Adaptation and Facilitation in the Barnacle Photoreceptor

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ABSTRACT The barnacle photoreceptor sensitivity may either decrease (light adaptation) or increase (facilitation) after exposure to a conditioning light. The balance between adaptation and facilitation is influenced by at least three factors: initial sensitivity state of the cell, external calcium concentration, and conditioning intensity. Cells of very high sensitivity show mainly adaptation, which appears only for higher conditioning intensities and is suppressed in low-calcium media. Less sensitive cells, or those whose sensitivity is reduced by injury or metabolic decay, exhibit facilitation, especially in low-calcium media and at intermediate conditioning intensities. Both phenomena show recovery time-courses of seconds-to-minutes. Models are proposed which relate light adaptation, as previously suggested, to increased internal calcium concentration, and facilitation either to decreased internal calcium concentration or to decreased activation "affinity" of ion-channel-blocking sites.

INTRODUCTION

One of the most interesting and functionally important properties of sensory receptors is their ability to change their characteristics temporarily, in particular their sensitivities, as a result of sensory stimulation. In photoreceptors, as in other receptors, the strongest and most obviously physiologically useful effect is *adaptation,* the reduction of sensitivity after intense illumination (light adaptation) and the subsequent recovery (dark adaptation).[1] However, *facilitation,* the enhancement of sensitivity after illumination, has also occasionally been seen in some preparations. Because of its relative rarity and apparent capriciousness, there have been far fewer investigations of facilitation than of adaptation, none of them systematic.

Adapatation has been seen in all photoreceptors in which it has been sought. Normann and Werblin (1974) review the literature to date in vertebrates, and Fein and DeVoe (1973) and Srebro and Behbehani (1974) in invertebrates.

Regarding the mechanism of adaptation, Lisman and Brown (1972) and Brown and Lisman (1974) have recently provided evidence that the internal Ca^{++} concentration may be closely involved in the (invertebrate) adaptation process. How light controls the $[Ca^{++}]_{in}$ is, however, unclear. Several authors have correlated the state of adaptation to the state of the pigment, suggesting a

[1] In this article, "adaptation" will generally be used to mean light adaptation, except where otherwise specified.

desensitizing effect of the photoproducts (Donner and Reuter, 1967; Sillman et al., 1972; Mainster and White, 1972). However, after illumination, the invertebrate visual pigment recovers to a state indistinguishable from the original state by early receptor potential measurements much more rapidly than the sensitivity recovers its "dark" value (Fein and DeVoe, 1973; Hillman et al., 1973).

Facilitation has been observed in electroretinogram (ERG) measurements and in extracellular and intracellular single-unit recordings (Ruck and Jahn, 1954; Stratten and Ogden, 1971; DeVoe, 1972; Shaw, 1972; Lange and Hartline, 1974), but no attempt has been made to determine possible mechanisms.

In this article we examine the balance between the two phenomena in a single preparation, the barnacle photoreceptor, in which both are apparently always present in varying degree. We demonstrate differential dependences of the two phenomena on three different parameters: the state of sensitivity of the dark-adapted cell, as determined by the intensity of a flash needed to elicit a small criterion response, external Ca^{++} concentration, and conditioning intensity. Finally, we sketch tentative models to encompass adaptation and facilitation, in the framework of the transduction process as a whole.

METHODS

The lateral ocelli of *Balanus amphitrite* were excised together with a short section of nerve. They were mounted in a chamber perfused either with "normal" barnacle Ringer (Brown et al., 1970) containing 10 or 20 mM Ca^{++}, or with a low-Ca^{++} Ringer containing 0.5 mM Ca^{++}, the rest of the Ca^{++} replaced by Mg^{++}. Mg^{++} does not appear to have any specific effect on a related photoreceptor (Millecchia and Mauro, 1969). Furthermore, very similar results were obtained by us in a few experiments in which the Ca^{++} was replaced by sucrose instead of Mg^{++}. In a few cases, the Ca^{++} concentration was buffered to below 10^{-6} M by EGTA; no substantial differences from the low-Ca^{++} Ringer were observed, except that the effects were sometimes less reversible. Before the experiments, the bath was perfused for 2 min with 1½% collagenase and 1½% protease in seawater. A corneal approach was then used for intracellular recording with 2 M KCl-filled micropipette electrodes. A quartz-iodide lamp provided white light whose unattenuated intensity was about 1×10^{16} photons/cm²/s/nm at 550 nm at the photoreceptor. This intensity corresponds to an absorption of about 100 photons per pigment molecule per second. Intensities are given as the logarithms of their ratios to this value. Calibrated neutral density filters and an electromagnetic shutter provided stimuli of the required intensity and duration. The sensitivity was examined by two alternative methods. In the first (Figs. 5 and 6), applicable where slow changes were to be followed and where the sensitivity change was large, the intensity of a 40-ms test flash needed to elicit a response 3.5 mV in amplitude was determined before and after a 400-ms conditioning "flash" of variable intensity. Use of other criterion responses (7 or 8 mV) did not alter the results. In the other method (Figs. 2–4 and 7), weak (40 ms, $\log I = -8.5$ to -5.0) test flashes of fixed strength were used, and the ratio R was measured of the response amplitudes at various times after the strong conditioning flash (here also 40 ms long) to the response amplitude before the conditioning flash. In a few experiments (see *Resistance Changes* section) voltage clamp was used and so current amplitude was recorded instead of voltage amplitude. The "*sensitivity state*" of a cell is defined as $S = -\log I$, where I is the intensity of a 40-ms test flash needed to elicit a 3.5-mV response in the cell *when dark adapted*. That is, it is the asymptote of the *sensitivity* after a conditioning flash. All test flashes were sufficiently weak as not to affect appreciably the cell sensitivity.

