Nonlocal Interactions in the Photoreceptor Transduction Process

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ABSTRACT We have recently demonstrated the dissection of the transduction process in the barnacle photoreceptor into antagonistic “excitor” and “inhibitor” processes. We now show that (a) the interaction between the two processes proceeds even when they are induced in different pigment molecules; (b) the excitor process appears to be slightly facilitated if those pigment molecules unaffected by the stimulus are in the stable metarhodopsin state or slightly inhibited if they are in the rhodopsin state; (c) there is a facilitatory interaction among the excitor processes induced in different pigment molecules. In case a, the interaction has a range of at least a few hundred angstroms, taking place in a time of less than a fraction of a second; in cases b and c, the range could be as little as “nearest neighbors” and the time as much as a few seconds. All these interactions could be intermediated by the “excitor” if it is a transmitter.

INTRODUCTION

The mechanism of transduction in photoreceptors is still unclear. Cone, 1973, and Yoshikami and Hagins, 1973, have proposed an internal transmitter intervening between the photon-initiated pigment cascade and the change in plasma membrane conductance. One approach to the internal transmitter problem is the spatial characterization of some of the processes involved: whether or not there is a spread of the influence of these processes within the photoreceptor cell.

We have recently suggested the existence of extrapigmental antagonistic “excitor” and “inhibitor” components in the coupling of the pigment cascade to the membrane conductance change in the photoreceptors of the lateral ocelli of the barnacle (Hillman et al., 1972; Hochstein et al., 1973) and the UV photoreceptors of the median eyes of Limulus (Minke et al., 1973b). In the barnacle (and presumably in Limulus) the visual pigment has two thermally stable states, interconnected by a network of thermally unstable states and thermal and phototransitions (Minke et al., 1974a). In the barnacle, the stable states have absorption peaks at 532 and 495 nm, respectively (Hillman et al., 1972 and Minke et al., 1973a) and the excitor and inhibitor processes are activated by photon-induced transfer of the pigment molecules from the 532- to the 495-nm state and vice versa, respectively.

Despite the lack of direct evidence for the identification of these states in this
preparation, Minke et al. (1973a and 1974a) suggested that the 532-nm state may be the rhodopsin state and the 495-nm state a metarhodopsin state (meta). Thus, net transfer of pigment from the rhodopsin to the meta state induces the excitor transduction process while reverse pigment transfer induces the inhibitor transduction process. The excitor process in isolation manifests itself in a prolonged depolarizing afterpotential (PDA) and the inhibitor process manifests itself in the depression or prevention of the PDA (anti-PDA). Saturating red stimulation (>600 nm) leaves nearly all of the pigment in the 495 meta state, while blue light (<550 nm) leaves the bulk in the 532 rhodopsin state. Thus red illumination of a blue-adapted cell induces a PDA and blue illumination of a red-adapted cell induces an anti-PDA. Neutral stimulation (of the same wavelength as the preceding adaptation) induces both equally, and therefore leaves no poststimulus effect.

According to our model, designed to fit the properties of the PDA and the anti-PDA (Hochstein et al., 1973), the two transduction processes have (different) long but finite lifetimes in isolation and neutralize each other when simultaneously present. Thus, whichever process is more weakly induced by a stimulus, disappears rapidly after its cessation. If, in addition, the neutralization is assumed rapid but not instantaneous, both processes will normally be present with finite strengths during the stimulus, giving rise to a stimulus-coincident response even where no PDA follows. However, other properties of the stimulus-coincident response are not predicted by the model in its present form (Hillman et al., 1972; Minke et al., 1974b).

In this paper we are interested in the spatial properties of the interactions among three elements: the excitor process, the inhibitor process, and the state (rhodopsin or meta) of those molecules not participating directly in the excitor or inhibitor processes, that is, which are in the same state before and after the stimulus. The particular interactions which we will treat here are: (a) the excitor-inhibitor interaction, (b) the excitor interaction with the nonparticipating molecules, and (c) the excitor-excitator interaction.

