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The Contribution of Pigment Transitions to Sensitivity Changes in the Barnacle Photoreceptor and the Correlation with the Prolonged Depolarizing Afterpotential

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Abstract. A conditioning light can cause a decrease (adaptation) or an increase (facilitation) in the sensitivity of barnacle photoreceptors, as measured by the amplitude of the late receptor potential (LRP). We show that a net transfer of visual pigment from the rhodopsin (R) to the metarhodopsin (M) state induces a large facilitation whereas the reverse transfer results in a much smaller facilitation or even an adaptation. These effects were not due to the response to the conditioning light but to the pigment reactions. When the conditioning light did not alter the pigment population (i.e., M → M, R → R) it was followed by an intermediate degree of facilitation. These conclusions are correct for cells which have relatively low sensitivity. In sensitive cells, all pigment transitions produce adaptation.

LRP facilitation and the prolonged depolarizing afterpotential (PDA) show several common characteristics with respect to pigment transitions: 1. Their magnitude increases with the amount of pigment transferred from R to M. 2. Both are depressed by the M → R transition. 3. Their production is impeded by the M → R transition. 4. The PDA itself is facilitated by the R → M transition and this facilitation decays with a time course comparable to that of LRP facilitation. These results suggest that there may be an underlying process common to LRP facilitation and PDA.

Key words: Photoreceptor – Visual pigment – Adaptation – Facilitation

Introduction

The most familiar after-effect of light on photoreceptors is a reduction of their sensitivity. We have recently shown that under certain circumstances light may instead enhance the sensitivity of a barnacle photoreceptor (Hanani and Hillman 1976). We found that the influence of a conditioning light on the sensitivity is

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pushed towards enhancement by reducing external Ca^{2+} concentration, for intermediate strengths of conditioning stimuli and for relatively insensitive cells. We suggested that the effect of a conditioning light on cell sensitivity is a balance between two opposing processes which we called "facilitation" and "light adaptation". In the present study we look at the effects of visual pigment state and change of state on this balance: Preliminary results (Hanani and Hillman 1979) suggested that pigment state does have an effect.

Both the rhodopsin (R) and the metarhodopsin (M) states of the visual pigment in the barnacle photoreceptor are thermally long-lived (Minke et al. 1973). The dark-adapted sensitivity has been shown in other invertebrate photoreceptors to depend linearly, or nearly linearly, on R population (Razmjoo and Hamdorf 1976), while in the barnacle it depends relatively weakly on this population (Atzmon et al. 1978). Pigment manipulation has also been found to have shorter-term effects. Hochstein et al. (1973) showed that substantial net transfer of pigment from R to M results in a prolonged depolarizing afterpotential (PDA) and M to R depresses the PDA or impedes its induction (anti-PDA). Hillman et al. (1976) also showed that "spectator" R molecules (those not absorbing photons) appear slightly to impede PDA induction or M molecules to enhance it.

In the present paper we extend our earlier study of pigment effects on the facilitation-adaptation balance and also present evidence that facilitation and the PDA share several properties.

Methods

The preparation of the excised eyes and the electronic apparatus were as previously described by us (Hanani and Hillman 1976).

The dependence of the pigment state on amount and wavelength of conditioning light was calibrated by using the early receptor potential as a linear measure of that state (Hillman et al. 1976). A quartz-iodide lamp provided white light whose unattenuated intensity was about 1×10^{16} quanta $\cdot$ cm^{-2} $\cdot$ s^{-1} $\cdot$ nm^{-1} at 550 nm at the photoreceptor. Intensities are given as the logarithms of their ratios to this value. The relative amount of light is expressed as $\log (I \cdot t)$, where I is the intensity and t is the flash duration; $\log (I \cdot t) = -1$ corresponds to a flash whose intensity is $\log I = -1$ and duration 40 ms. The filters used for the prior adaptation and for the conditioning stimuli were mostly Balzers 443 nm (blue) and K6 (red) interference filters. Saturating exposure to these left 70 ± 20% and 10 ± 5% of the pigment in the R state respectively (the R absorption is peaked around 532 nm and the M around 493 nm). For present purposes we call these impure states the "R" and "M" states of the pigment respectively. The *test* light was white, and of an intensity and duration such as not appreciably to change the state populations. Blue and red test flashes were found to give similar results.