OBSERVATIONS

Fig. 1 illustrates some observations of light adaptation (top row) and of facilitation (bottom row), in three fairly sensitive cells. Weak test flashes were presented at 5-s intervals, with one test flash replaced by a stronger conditioning flash. Traces, B, D, and F show that adaptation may be converted into facilitation by spontaneous decline of sensitivity, by reduced conditioning intensity, or by reduced external calcium concentration, respectively. We now examine these three types of observation more quantitatively.

Effects of Sensitivity State

Cells are found to have sensitivity states S (dark-adapted sensitivities) ranging over several log units. Since the highest S values are found in fresh cells carefully penetrated with fine electrodes (resistance over 15 MΩ, tip diameter less than 0.5 μm), most of this range may be due to injury and/or biochemical decay. S can decline spontaneously or can be reduced by mechanical injury or very bright illumination. S is found to be closely correlated with cell membrane potential; high S values are found only for $-V_m > 40$ mV, and are associated with strong poststimulus hyperpolarizations (as in Fig. 1). These hyperpolarizations are ascribed by Koike et al. (1971) to a metabolically driven electrogenic Na^+ pump. Accordingly, metabolic state is probably a major determinant of S. Since internal calcium concentration is also known to control S (Lisman and Brown, 1972; Brown and Lisman, 1974), the metabolic state may be acting on S through $[Ca^{++}]_{in}$. As a partial check on this, we perfused the preparation chamber with a very low concentration (5 μM) of ruthenium red. Ruthenium red inhibits Ca^{++} uptake by mitochondria (Moore, 1971) without apparently directly affecting metabolic state. S is reduced by some 3 log units a few minutes after application

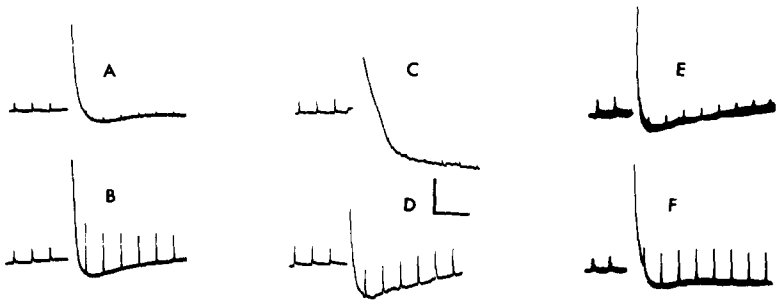

FIGURE 1. Samples of facilitation and adaptation. Intracellular recordings from *B. amphitrite* at 22 ± 1°C. Test flashes (40-ms duration, log intensity −7.5 for A and C–F, −5.0 for B) were presented every 5 s; one was replaced by a conditioning flash (40 ms, log I = −1.0 for A–C and −3.0 for D–F). The top traces show adaptation in three fairly sensitive cells for quite strong conditioning flashes, all in normal-calcium media (20 mM). The bottom traces illustrate facilitation in the same three cells brought about, respectively, by a spontaneous decline in cell sensitivity (B), a decrease by ×100 in the conditioning intensity (D), and a decrease in the Ca^{++} concentration of the medium to 0.5 mM (F). Calibration bars: horizontal, 10 s; vertical, 10 mV for A, B, and D–F, 20 mV for C.

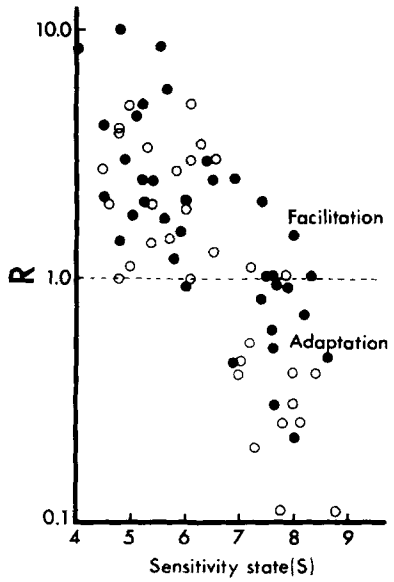

FIGURE 2. The correlation of the facilitation-adaptation balance with the sensitivity state S of the cell. S is defined by $S = -\log I$, where I is the intensity of a 40-ms flash needed to produce a 3.5-mV response in the dark-adapted cell. R is the ratio of the amplitude of the response to a weak test flash 10 s after a conditioning flash (here 40 ms, $\log I = -2$) to the amplitude before the conditioning flash (dark adapted). Note the log scale for R. $R < 1$ is adaptation, $R > 1$ is facilitation. The data are from 45 cells. The open and filled circles are for $[Ca^{++}]_{out} = 20$ and 10 mM, respectively.

of the ruthenium red. The effect is partially reversible. $[Ca^{++}]_{in}$ may thus be the major common pathway of S control.