(a) We have previously shown the existence of an excitor-inhibitor interaction (Hillman et al., 1972; Hochstein et al., 1973) manifested, for example, in the depression of the PDA by blue stimuli, and here demonstrate that the inhibitor acts on the excitor even when the two processes are induced in (statistically) largely different sets of molecules. We do this by using sufficiently weak stimuli so that only a fairly small fraction of the pigment is affected by each stimulus. (b) We find a marginal dependence of the PDA on the state of the nonparticipating molecules by showing that a given amount of rhodopsin to meta pigment transfer results in slightly different PDA's depending on the state (rhodopsin or meta) of the remaining pigment molecules. (c) We establish the existence of an excitor-excitator interaction by showing that the membrane potential, and hence conductance, during the PDA, depend nonlinearly on the amount of pigment transferred from the rhodopsin state to the meta state.

All of the quantitative observations reported here relate to the PDA and not to the stimulus-coincident response: For the second and third problems set out above, the excitor interaction with the nonparticipating molecules and the
excitor-excitator interaction, it is desirable to deal with the isolated excitor process, that is, with the PDA. In the first problem also, that of the excitor-inhibitor interaction, the simplest measure of the degree of mutual neutralization is the PDA. However, the use of the PDA has a disadvantage: Since it only appears for activation of an appreciable fraction of the pigment, the average distance between the molecules affected by successive irradiations cannot be made larger than a few intermolecular distances (see below). The technique is suitable, therefore, for examining ranges up to a few hundred angstroms only; since the results here are all lower limits, we are proceeding with a small-spot technique for examining the few-micron-to-whole cell range, and will report later on this approach. We report only our barnacle experiments, although similar results were obtained for the excitor-inhibitor and excitor-excitator interaction in the *Limulus* median eye. Preliminary results were presented by Hochstein et al., 1974.

**METHODS**

**Techniques**

The preparation, the techniques of intracellular recording, and the optical apparatus have been described previously (Hillman et al., 1973; Minke et al., 1973a) except that besides the quartz iodide lamp a He-Ne laser was used in part of this study for the red stimulation and a Bausch and Lomb Xenon-arc lamp was used in a second part for all lights (Bausch & Lomb, Inc., Rochester, N. Y.).

The "blue" lights were via a Balzers 447-nm narrow band filter (Aktiengesellschaft, Fürstentum, Liechtenstein) or a Corning 5-74 filter (Corning Glass Works, Corning, N. Y.) ($\lambda_{\text{max}} = 435$ nm) with intensities at the photoreceptor of $7 \times 10^{15}$ and $1 \times 10^{16}$ photons/cm$^2$/s, respectively; the nonlaser "red" lights were via a broad band Balzers K6 filter ($\lambda_{\text{max}} = 650$ nm) or a Kodak #26 filter (Eastman Kodak Co., Rochester, N. Y.) (low-cut at 600 nm) with intensities (<700 nm) at the photoreceptor of $4 \times 10^{15}$ and $5.5 \times 10^{17}$ photons/cm$^2$/s, respectively; the laser light was at 632.8 nm, intensity $3 \times 10^{14}$ photons/cm$^2$/s. Illumination of all three photoreceptors in any eye was probably nearly equal, especially in view of the tapetal reflection, so their being electrically coupled (Shaw, 1972) should introduce no distortions into the experiments.

**Use of the Early Receptor Potential to Determine Pigment Distributions**

The early receptor potential is believed to be a manifestation of conformation changes in the pigment molecules. The molecules apparently act independently and their responses sum linearly so that the ERP is linear with the number of molecules affected. The response to brief flashes of light has frequently been used as a quantitative measure of the amount of pigment in a pigment state (see for instance Berson and Goldstein, 1970 and Hillman et al., 1973, for the complete list). The technique remains valid for long, bright test illuminations and for systems with more than one stable state as follows: For a given light stimulus (fixed intensity, duration, and wavelength), the succession of pigment states is fixed and so the ERP due to molecules beginning in a particular state is fixed. This is true just as well for long as for short stimuli, since it also applies to responses due to further phototransitions including those from stable or unstable photoproducts and also returns to the original state and additional exits from it. Therefore, the response to the molecules in a particular state is proportional to the initial number of molecules in that state. That is, the response of the molecules initially in a given state always has the same
shape with an amplitude which is proportional to the number of molecules initially in that state. This has been demonstrated for each of the two stable states of the barnacle photopigment (see Figs. 2 and 3 in Minke et al., 1974a). Although the dependence on intensity is only linear for lights which do not affect most of the molecules in a particular state, the dependence on population of the state for given intensity is always linear.