A cell was initially adapted to saturating blue or red light. After the resulting PDA, anti-PDA, adaptation or facilitation had died away and the cell sensitivity had reached a plateau, test flashes were presented every 5 s, and the effect of a

conditioning blue or red flash on the response (the late receptor potential, or LRP) to these test flashes was observed. If both adapting and conditioning lights were red, the experiment was "M → M" ("neutral" conditioning) and so on. The parameter measured, r, is the ratio of the test flash amplitude after the conditioning flash to that before it. A typical trace from which r, was measured is shown in Fig. 4.

Results

The experiments described in this paper lasted several hours during which some changes in the resting sensitivity of the cells took place (both increase and decrease). In order to maintain the dark adapted response constant (within the range of 2–5 mV) it was necessary sometimes to alter the test flash intensity. In most cases this adjustment did not exceed ± 0.5 log units. Recently it was found that the measured light-induced "sensitivity change" is not always independent of the test flash intensity (Atzmon 1980). The sensitivity-change parameter was sometimes appreciably different for bright and dim test flashes. However, more detailed study (Hanani and Hillman, unpublished results) showed that this phenomenon occurs only in cells with relatively high sensitivity and such cells were excluded from the present study. Figure 1 shows that in a medium-sen-

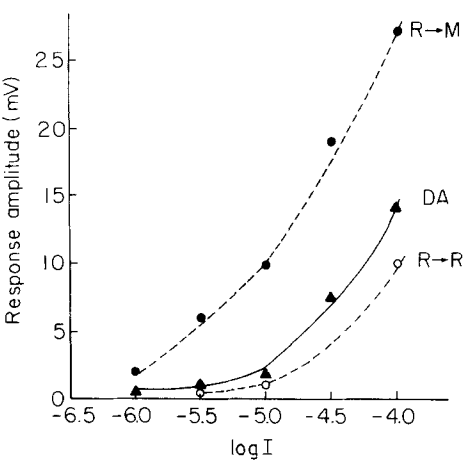

Fig. 1. Demonstration that the choice of test stimulus intensity is not critical in determining the effect of a conditioning light on cell sensitivity. The amplitudes of responses to 40 ms flashes of white light test stimuli are plotted as functions of the test intensity. The pigment was initially in the R state. The middle curve was measured in the dark-adapted (DA) state and serves as a reference. The lower curve was measured after the cell was illuminated with a blue light (λ_{max} = 443 nm, 4 s duration). The upper curve was measured after illumination with red light (K6 filter, λ_{max} = 650 nm, 4 s duration). It is clear that the effect of a conditioning light, measured as after/before test response amplitude ratio, is not systematically dependent on the test flash intensity. This behaviour was observed in all cells displaying low to moderate sensitivity; deviations were observed in very sensitive cells