Whatever the source of variation in S, there appears a clear correlation between the facilitation-adaptation balance and S, for fixed $[Ca^{++}]_{out}$. Fig. 2 shows measurements of R (see Methods) for 45 cells in two near-normal external Ca^{++} concentrations, for a fixed conditioning intensity. R shows a strong downward trend, from facilitation to adaptation, with increasing S.

Effects of Conditioning Intensity

The dependence of R on log conditioning intensity is displayed for a fairly sensitive cell ($S = 7.2$) in a normal medium in the lower curve of Fig. 3 A. With time, the sensitivity of this cell declined, and with S at 4.9, the upper curve of Fig. 3 A was obtained. The adaptation seen in the cell in the high-S state appears to have an intensity dependence different from (and not only of reversed direction to) that of the facilitation in the low-S state. This is confirmed by our sometimes finding "biphasic" dependences of R on intensity in cells with intermediate S, as in Figs 3 B(3) and C (upper).

Similar changes were observed with similar shifts in S induced by application of ruthenium red to a high-S cell. Fig. 3 B shows the initial intensity dependence

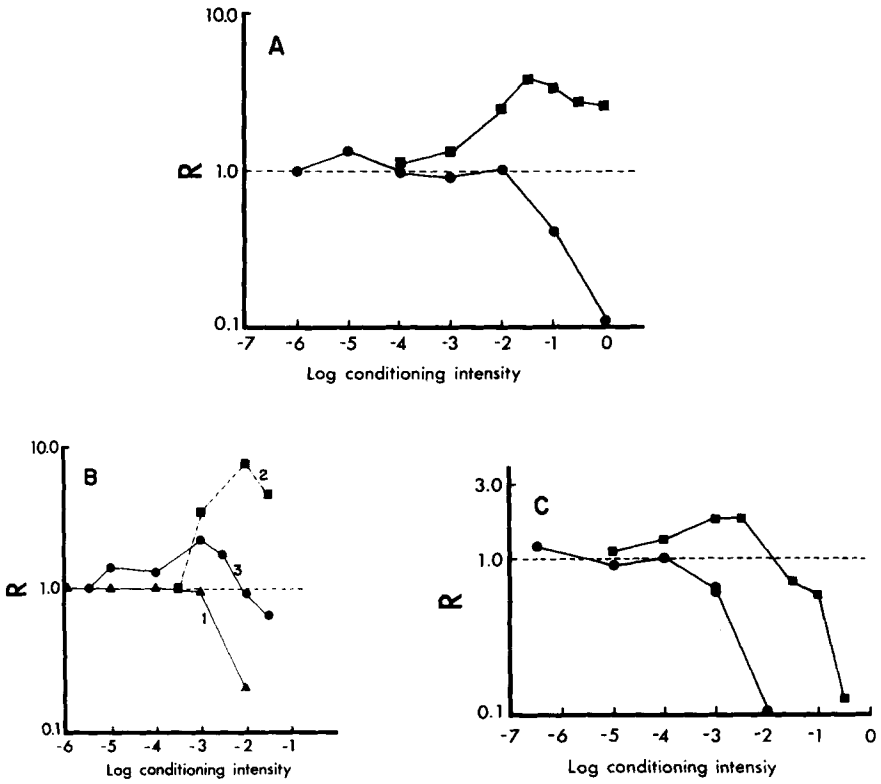

FIGURE 3. The dependence of facilitation/adaptation on conditioning intensity under various conditions affecting the sensitivity state S. Conditioning flash duration 40 ms. $[Ca^{++}]_{out}$ was 10 mM (A and B) or 20 mM (C). (A) The effect of spontaneous decay. The lower curve is for a cell of intermediate-high sensitivity (S = 7.2) and shows mainly adaptation. When the sensitivity of the cell had decayed some hours later to 4.9 the cell only facilitated (upper curve). (B) The effect of ruthenium red (a Ca^{++} decoupler). Curve 1, S = 8.0, was transformed into curve 2, S = 4.0 a few minutes after addition of 5 μM ruthenium red to the medium. Partial recovery to curve 3, S = 6.0, occurred after washing with normal Ringer. (C) The effect of injury. A very sensitive cell (lower curve, S = 8.8) was injured by withdrawal and reinsertion of the electrode (upper curve, S = 7.0).

of R (curve 1, S = 8.0), the effect of the ruthenium red application (curve 2, S = 4.0) and the partial recovery of the R curve after washing in normal Ringer solution (curve 3, S = 6.0).