If more than one state is initially populated, the linearity will apply to each of the populated states. For a pigment system with two stable states (e.g., rhodopsin and metarhodopsin) the total response is the sum of the responses due to the initial populations of the two states. Therefore the difference between the responses for different initial distributions will be proportional to the change in initial population of either of the states. (The changes for the two states are equal and opposite for a conservative system where the sum of the populations is a constant.) That is, the difference between the responses for the different initial conditions is just the difference between the response of one molecule in one of the two states and the response of a molecule in the other state, times the number of molecules by which the population of one state has gone up and the other down. Thus the difference between the responses of the system in two initial states to a particular stimulus will always have the same shape, and an amplitude proportional to the population difference between the two states. It is therefore possible to use the amplitude of this difference at any fixed time during the response as a linear measure of pigment population change, and this is what we do in this article. In each case we record the ERP response to a white test stimulus from the cell when (a) fully red-adapted (Fig. 1 A top trace); (b) fully blue-adapted (Fig. 1 A bottom trace); (c) the fully red-adapted cell had been exposed to various amounts of blue light (other traces of Fig. 1 A, amounts B of blue light indicated at the left of each trace).

We represent the pigment fraction in the rhodopsin state after full red adaptation as \( F(Bo) \), and the net fraction moved from the meta state to the rhodopsin state by a following blue light of amount B as \( P(B) \), or by a saturating blue light as \( P(B_s) \). Thus, the fractions of the pigment in the rhodopsin state for cases a, b, and c above at the start of the white test stimulus are \( F(Bo) \); \( F(Bo) + P(B_s) \); and \( F(Bo) + P(B) \), respectively. Since all the pigment in the dark must be either in the rhodopsin state or the metarhodopsin state, the fractions in the metarhodopsin state at the start of the white test stimulus are \( 1 - F(Bo) \); \( 1 - F(Bo) - P(B_s) \); and \( 1 - F(Bo) - P(B) \), respectively. We represent the amplitudes of the ERP responses at any one fixed time during these responses in the three cases as \( a \), \( b \), and \( c \), respectively. Then, since the amplitudes of these responses vary linearly with the initial stable-state population fractions, we have for the three cases:

\[
\begin{align*}
a &= m[F(Bo)] + n[1 - F(Bo)] \\
b &= m[F(Bo) + P(B_s)] + n[1 - F(Bo) - P(B_s)] \\
c &= m[F(Bo) + P(B)] + n[1 - F(Bo) - P(B)]
\end{align*}
\]

where \( m \) and \( n \) are constants. Thus,

\[
(c-a)/(b-a) = P(B)/P(B_s).
\]

It is in this manner that we determine in the following sections the amount of pigment shifted by a particular stimulus \( (P(B)) \) as a fraction of the total amount which can be shifted by a saturating stimulus of the same wavelength \( (P(B_s)) \). Similarly, we may determine the relative amounts shifted by two stimuli \( (P(B_1))/P(B_s) \).
In Fig. 1 B we plot log \(1 - \frac{(c-a)}{(b-a)}\) vs. B for the ERP responses of Fig. 1 A. The change in population of either stable state is exponential with the amount of light and therefore a straight line is expected on a semilog presentation; see Hochstein, Hillman, Minke, and Knight (in preparation).

**Figure 1.** The use of the early receptor potential (ERP) to determine the fraction of pigment transferred from one pigment state to another. All traces in Figs. 1-5 are intracellular recordings in photoreceptors of the barnacles *B. amphitrite* or *B. eburneus* at 24°C. Light durations are indicated by bars which appear below the responses. Recording of the ERP without late receptor potential (LRP) interference was made possible by spontaneous or ouabain-induced disappearance of the LRP. (A) A cell was first fully adapted to red light (bringing nearly all the pigment molecules into the 495-nm metarhodopsin state). Then the cell was stimulated by a number (shown at left of traces) of blue light pulses. (Each pulse had \(7.5 \times 10^{14}\) photons/cm\(^2\) of 435-nm light at the photoreceptor. Bottom trace followed “saturating” blue light.) This transferred some of the pigment molecules from the metarhodopsin state to the rhodopsin state, and it is this pigment distribution which we would like to measure. To do this a fixed maximal-intensity white-light stimulus was presented to the cell which induces the ERP responses of the figure. If c is the amplitude of the ERP with a (top trace) and b (bottom trace) its extreme values, we show in the text that \(\frac{(c-a)}{(b-a)}\) is the amount of pigment transferred by a blue stimulation of amount B, divided by the total amount of pigment transferable by saturating blue stimulation. Thus the amplitudes of the two responses may be used as a measure of the difference in initial pigment populations. Calibration pulse 1 mV and 50 ms. (B) The relative ERP amplitude change, equal to the relative pigment population change, is plotted on a logarithmic scale against the amount of light B, for the data of Fig. 1A.

