potential in terms of ionic processes. Cold Spr. Harb. Symp. quant. Biol. **30,** 451–456 (1965)

Stieve, H.: On the ionic mechanisms responsible for the generation of the electrical response of light sensitive cells. In: Biochemistry of Sensory Functions (ed. L. Jaenicke). Berlin-Heidelberg-New York: Springer 1974

Stieve, H., Bruns, M., Gaube, H.: Simultaneous recording by extra and intracellular electrodes of light responses in the crayfish retina. Vision Res. (in press, 1978)

Stieve, H., Gaube, H., Malinowska, T.: The effect of tetrodotoxin, veratridine and tetraethylammonium chloride on the receptor potential of the crayfish photoreceptor cell. Z. Naturforsch. **28c,** 149–156 (1972)

Stieve, H., Wirth, C.: Über die Ionenabhängigkeit des Rezeptorpotentials der Retina von *Astacus leptodactylus*. Z. Naturforsch. **26b,** 457–470 (1971)

Stinnacre, J., Tauc, L.: Calcium influx in active *Aplysia* neurones detected by injected aequorin. Nature (Lond.) New Biol. **242,** 113–115 (1973)

Tomita, T.: The electroretinogram, as analysed by microelectrode studies. In: Handbook of Sensory Physiology, vol. 7 (2) (ed. M. G. F. Fuortes), pp. 635–665. Berlin-Heidelberg-New York: Springer 1972

Weeks, F. I., Duncan, G.: Photoreception by a cephalopod retina: response dynamics. Exp. Eye Res. **19,** 483–509 (1974)

Werblin, F. S.: Regenerative hyperpolarisation in rods. J. Physiol. (Lond.) **244,** 53–81 (1975)

Wulff, V. J.: The effect of sodium, potassium and calcium on *Limulus* lateral eye retinular cells. Vision Res. **13,** 2309–2326 (1973)

Wulff, V. J., Mendez, C.: Effect of manganous chloride and tetrodotoxin on *Limulus* lateral eye retinular cell. Vision Res. **13,** 2327–2333 (1973)
Wulff, V. J., Mueller, W. J.: On the origin of the receptor potential in the lateral eye of *Limulus*. Vision Res. **13,** 661–671 (1973)
Wulff, V. J., Stieve, H., Fahy, J. L.: Dark adaptation and sodium pump activity in *Limulus* lateral eye retinular cells. Vision Res. **15,** 756–765 (1975)
Yamamoto, T., Tasaki, K., Sugawara, Y., Tonosaki, A.: Fine structure of the octopus retina. J. Cell Biol. **25,** 345–359 (1965)
Young, J. Z.: Regularities in the retina and optic lobes of *Octopus* in relation of form discrimination. Nature (Lond.) **186,** 836–839 (1960)
Young, J. Z.: The retina of cephalopods and its degeneration after optic nerve section. Phil. Trans. B. **245,** 1–18 (1962)
Zonana, H. V.: Fine structure of the squid retina. Bull. Johns Hopk. Hosp. **109,** 185–205 (1961)

Received August 12, 1977/Accepted January 24, 1978