Similar changes in the R curves also occur for similar shifts in S induced by injury. In Fig. 3 C, the lower curve was recorded in a cell with a high S = 8.8. The electrode was then removed and reinserted; S was now found to be 7.0 and the upper curve was observed.

In summary: In sensitive cells, one sees mainly adaptation ($R < 1$), and only for high conditioning intensities. If S is reduced by decay with time, by application of ruthenium red, or by injury, mainly facilitation ($R > 1$) is seen. This

facilitation appears at lower conditioning intensities than the adaptation, and increases less abruptly.

The time dependence of the recovery from light adaptation (dark adaptation) and from facilitation are shown in Figs. 4 and 5. The lower curve of Fig. 4 is the dark adaptation of a fairly sensitive cell (S = 7.2) and the upper curve the recovery from facilitation in the *same* cell following the *same* conditioning intensity after S had spontaneously declined to 5.0.

For cells of very high sensitivity and for strong conditioning stimuli, the light-induced suppression of sensitivity is so large that the response to a fixed test flash is depressed far below noise, and the R method is inapplicable. The more laborious criterion-response approach is therefore used (see Methods). Fig. 5 shows the initial light adaptation and the time-course of the dark adaptation for a sensitive cell after conditioning flashes of four different intensities. Both the degree of initial sensitivity suppression and the time scale of the recovery are graded with conditioning intensity.

External Calcium Concentration

Fig. 6 demonstrates the effect of decreasing external Ca^{++} concentration $[Ca^{++}]_{out}$ on the adaptation in a sensitive cell. An initially dark-adapted cell in normal Ca^{++} Ringer (sensitivity indicated to left of zero time) was exposed, at times indicated by downward arrows, to strong conditioning flashes. The perfusion fluid was changed from normal-Ca^{++} Ringer to low-Ca^{++} Ringer and back at the upward arrows as indicated. The third conditioning flash *preceded* by a few seconds the return to normal-Ca^{++} Ringer. There is an overall drift to increased sensitivity with time and a slight depression of the sensitivity in low Ca^{++}; but the most striking effect is the much reduced depression, and more rapid recovery, of the sensitivity after the conditioning light in low Ca^{++}. Furthermore, the smallness of the light adaptation after the third conditioning flash shows that it is only $[Ca^{++}]_{out}$ *during* the stimulus, and not after it, that matters for this purpose.

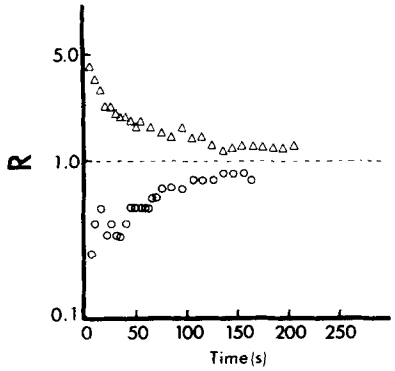

FIGURE 4. Time-courses of recovery from light adaptation (dark adaptation) and from facilitation. This is the same cell as that of Fig. 3 A. In the cell's initial sensitive state (S = 7.2, lower curve of Fig. 3 A) the lower curve of this figure was recorded (dark adaptation). After spontaneous decline of S to 4.9 (upper curve of Fig. 3 A), the upper curve here was recorded (recovery from facilitation).

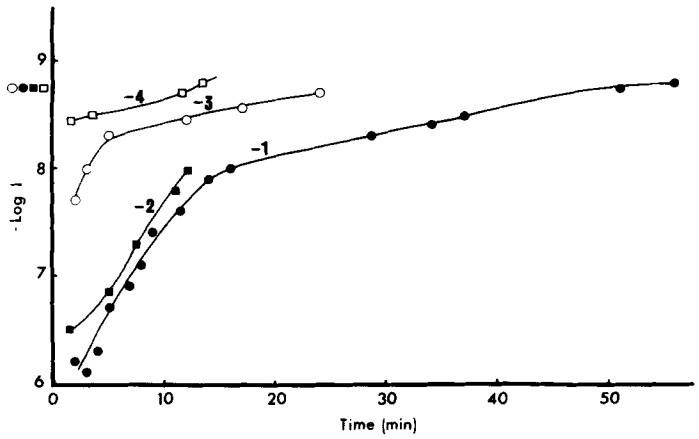

FIGURE 5. The time-courses of dark adaptation at four conditioning light intensities in a sensitive cell (S = 8.7). The ordinate is the negative logarithm of the intensity of a 40-ms test flash required to produce a criterion 3.5-mV response. (This method is used here and in Fig. 6 because of the relatively large changes in sensitivity.) This is plotted as a function of time after a 400-ms conditioning flash applied to a dark-adapted cell. The curves are labeled with the logarithms of the respective conditioning intensities. The test flash intensities for criterion response immediately before the respective conditioning flashes are shown to the left of zero time (all equal in this case). The first data points appear at a minute or so because it took that long to carry out this type of measurement. However, we have no indication of large changes occuring in the first minute. The dark adaptations in this and in the following figure are much slower than in Fig. 4 because of the much brighter conditioning flashes used.