**EXPERIMENTS AND RESULTS**

**(A) The Excitor-Inhibitor Interaction**

The principle of the excitor-inhibitor experiment is the induction of a PDA in a certain number of molecules and of an anti-PDA in an equal number of molecules of a statistically independent set. Under these conditions, if the
interaction is local, depression of the PDA by the anti-PDA should result only from the overlap of the two sets of molecules, which may be kept small, but complete suppression should occur if the interaction is nonlocal and has a range which is long compared with the average distance between a molecule of one set and the nearest molecule of the other set.\(^1\)

The equality of pigment transfer in the two directions, and therefore of the PDA and anti-PDA induction, is ensured if the states of pigment adaptation of the cell before and after the induction of the PDA and its suppression are the same. In the chosen paradigm (see Table I), both adaptation states are of a fully red-adapted cell which was exposed to a fixed amount of blue light. Accordingly, the experiment was carried out as follows (each step corresponds to a column in Table I): A cell was initially fully red adapted, that is, almost all of the pigment was brought to the 495-nm meta state which absorbs red light only very weakly. This caused a PDA whose magnitude depended on the previous history of the cell. The cell was then completely dark adapted, that is, a sufficiently long period of darkness was allowed to elapse for the PDA to decline completely. (To make sure that the dark period was sufficiently long, we tried longer dark periods and saw no change in the PDA response to the following stimuli.) Then the cell was exposed to a small amount \(B\) of blue light. This transferred into the 532-nm rhodopsin state a fraction \(P(B)\) of the pigment molecules. Using the early receptor potential (see Methods), \(P(B)/P(B_a)\) under the conditions of Fig. 2A, for example, was shown to be about 0.14. Since \(P(B_a) < 1\), \(P(B) < 0.14.\(^2\)

(The numbers in Table I arbitrarily correspond to the mean value of \(P(B_a) = 0.7\).) Next, the cell was again completely dark adapted, allowing the anti-PDA induced by this blue light to decay. Its complete decay was ensured by showing that longer dark periods did not affect the results. Finally, the last two stimuli were presented without a long dark period between them. A saturating red light induced a partial PDA as shown in the traces on the extreme left of Fig. 2. Since red light virtually empties the rhodopsin state, this PDA should correspond to transferring back into the meta state the fraction \(P(B)\) of the pigment which was in the rhodopsin state. In the left-most traces of Fig. 2 the PDA was allowed to decline unmolested. In the traces of the middle column, a blue light of amount \(B\) was again presented, closely following the red light. This again transferred into the rhodopsin state a fraction \(P(B)\) of the molecules, and so induced in that fraction an anti-PDA. Thus a PDA and an anti-PDA are induced in a fraction \([P(B)]^2\) of the molecules (<0.02 in row A of Fig. 2) leaving a fraction \(P(B)[1 - P(B)]\) of the molecules (>0.86 \(\times\) \(P(B)\)) in which a PDA was induced but in which an anti-PDA was not directly induced. If the neutralization were local, that is, only in the \([P(B)]^2\) molecules, we should be left with a PDA in the other \(P(B)[1 - P(B)]\) molecules. The middle trace of Fig. 2A shows that the PDA was neverthe-

\(^1\) The "PDA sensitivity," that is, the PDA height and duration for given pigment transfer, varied greatly from cell to cell (compare Figs. 2A and 5). All of the conclusions of this article held over a wide range of such sensitivities.