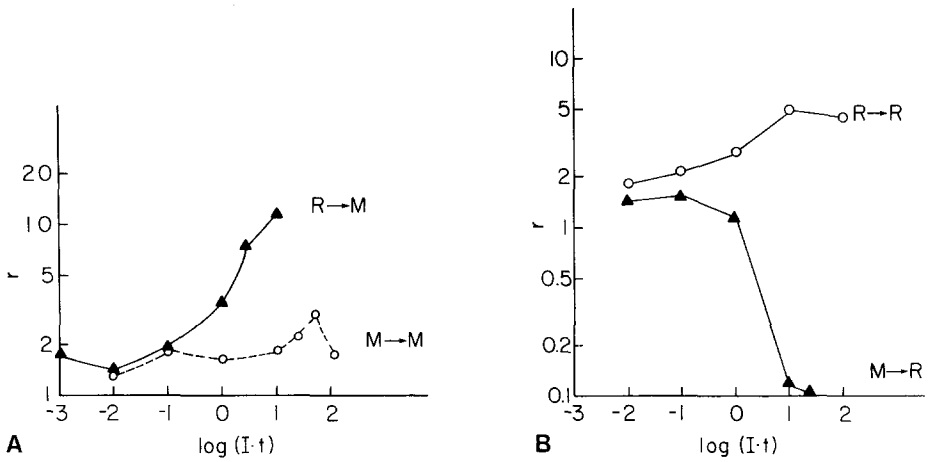

Fig. 2 A and B. The dependence of the effect of a conditioning light on its wavelength and intensity and on the pigment state. The ratio of the amplitudes of the responses to test flashes after/before a conditioning illumination is plotted against the logarithm of the amount of conditioning light. In **A** the conditioning light was red, leaving the pigment in the M state, and before the conditioning the pigment was initially in the R state (upper curve, R → M) or M state (lower curve, M → M). The R → M transition is followed by a large facilitation, whereas the facilitation caused by M → M conditioning is relatively small. **B** is from the same cell, but the conditioning light was blue, leaving the pigment in the R state. The R → R conditioning produces moderate facilitation, similar to, though perhaps a little larger than, M → M (**A**); the M → R transition causes strong adaptation. These differences appear in all cells of low and moderate sensitivity

sitivity cell the effect is negligible. Figure 1 displays the dependence of sensitivity changes following a conditioning light as function of the test flash intensity. The intensity–response curves were obtained as following: The cell was adapted to 443 nm light, and after over 30 min in the dark, the dark-adapted curve was measured. The effect of a conditioning light was tested by illuminating the cell with a bright red light (K6, log $I \cdot t = 1$) and then quickly measuring the intensity–response curve (dashed line). The same procedure was done for a blue (443 nm) conditioning light. Clearly the effect of the conditioning lights was qualitatively the same for all test intensities used. Such results were always obtained for cells with low or intermediate sensitivity (see Hanani and Hillman 1976, for definition of sensitivity).

The mechanism of the dependence of sensitivity changes on the test flash intensity is not yet clear, but it thus seems unlikely that this effect could seriously distort our results, because most of the experiments were done on cells with relatively low sensitivity, and because the variation of test flash intensity was small and not systematic.

A. Effect of Pigment Transfer on the Facilitation-Adaptation Balance

Figures 2A and B display the sensitivity-change parameter r (see Methods) as a function of the logarithm of the amount of the conditioning stimulus in a single

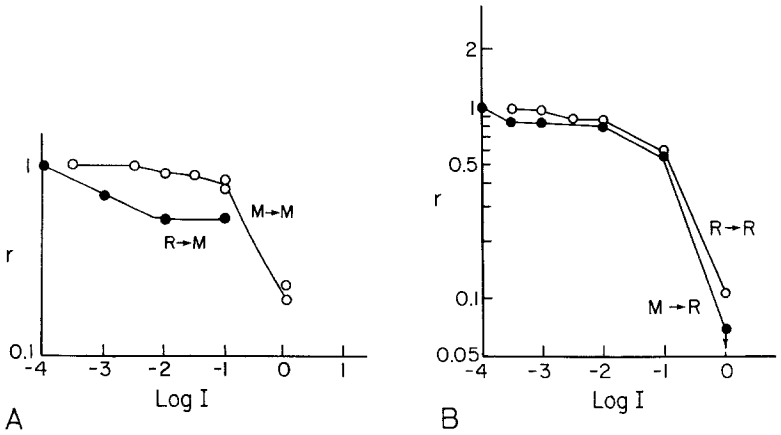

Fig. 3 A and B. The dependence of the effect of a conditioning light on its wavelength and intensity and on pigment state in a very sensitive cell. As in Fig. 2 but for a cell of high sensitivity; the differences among the curves for different pigment states and transitions are much reduced and no increases in r (facilitation) are observed. The apparent difference between the R → M and M → M curves in **A** does not seem to be reproducible in other sensitive cells

cell. The four sets of points correspond, as labeled, to conditioning lights that transfer net pigment from R to M or M to R, or transfer no net pigment but maintain the bulk in R or M (see Methods). These results are typical for cells with low to moderate sensitivity (see Hanani and Hillman 1976). However, cells of especially high sensitivity tended to show less pigment dependence (and less enhancement). Observations on such a cell are illustrated in Fig. 3.