(That the normal-Ca^{++} Ringer reaches the cells in less than a few tens of seconds was shown by demonstrating, in another cell, that changing from low Ca^{++} to normal Ca^{++} a few tens of seconds *before* the conditioning flash resulted in a greatly increased sensitivity depression.) When the preparation was stable the effects of changing $[Ca^{++}]_{out}$ (at least down to 0.1 mM) were always reversible, as in Fig. 6. The effects of extracellular calcium appear to be graded smoothly with concentration, but the detailed dependences were not systematically investigated.

The cell of Fig. 6 was very sensitive and exhibited reduced adaptation in low $[Ca^{++}]_{out}$ but little or no facilitation. In many cells, generally those of lower sensitivity, lowering $[Ca^{++}]_{out}$ not only reduced light adaptation but converted it into facilitation. Fig. 7 shows the dependence of R on intensity for such a cell, a little less sensitive than that of Fig. 6, in normal and low $[Ca^{++}]_{out}$. The time-courses of recovery from facilitation and from light adaptation in this cell were very similar to those shown in Fig. 4.

Some effect of $[Ca^{++}]_{out}$ on S is seen in Fig. 6. However, this effect was small and variable, and in any case the change in the facilitation/adaptation balance in the cell of Fig. 6, induced by changing $[Ca^{++}]_{out}$, is much larger than that which would result from comparable changes in S arising from other sources as detailed in the preceding two sections.

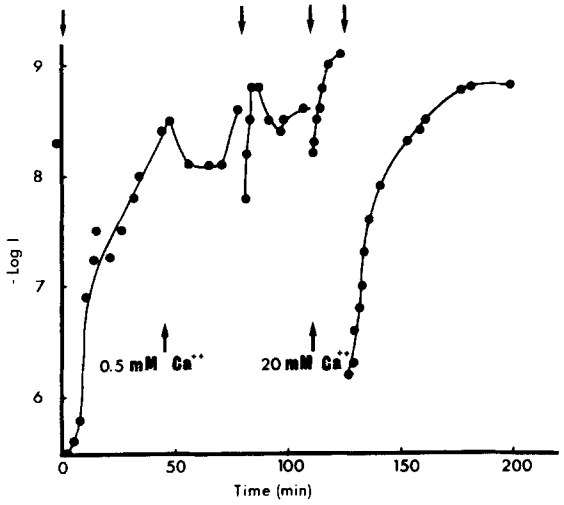

FIGURE 6

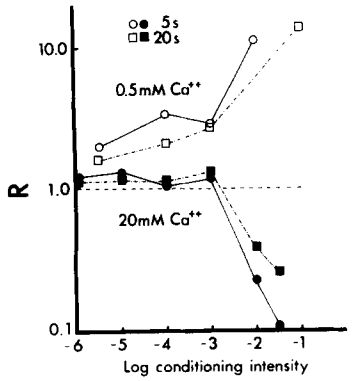

FIGURE 7

FIGURE 6. The effect of external calcium concentration on the adaptation in a sensitive cell (S = 8.3–9.1), as in Fig. 5 but one continuous record. Equal strong conditioning flashes (400 ms, $\log I = -1$) were presented at the downward arrows, and the bath fluid was changed from normal Ca^{++} (20 mM) to low Ca^{++} (0.5 mM) and back at the upward arrows as indicated. The third conditioning flash was presented a few seconds *before* the return to normal Ca^{++} indicated at about the same time. Low Ca^{++}, when present during the conditioning flash, strongly suppresses the light adaptation and speeds up the recovery (dark adaptation). Some of the rapid fluctuations seen in the low-Ca^{++} medium are probably due to irregularities noted in the flow system in this experiment.

FIGURE 7. The effect of external calcium concentration on the intensity dependence of the facilitation/adaptation in a fairly sensitive cell (S = 7.5 for *both* media). The test responses were observed 5 and 20 s after the conditioning flash (instead of the usual 10 s) as indicated. S changed only slightly when the medium was changed. Qualitatively similar results were observed in all cells except the most sensitive (which showed little facilitation, even in low Ca^{++}, as in Fig. 6) and the more insensitive (with weakened or absent adaptation, even in normal Ca^{++}, as in Fig. 3 A, upper curve).