\(^2\) In several cases, \(P(B_a)\) was actually measured using the ERP and was found always to be in the range 0.5-0.9.
| Succession of stimuli: | Test | Control | Expected PDA |
|------------------------|------|---------|--------------|
| Saturating red | Saturating dark | B blue | Saturating dark | Saturating red | B blue | if: | Local | Nonlocal |
| $F(B_0)$ | $F(B_0)$ | $P(B)$ | $P(B)$ | $P(B)^2$ | $1-P(B)(1-P(B))$ | 0 |
| 0.00 | 0.00 | 0.10 | 0.10 | 0.01 | 0.09 | 0.00 |

| Succession of stimuli: | Rhodopsin state | Metarhodopsin state |
|------------------------|-----------------|---------------------|
| Saturating red | Saturating dark | B/3 blue | Saturating dark | Saturating red |
| $F(B_0)$ | $F(B_0)$ | $P(B/2)$ | $P(B/2)$ | $P(B/2)$ |
| 0.00 | 0.00 | 0.05 | 0.05 | 0.05 |

The algebraic expressions give the fractions of the pigment in each state after the stimulus at the head of each column. "Saturating" means that more of the same would make no difference. (Since $F(B_0)$ is always near zero it is not carried through after the first two columns.) The number under each expression indicates its approximate value for the experiment of Fig. 2A, assuming $P(B/2) = 0.7$. Transitions indicated by down-sloping arrows (rhodopsin to metarhodopsin transitions) result in PDA's (ovals). Those with up-sloping arrows result in anti-PDA's (rectangles). Horizontal arrows result in neither. The dashed ovals mean that the degree of PDA induction depends on prior conditions. If up and down transitions occur in rapid succession in the same molecules, the effects cancel. If they occur in different molecules, the effects cancel only if the interaction is nonlocal.
less apparently completely suppressed by the appropriate blue light, indicating a nonlocal interaction between the excitor and inhibitor processes.

As a control, we demonstrated that induction of a PDA directly into a fraction less than $P(B)(1 - P(B))$ of the molecules resulted in a much larger PDA than that

![Diagram](https://example.com/diagram.png)

**Figure 2.** The interaction between the excitor and the inhibitor processes. Late receptor potentials (LRP's). The traces of the center column show that a PDA (prolonged depolarizing afterpotential) whose unmolested course appears in the left trace is completely suppressed by an equal anti-PDA, when the two are induced in largely nonoverlapping sets of molecules. The right traces show that the degree of overlap of the sets is insufficient to explain this suppression: Each PDA in the right traces arises from a smaller number of pigment molecules than are in the part of the PDA population in which anti-PDA is not directly induced, in the center traces; and yet the PDA's in the right traces are larger than those in the center traces (see text). The cells in the center and left traces were prepared by saturating ("full") red illumination followed by an amount $B$ of blue light and then 5 min of darkness. The responses shown are to 10 s red light, followed, in the center traces, by a further brief blue exposure of amount $B$, (indicated by dots after light bars). In the right traces the preparation was full red/0.5 $B$ blue/5 min darkness, and the stimuli again were 10-s red. The calibration bar represents 20 mV for rows A and C, 5 mV for row B. Row A is for a cell with strong pump postillumination hyperpolarization (PIH) ($B. eburneus$); row B is for a cell in which the PIH was weak ($B. amphitrite$); and rows C are sequential responses 10–20 min after application of ouabain in a cell with a strong PIH originally ($B. eburneus$). For each row the procedure was Sat. $R/B$ blue/3 min dark/10 s $R$-blue (center response)/3 min dark/10 s $R$ (left response)/0.5 $B$ blue/3 min dark/10 s $R$ (right response).

of the middle trace of Fig. 2 A (if any). Since the PDA amplitude is a monotonic function of pigment transferred, this shows that the PDA of the middle trace (if any) corresponds to a number of PDA molecules smaller than the number of those not directly cancelled by the anti-PDA; that is, at least part of the PDA
molecules must be cancelled by remote anti-PDA's. The control experiment consisted of the same paradigm as the main experiment but with half as much blue light and without the second blue exposure (see Table I, lower half). Thus a PDA was induced in a fraction \( P(B/2) \) of the molecules. We used the early receptor potential to demonstrate that, for this cell and these experimental parameters,

\[
P(B/2)/P(B_a) \sim 0.07,
\]

and

\[
[P(B)/P(B_a)] \times [1 - \{P(B)/P(B_a)\}] \sim 0.12.
\]