Over most of the range of amounts of conditioning stimulus in Fig. 2, the amounts of R → M and M → R pigment transfer were too small to elicit detectable PDAs and anti-PDAs. These responses are small below about 20% pigment transfer (Hillman et al. 1976), while log $(I \cdot t) = 1$ corresponds to an estimated 5% transfer only. For R → M, r is not plotted for values of log $(I \cdot t) > 1$ because the resulting PDAs correspond to reduced cell resistances and hence to r's depressed from their true (conductance) values. However, the trends of the $r-I$ dependence do not seem to be radically affected by the appearance of the PDA (see Fig. 5). As seen in Fig. 2A, the trend to increasing facilitation for higher $I \cdot t$ is clear even without correcting for increasing cell conductance.

The possibility should be considered that the effects of conditioning lights corresponding to different pigment transfer directions differ because they induce different responses (see Bolsover 1980). In Fig. 4, typical of several checks, this is shown to be improbable: Two conditioning stimuli corresponding to M → M and M → R pigment transfer are seen to induce nearly identical responses but very different r's. These responses are substantially below saturation, so the conductances are also very similar.

If the conditioning stimulus is divided into portions presented successively, is the effect on the sensitivity change parameter r modified? That is, does the process include short-lived interactions? Figure 5 suggests that it does not.

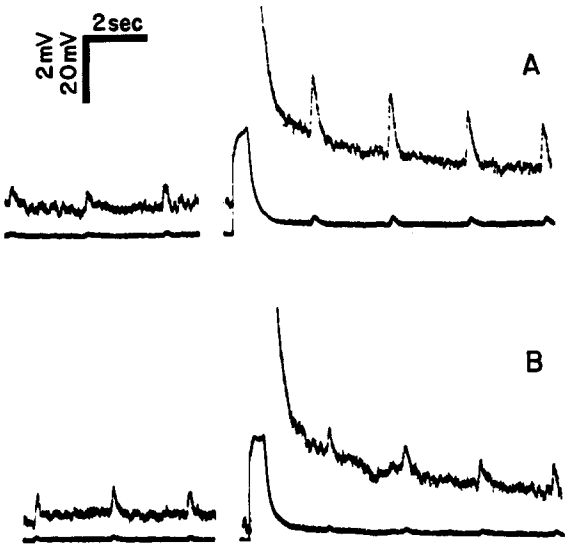

Fig. 4 A and B. Demonstration that the effect of the conditioning on the response to the test flash is not due to differences in the response to the conditioning light. In **A** and **B** the pigment was in the M state and the conditioning lights were red and blue respectively; their duration was 500 ms. The test flash was a white light of 60 ms duration and $\log I = -4.5$. Note that although the responses to the conditioning lights are similar in amplitude and quite similar in form their effects on the response to the test flash are very different. The conditioning responses are far from saturation, so the voltage records sensitively reflect conductance changes

Figure 5A shows that the second of two R → R stimuli hardly changes the facilitation. This is in line with the weak intensity dependence for neutral conditioning stimuli seen in Fig. 2; a factor of two increase in light amount changes r only slightly. Similarly, the second of two R → M stimuli, each transferring only a few percent of the pigment, appreciably increases r (Fig. 5B); this corresponds to the sharply rising intensity dependence seen in Fig. 2. The most interesting result, however, is Fig. 5C. A neutral stimulus is equivalent to equal R → M and M → R transfers. One sees that when these transfers occur separately in time, they give a strong facilitation and a strong adaptation as expected. These largely cancel each other and a small net facilitation (with respect to the original sensitivity) remains, as expected for a neutral stimulus. The temporal separation of the two components of the neutral stimulus has not changed its effect on r.