Resistance Changes during Adaptation and Facilitation

Using a bridge method, we found that the cell resistance generally changed considerably after the conditioning flash. The resistance almost always increased (sometimes by up to 10–15 MΩ), and recovered with a time-course of up to minutes. These changes were greatly reduced (to less than 2–3 MΩ) in low $[Ca^{++}]_{out}$.[2]

Because of these changes, the observed voltage responses cannot be directly

[2] Lisman and Brown (1971) observed a similar effect in ventral photoreceptors of *Limulus* (in normal $[Ca^{++}]_{out}$), and pointed out that this would cause an apparent increase in voltage sensitivity after exposure to bright lights.

interpreted as current responses. How much the interpretation must be corrected for these changes depends on the resting membrane resistance. Brown et al. (1970) quote an average voltage-clamp value of 1.65 MΩ, but we believe the "undamaged" value to be generally much higher than this, perhaps 10 MΩ, in most of our cells (our resting potentials are also larger than those of Brown et al.). This is based on a number of experiments in which a resistance decrease of 3–5 MΩ was observed during illumination, one in which repeated electrode penetrations into and withdrawals from the same cell showed apparent 10-MΩ resistance changes and a few direct two-electrode measurements. But even if the resting resistance is 10 MΩ or more, considerable care is needed in any quantitative interpretation of our results in terms of currents.

However, from these figures it appears that facilitations as large as those of Fig. 7, for instance, cannot be artifacts of these resistance changes. To verify further the existence of current facilitation, a few voltage-clamp experiments were carried out. These directly measure the current responses to the test flashes, independent of the resistance state of the cell. Fig. 8 illustrates such a measurement. The clear current facilitation observed shows that cell resistance changes are not a dominant cause of facilitation, nor (since these changes are generally increases) of adaptation.

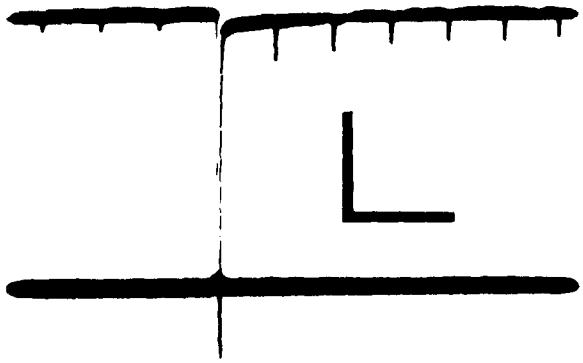

FIGURE 8. Voltage-clamp demonstration of current-response facilitation. A cell in a low-Ca^{++} medium (0.5 mM) was exposed to test flashes (50-ms duration, $\log I = -4$) every 5 s; one test flash was replaced by a conditioning flash (50 ms, $\log I = -2$). The cell was clamped at its dark-resting potential of about −45 mV. The upper trace shows the current response, the lower trace the (clamped) voltage. The current responses to the fixed tests stimuli are clearly increased after the conditioning stimulus. The time calibration bar is 10 s; the vertical bar represents 20 nA for the current trace and 40 mV for the voltage trace. This experiment was carried out by C. Shaw.

DISCUSSION

Facilitation is observed mainly in cells whose sensitivity has been reduced biochemically or by decay or mechanical damage. Decreased $[Ca^{++}]_{out}$ often induces facilitation even in cells which are, and remain, fairly sensitive, but on the whole the degree of facilitation decreases with increasing cell sensitivity. Thus it ap-

pears likely that cells cannot be facilitated beyond some intrinsic ceiling, which is probably the sensitivity of a normal very sensitive cell when dark adapted.

The current-response sensitivity changes discussed above could arise from changes either in the conductance response or in the transmembrane gradient of the current-carrying ions. Most of the current is carried by Na^+, and it seems highly unlikely that intracellular (or extracellular) Na^+ concentrations could follow the very large and rapid sensitivity changes observed (Lisman and Brown, 1972). We conclude that these changes arise predominantly from modifications of the conductance response to test stimuli.

Facilitation and adaptation could be either two separate and opposing processes, or the manifestation of the variation in strength of a single influence, which, under different conditions, has opposing effects. The explanation as two separate processes seems easier to handle. Assuming two processes, we attempt to specify their separate characteristics: (*a*) Sensitivity state: The shift towards facilitation arising from cell damage and/or metabolic deterioration could be due either to a depression of adaptation or to an enhancement of facilitation or both. (*b*) Conditioning intensity: The curves of Fig. 3, especially those showing a transition from facilitation at intermediate intensities to adaptation at high intensities, indicate that adaptation has a dependence on intensity much sharper than that of facilitation. (*c*) External Ca^{++} concentration: Removal of most of the external Ca^{++} suppresses the adaptation and/or enhances the facilitation (Figs. 6 and 7). Given the preceding conclusion, that the adaptation depends more sharply on light intensity than does facilitation, the absence of a dip at the high-intensity end of the 0.5 mM Ca^{++} curve of Fig. 7 indicates that suppression of the adaptation is the dominant effect.