But

\[
[P(B)/P(B_a)] \times [1 - P(B)] > [P(B)/P(B_a)] \times [1 - P(B)/P(B_a)],
\]

so

\[
P(B/2)/P(B_a) < [P(B)/P(B_a)] \times [1 - P(B)],
\]

or

\[
P(B/2) < P(B)[1 - P(B)],
\]

and the control condition is fulfilled. The right trace of Fig. 2 A shows that the result was a PDA clearly larger than the residual PDA in the test experiment. The experiment and control were repeated for several values of \( B \) and in several cells, with similar results.

The postillumination hyperpolarization (PIH) due to the electrogenic pump in this preparation should not be a substantial factor in the test-control comparison of this experiment, since the pump apparently depends on the response of the cell (Koike et al., 1971) which is very similar in the three cases (Fig. 2 A).

As an added precaution we performed this set of experiments also under conditions where the pump potential could not affect the result. The second row of traces (Fig. 2 B) shows responses for a cell where the PIH was extremely weak, presumably because of metabolic decay (See Hanani and Hillman, 1976). This is apparent in the middle trace, as it was for all conditions of stimulation in this cell. The stimulus-coincident response and the PDA, under these conditions, though somewhat reduced in amplitude, were in every other respect identical to the responses recorded when the PIH was strong. Comparison of the PDA in the middle trace with those in the other two traces of Fig. 2 B clearly shows that there is less PDA for the "test" middle trace than for either "control." In this case, by ERP test, we found

\[
P(B)/P(B_a) = 0.070,
\]

\[
P(B/2)/P(B_a) = 0.035,
\]

and

\[
P(B)/P(B_a)[1 - P(B)/P(B_a)] = 0.065,
\]

so that

\[
P(B/2)/P(B_a) \ll P(B)/P(B_a) [1 - P(B)/P(B_a)] < P(B)/P(B_a)[1 - P(B)],
\]
The PDA on the middle trace of row B is nevertheless clearly close to zero, and certainly smaller than that in the right trace.

The same experiment was also performed after the application of 10^{-5} M ouabain to a cell with a strong pump hyperpolarization. The final two rows of Fig. 2 show responses recorded sequentially beginning 10 min after perfusion with ouabain. For each row the order was center, left, right trace, with 3-min dark between each two stimuli. Despite the gradual, continuous decline in response amplitude (Koike et al., 1971), it is clear that the PDA, if any, on the middle traces is smaller than those on the right traces. Here, by ERP measurement, we found

\[ P(B)/P(B) = 0.48, \]
\[ P(B/2)/P(B) = 0.28, \]

and

\[ P(B)/P(B) [1 - P(B)/P(B)] = 0.25, \]

so that

\[ P(B/2) \leq P(B)[1 - P(B)]. \]

Thus, locality would predict that the middle traces have PDA's approximately equal to or greater than those of the right traces. Rather, the PDA's, if any, are smaller.

A lower limit on the range (distance) of the interaction can be derived from the upper limit on the value of \( P(B) \). The number of molecules which must lie within the range must be at least of the order of \( 1/P(B) \), if substantial excitor-inhibitor cancellation is to occur. The smallest \( P(B) \) for which the measurement could be carried out on any cell was about 0.02, so \( P(B)/P(B) = 0.09 \) as measured with the ERP, and no sign of a departure from complete suppression was seen. At least the 50 neighbors near each molecule must therefore be within its interaction range.

(B) Interaction of the Excitor Process with the Nonparticipating Molecules

Is the PDA, induced by transferring a fixed number of pigment molecules from the rhodopsin state to the meta state, influenced by the state (rhodopsin or meta) of those molecules which are not transferred either way (and so are not directly responsible for any PDA or anti-PDA)? This was investigated as follows: Trace 1 of Fig. 3 A shows a partial PDA induced by a certain amount \( R \) of red light in a fully blue-adapted cell. When this PDA had decayed in the dark, a further, saturating, red light (\( R_a \)) was presented, inducing a further PDA: trace 2. In each case there was a period of darkness between each two stimuli and we made sure this period was “saturating” by seeing that longer periods had no further effects.