Figure 5D shows that the result of Fig. 5C is valid even when the R → M stimulus is strong enough to induce a large PDA and the M → R to suppress the PDA completely.

B. Similarity between Late Receptor Potential (LRP), Facilitation, and PDA

The results above show that there is a greater tendency towards facilitation after a conditioning light which transfers a net amount of pigment from R to M, and

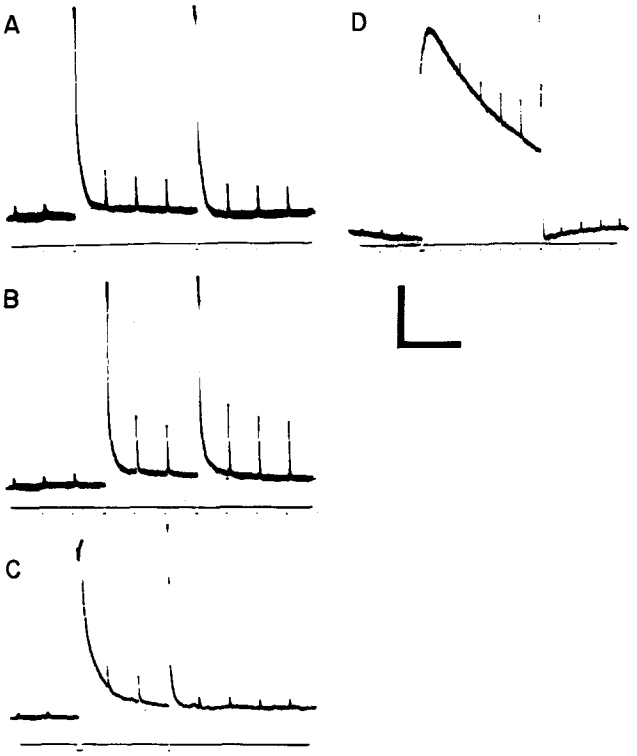

Fig. 5 A–D. Demonstration that dividing the conditioning stimulus into time-separated parts does not change the overall effect on cell sensitivity. Prior adaptation was to blue light in all cases. In **A**, a blue (R → R) stimulus induces some facilitation; a second blue stimulus slightly increases the facilitation – in agreement with the slowly rising R → R curve of Fig. 2B. In **B**, a red (R → M) stimulus induces a stronger facilitation which is appreciably enhanced by a second red stimulus – compare the rapidly rising R → M curve of Fig. 1A. In **C** a red (R → M) stimulus induces a strong facilitation which is largely canceled by a following blue (M → R) stimulus, leaving a small facilitation, as expected for a overall R → R stimulus (Fig. 2B). **D** (in another cell) is like C except that a longer red stimulus (1 s) was used, inducing a PDA. The blue stimulus depresses both the PDA and the facilitation. [Note that this blue stimulus alone, not preceded by red, would have *facilitated* the cell **(A)**]. Thus, in all cases the effects of the time-separated pairs of conditioning stimuli are similar to those which simultaneous presentation would have given, suggesting the absence of short-term interactions in the sensitivity-modifying process. All conditioning stimuli were 400 ms long except as noted. Test flashes were 40 ms long and of intensity log $I = -5.0$ (**A, B**); -4.8 (**C**); and -4.3 (**D**). Calibration bars: Time 10 s for **A–C**, 15 s for **D**, amplitude 10 mV

towards adaptation after the reverse transfer. The same transitions (but with larger pigment amounts) produce PDA and anti-PDA respectively. In this section we examine in more detail the correlation between facilitation and PDA.