The fact that Ca^{++} plays a role in adaptation is strongly suggested by previous studies. $[Ca^{++}]_{in}$ is known to be normally very low in nervous tissue (summarized by Baker, 1972). Internal Ca^{++} apparently blocks, or intermediates a blockage of, inward Na^{++} conductance in squid giant axon (Frankenhaeuser and Hodgkin, 1957), probably in barnacle photoreceptors (Brown et al., 1970), and also in vertebrate rods (Yoshikami and Hagins, 1973). Light increases $[Ca^{++}]_{in}$ in barnacle and *Limulus* photoreceptors (Brown and Blinks, 1974) and apparently (from intrasac Ca^{++}) in vertebrate photoreceptors (Szuts and Cone, 1973; Hendricks and Bonting, 1973). Furthermore, Brown and Lisman (1974) and Fein and Lisman (1975) have shown that injection of Ca^{++} into *Limulus* photoreceptors mimics the adaptation effect. This fits with our observations on the effect of ruthenium red. This chemical increases $[Ca^{++}]_{in}$ by inhibiting Ca^{++} uptake by mitochondria, and its addition to the medium results in a 10,000-fold drop in sensitivity. Internal Ca^{++} is known to increase K^+ conductance in some tissues, but this is not the dominant mechanism of adaptation in this preparation, since adaptation is generally accompanied here by a membrane conductance *decrease* (preceding section).

Light adaptation may, therefore, be due to a light-induced influx of Ca^{++} into the cell. If so, light adaptation should be suppressed either by decreasing $[Ca^{++}]_{out}$ (because of the reduced driving force and available Ca^{++}) or by increas-

ing $[Ca^{++}]_{in}$ (because the driving force is reduced and the influx in any case is less significant). We indeed observe reduced light adaptation in low $[Ca^{++}]_{out}$. We also observe reduced light adaptation in cells of low sensitivity, and suggest that such cells have high $[Ca^{++}]_{in}$, due to either injury (increased leakage of ions), weakened metabolic activity (reduced extrusion through the membrane and/or reduced sequestration by mitochondria [see Baker, 1972 and Brinley, 1973]), or ruthenium-red decoupling of Ca^{++} binding to mitochondria. Such cells may be said to be in a permanently "light-adapted" state of high $[Ca^{++}]_{in}$.

Just as adaptation is supposed to result from an influx of Ca^{++} during the response to light, we speculate that facilitation could be due to an efflux of Ca^{++} during the response. The facilitation-adaptation balance would then be simply related to the Ca^{++} efflux-influx balance. In sensitive cells in normal $[Ca^{++}]_{out}$, $[Ca^{++}]_{in}$ would be very low, and (assuming the efflux to depend on $[Ca^{++}]_{in}$) the influx, and hence adaptation, would dominate. In less sensitive cells with higher $[Ca^{++}]_{in}$ (due to injury, decay, or ruthenium-red treatment) Ca^{++} efflux, and hence facilitation, would be relatively enhanced, especially if $[Ca^{++}]_{out}$ were also reduced. One additional hypothesis is necessary in order to explain the "biphasic" curves of Figs. 3 B and C: That the efflux saturates, while the influx does not (or does so at a higher level). These predictions then fit the observations very well.

No light-dependent Ca^{++}-extrusion process is known, but one could speculate about a Na^+-driven Na^+-Ca^{++} exchange related to (part of) the light-induced Na^+ influx. Such an exchange exists in various nerve and muscle membranes (Baker, 1972; Blaustein, 1974) but has not been shown in any sensory receptor, although Lisman and Brown (1972) speculated about a Na^+-Ca^{++} exchange contribution to Ca^{++} influx. It is clearly desirable to see whether there is a light-dependent Ca^{++} efflux in such preparations.,

A completely different speculative model for facilitation is based on hypothetical light-induced changes in the "affinity" for Ca^{++} of the sites from which the Ca^{++} must be removed to open an ion channel (or group of channels) in the membrane. A light-induced decrease in Ca^{++} affinity has in fact been seen by Hemminki (1975) in isolated bovine photoreceptors. The present model proposes that the channel(s) can only be opened if both of a pair of sites are unoccupied, that light removes Ca^{++} ions one at a time and randomly, and that removal of a Ca^{++} ion from a site by light is followed by a period of reduced affinity for Ca^{++}. Exposure to light would then leave many unpaired unoccupied sites, and further light would have to remove only the second Ca^{++} ion of each pair to open the channel(s). The response to a given light would be larger than if all the sites were initially occupied, that is, the response would be *facilitated*.

Thus light exposure, or site activation, would have two opposing aftereffects: Paired activated sites would lead to channel opening and so to an increased $[Ca^{++}]_{in}$, resulting in light adaptation; and unpaired activated sites would constitute facilitation. The dependence on intensity of the number of unpaired activated sites should be linear, and that for paired activated sites quadratic (for weak activations). Thus facilitation should depend less sharply on light inten-

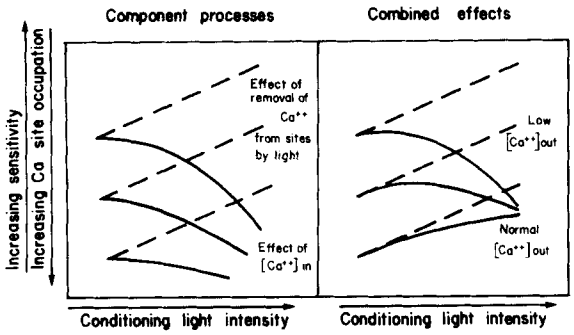

FIGURE 9. The affinity model for facilitation and adaptation. See Appendix text.

sity than adaptation, in agreement with the observations. The predictions of this model under various conditions are summarized in the Appendix and in Fig. 9.