Figure 6 illustrates qualitatively the dependence of facilitation and PDA on the amount of pigment transferred from R to M. In A the cell was in the R state and a red light transferred about 50% of the pigment to M. A PDA is induced,

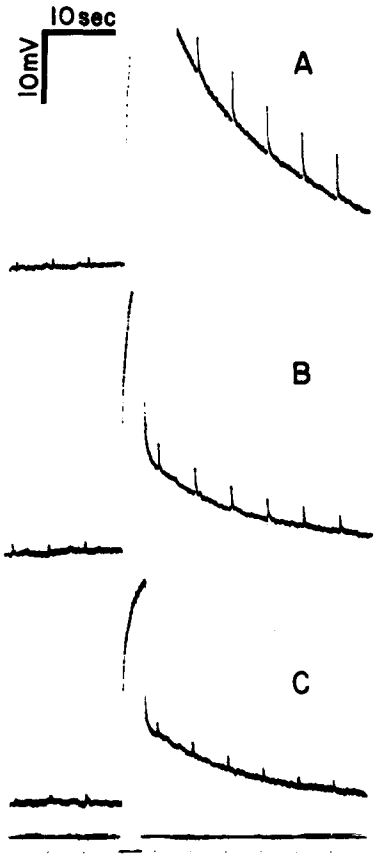

Fig. 6 A–C. Demonstration that the pigment dependences of the LRP facilitation and of the PDA are similar. The pigment was initially in the R state. In **A**, a red stimulus transferred approximately 50% of the transferrable pigment to M, inducing a partial PDA and some LRP facilitation. After the PDA declined completely the same red stimulus transferred 50% of the *remaining* pigment (**B**); the resulting PDA and the LRP facilitation are both considerably, and comparably, smaller. The same procedure was repeated in **C** and a further comparable reduction in the PDA and the LRP facilitation is evident. Thus, PDA amplitude or duration and LRP facilitation seem closely correlated. Test flash duration 40 ms, intensity log $I = -4.0$

and superimposed on it a large facilitation is observed. After the PDA and facilitation decayed the same conditioning light was presented again (B) and transferred 50% of the remaining R to M. The resulting PDA and facilitation are much reduced. The same procedure was repeated in C. It is evident from this Figure that both PDA and facilitation decrease with decreasing amount of pigment transfer from R to M, with roughly comparable dependences.

In addition to the facilitatory effect of an R → M conditioning light on the cell sensitivity for LRP, a PDA-inducing R → M stimulus also facilitates the PDA-induction by a later R → M stimulus, and the time courses of decline of these facilitations are also similar. This is illustrated in Fig. 7. An initially blue-adapted cell was exposed to two successive red stimuli which transferred about 50% and 25% of the transferrable pigment from R to M respectively, inducing partial PDAs. The Figure shows that the amplitude of the PDA due to the second stimulus depends strongly on the time interval between the stimuli (Traces A-C). The PDA is large for short intervals (5 s) and very small when the interval is 40 s. In the same cell and under similar experimental conditions (blue adapted) a red conditioning light induces facilitation (Trace D). This facilitation

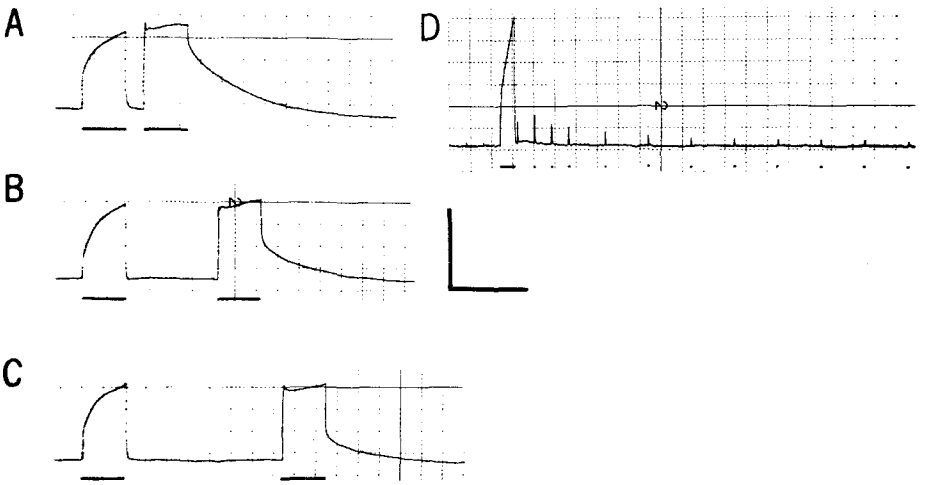

Fig. 7 A–D. Demonstration that the time courses of the decays of the facilitating processes are similar for the PDA and for the LRP. In **A, B,** and **C** a pair of equal R → M stimuli is presented; when the second stimulus closely follows the first, as in **A,** it induces a strong PDA even though the first did not. This facilitatory effect declines with time between the stimuli (**B, C**). In **D**, in the same cell, facilitation of the LRP response to brief test stimuli is demonstrated. Decline of this facilitation is seen to follow a similar time course, suggesting a relation to the PDA facilitation. Calibrations: Time 30 s, amplitude 50 mV for **A–C**, 20 mV for **D**

decays in 40–60 s. That is, both the LRP and the PDA are facilitated by LRP-inducing conditioning lights and the facilitations of both die away in about 60 s. This decay time course is similar to that of the full (saturated) PDA itself in this cell (not illustrated).

A final observation relating the PDA and LRP phenomenologies refers to the anti-PDA: During the course of an anti-PDA, induction of a PDA is impeded (this is, of course, the definition of an anti-PDA); and we find that the LRP facilitation is *also* depressed during an anti-PDA. That is, following the induction of an anti-PDA, induction of a PDA and of LRP facilitation are both impeded for comparable times. To show this, a full anti-PDA was induced by strong blue illumination of a red-adapted cell. At various times later a further red stimulus was presented – either a long stimulus to induce a PDA or a short one to induce facilitation without visible PDA. Figure 8 shows the similar dependences of the facilitation parameter r and the PDA strength parameter time-to-half-height on the interval between the blue anti-PDA-inducing light and the red PDA- or facilitation-inducing stimulus.

Discussion

One may differentiate between the influences of the *presence* of M and *light absorption* by M. Hillman et al. (1976) reported a marginal effect of the *presence*

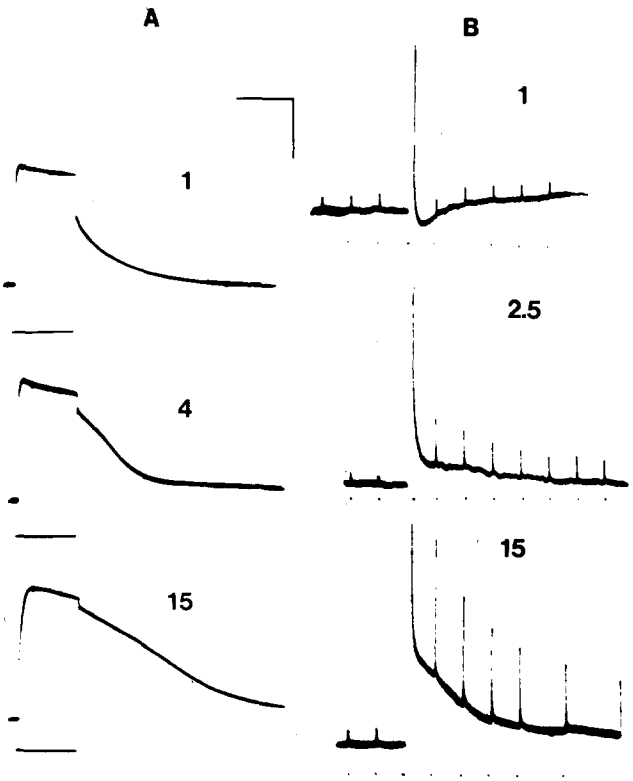

Fig. 8 A and B. An M → R conditioning stimulus (inducing an "anti-PDA") impedes both PDA and LRP facilitation. In **A**, PDAs were induced by identical R → M stimuli at various times (indicated in minutes for each trace) after M → R "anti-PDA-inducing" stimuli; the "anti-PDA" dies away over 15 min. In **B**, in the same cell, briefer identical R → M stimuli, also at various times after M → R stimuli as indicated, cause LRP facilitation which is suppressed for short times. This suppression also dies away over 15 min. Thus, the "anti-PDA" and the "anti-LRP-facilitation" have similar time courses. Calibration bars: Time 20 s, amplitude 20 mV for **A**, 10 mV for **B**