The model can clearly be generalized to any system in which more than one light-induced event is required for channel opening. Some support for the idea that more than one such event may be required comes from the observation (Hillman et al.)[3] that the strength of the excitatory component of the transduction process in this photoreceptor (Hochstein et al., 1973) depends more than linearly on light amount.

CONCLUSION

We suggest that facilitation in photoreceptors may well be as widespread a phenomenon, at least in invertebrates, as adaptation. It has not been seen more often presumably because in most preparations and conditions it is dominated by adaptation. It may be possible to unmask it (or enhance it into visibility) in other preparations by manipulating parameters such as those found effective in the barnacle, conditioning intensity, sensitivity state, and external calcium concentration. It seems clear that facilitation is a significant aspect of the transduction process and should provide a useful additional handle for its study.

APPENDIX

A Qualitative Illustration of the Possible Parametric Relations Underlying the Adaptation/Facilitation Balance on the Affinity Model

The basic assumptions of this model are that two (or more) sites have to be activated to open an ion channel or group of channels, and that activation of a site consists in removal of a Ca^{++} ion from that site. An ion may leave the site, or occupy it, spontaneously, in equilibrium with $[Ca^{++}]_{in}$, or it may be removed by light. In the latter case the affinity of the site is assumed to be reduced for a certain time. Thus the site occupation depends on the recent light history and on $[Ca^{++}]_{in}$ (which in turn also depends partly on the light

[3] Hillman, P., S. Hochstein, and B. Minke. Non-local interactions in the photoreceptor transduction process. Manuscript submitted for publication.

history). Site occupation determines sensitivity, since if one of a pair of sites is already unoccupied, light has only to remove the other to open the channel(s). Light then has two opposing effects on sensitivity: (*a*) It increases the number of unoccupied sites by direct ion removal, and (*b*) it decreases the number of unoccupied sites by opening Na^+-Ca^{++} channels and so increasing $[Ca^{++}]_{in}$. The number of sites from which an ion has been removed by light depends linearly on light intensity (for nonsaturating lights), as in the dashed lines in the left panel of Fig. 9. The number of opened channels is the number of unoccupied *pairs* of sites, and this is proportional to the *square* of the light intensity (if not too many sites were already unoccupied); this effect is depicted in the continuous curves in the left panel of the figure. The initial sensitivity depends on the initial $[Ca^{++}]_{in}$, and three different sensitivities are shown for each effect and in each panel of the figure.

On the right, the combined results of the two opposing effects of light on the sensitivity dependence are shown. In low $[Ca^{++}]_{out}$, little Ca^{++} flows in during light, $[Ca^{++}]_{in}$ is unchanged, and the dominant effect of light is the direct removal process (*a*). The expected intensity dependence of the sensitivity is then just that for the direct removal process shown in the left panel, and is shown by similar dashed lines in the right panel. These lines are to be compared with the experimental results under these conditions, which are shown in the upper curves of Fig. 7. (Note that the experimental figures display the conditioning intensity on a logarithmic scale.)

In normal $[Ca^{++}]_{out}$, the behavior of the curves is expected to depend on the initial sensitivity, that is, on the initial $[Ca^{++}]_{in}$. If $[Ca^{++}]_{in}$ is high (low sensitivity cell), the light-induced Ca^{++} influx has little effect, and the curve is again nearly that for the direct removal process (*a*) only (bottom continuous curve, right panel), giving facilitation. Compare the experimental results: the top curves of Fig. 3 A and B. If $[Ca^{++}]_{in}$ is initially low (high sensitivity cell), the effect of the light-induced influx of Ca^{++} is dominant, and the curve is nearly that for the $[Ca^{++}]_{in}$ process (*b*) (top continuous curve of left panel), giving mainly adaptation (top continuous curve of right panel; to be compared with the lower curves of Figs. 3 A, B, C and 7). Finally, with intermediate initial $[Ca^{++}]_{in}$ the expected curve is biphasic, showing facilitation at intermediate conditioning intensities and adaptation at high intensities, as is seen in the experimental middle curve of Fig. 3 B and upper curve of Fig. 3 C.

We thank Mr. C. Shaw for carrying out the voltage-clamp measurements, Miss S. Ashkenazy and Miss Ada Dorman for technical assistance, and Professor R. Werman and Dr. S. Hochstein for helpful comments on the manuscript.

The work was supported in part by grants from the Israel Commission for Basic Research and from the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel.

Received for publication 31 July 1974.

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