of M on the induction of the PDA. Lisman and Strong (1979) showed that the presence of M has little effect on the LRP sensitivity of *Limulus* cells for low and intermediate stimulus amounts. Hanani and Hillman (1979) showed that the facilitation induced by R → R is similar to, but may be a little larger than, that induced by M → M (compare Fig. 2). This suggests that the presence of M does not greatly influence the modulation of the LRP sensitivity by a conditioning light, though a small enhancement by R presence or depression by M presence is not excluded.

There are strong indications that for small stimulus amounts, *absorption of light* by metarhodopsin (M) also does not contribute to or modulate the sensitivity of *Limulus* ventral photoreceptors (Lisman and Strong 1979) or of barnacle photoreceptors (Atzmon et al. 1978; Strong and Lisman 1978). Atzmon et al. and Strong and Lisman showed that the barnacle LRP excitation and adaptation action spectra agree with the absorption spectrum of R even when

much M absorption occurs; and Lisman and Strong demonstrated that in *Limulus* the sensitivity is directly proportional to the amount of R present and unmodulated by M absorption. On the other hand, M absorption clearly *does* have an effect on the cell response: A PDA is depressed or its induction impeded by net photo-transfer of pigment from M to R (anti-PDA). If M absorption were found to influence *only* the PDA phenomenology, one would conclude that at least one component of the PDA process does not contribute to the LRP.

In this article we have explored the effect of M absorption on the LRP over a wide range of stimulus amounts, and found that its effect is, under certain circumstances, also significant at intermediate-to-high intensities well below those at which PDAs and anti-PDAs become visible. Specifically, we have shown that two conditioning lights inducing very similar responses in the cell can result in quite different modulations of the cell sensitivity, depending, presumably, on the degree of M excitation involved.

This article deals in particular, with the pigment contribution to the effects of conditioning lights on the *ensuing* sensitivity of the cell to weak test lights; the self-effect on the conditioning LRP will be dealt with in a separate investigation. However, as already noted, the results of Fig. 4 suggest that the present results do not depend on the self-effect of the conditioning LRP but arise more directly from the pigment.

We have shown (Fig. 5) that the division of a conditioning light into two stimuli separated by ten or more seconds does not change its effect – even when the stimuli are arranged to result in opposite net transfers of pigment. If the sensitivity-modulating process were composed of successive non-linearly related stages with time-constants of up to tens of seconds, a non-additivity would have been expected. Accordingly, the modulating process is a single-stage process, and/or its stages are linearly coupled, and/or its time constants are longer than tens of seconds.

The results suggest a possible link between the LRP facilitation process and the PDA process – implying the existence of a "sub-liminal" or sub-threshold PDA process in that region of stimulus strength where strong, and pigment-dependent, LRP facilitation is seen, but little or no PDA. However, the existence of a residual LRP facilitation following neutral stimuli suggests that PDA-LRP facilitation link is not absolute.

Conclusion

The role of metarhodopsin (M) in the transduction process in invertebrate photoreceptors has become the center of considerable recent interest with a distinction being drawn between its now well-known effects on the prolonged depolarizing afterpotential (PDA) and its apparent lack of influence on the stimulus-coincident response (LRP).

The absorption of light by M has a controlling effect (the "anti-PDA") on the PDA. Until the present study, however, it seemed to have no effect on the LRP. In the present article we have demonstrated that for medium-to-high stimulus amounts – but well below the threshold for measurable PDAs and anti-PDAs,

as well as in their presence – M absorption substantially influences the modulation of the LRP sensitivity by a conditioning light. A small effect of the *presence* of M may also be indicated.

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