The initial amount \( R \) of red light had been adjusted so that the PDA's resulting from the two red exposures would be nearly identical. An initial 0.8 \( R \) red light
resulted in a PDA very near to that of trace 2 (Fig. 3A) and the following $R_a$ in a PDA close to that of trace 1 (Fig. 3A), i.e. with a reversed difference between the two PDA's so that the amount of initial red light $R_a$ for which the two PDA's were equal was about 0.9 $R$. (A square dependence of PDA on light amount, for example, would make this 0.906 $R$.) If the state of the remaining molecules is irrelevant, each of the two PDA's should represent the transfer into the meta state of half of the initially transferable pigment. The nonparticipating molecules are mainly in the rhodopsin state during the first PDA and almost entirely

Figure 3 The interaction between the excitor process and the state of those pigment molecules not participating directly in the PDA induction. B. amphitrite. (A) LRP's. Trace 1 was preceded by full blue adaptation and 5 min of darkness. Trace 2 was preceded by trace 1 and 3 min of dark. In trace 1 the stimulus was a limited amount $R$ of red light, and in trace 2, a full red light. For an amount 0.8 $R$ of red light, the ratio of the heights of these two PDA's was approximately reversed. That is, about 0.9 $R$ of red light is needed to transfer from the 552 rhodopsin to the 495 meta state such a fraction of the pigment population that transferring the remainder induces a similar PDA. Traces 3 and 4 are like 1 and 2, respectively, except that the stimulus in 3 was a full red light, so that trace 3 shows a full PDA and trace 4 a "zero," or no-pigment change, PDA, for comparison. Dashed lines are added to guide the eye where the trace was too weak to appear in the photograph. The light duration bars relate, from top to bottom, to traces 3, 1, 2, and 4, respectively. (B) Traces show the ERP responses to a white test stimulus in the same cell. The central trace is for the cell after 0.9 $R$ red light, and is not halfway between the responses for the fully blue-adapted state (bottom trace) and the fully red-adapted state (top trace), indicating that 0.9 $R$ transfers more than half (about 0.63) of the transferable pigment. The simplest explanation is that nonparticipating molecules affect the PDA. In trace 1 they are largely in the 552 rhodopsin state and in trace 2 in the 495 meta state. The calibration bars represent 20 mV and 10 s for the traces in Fig. 3A and 1 mV and 40 ms for the traces in Fig. 3B.
in the meta state during the second. (Note that all the molecules have reached these states in this preparation within seconds after any stimulus [Minke et al., 1974].)

To check whether the two equal PDA's correspond to equal pigment transfers, in which case the nonparticipating molecules have no effect, we exploit the linear dependence of the early receptor potential (ERP) on pigment change, as we did above (see Methods). The traces of Fig. 3 B show, from bottom to top, the ERP responses to white test flashes of the cell: (a) in the fully blue-adapted state (fraction of pigment in rhodopsin state = $F(R_o) = F(B_o) + P(B_o)$), (b) after $R_h$ red light (rhodopsin fraction = $F(R_o) + P(R_h)$), and (c) in the fully red-adapted state (rhodopsin fraction = $F(R_o) + P(R_o) = F(B_o)$). (Note that $P(R_h)$ and $P(R_o)$ are negative numbers here as pigment is transferred from rhodopsin to meta.) If $R_h$ really transfers to the meta state half the pigment transferable by saturating red light, $P(R_h) = 0.5$, and the middle response should be exactly halfway between the top and bottom responses at all times. That is, presenting the response amplitudes at any time $t$ by $a$, $b$, and $c$, $(b-a)/(c-a) = P(R_h)/P(R_o)$ should be 0.5. (This equality can be formally derived in the same way as that used in the Methods section.)

The measured value of $(b-a)/(c-a)$ in this cell is 0.63; values in three other cells ranged from 0.59 to 0.67. Thus there appears to be a small influence of the nonparticipating molecules on the PDA in the direction that the PDA is facilitated by interaction with the nonparticipating molecules in the meta state and/or inhibited by those in the rhodopsin state.

(C) The Excitor-Excitor Interaction

Two demonstrations of the existence of a facilitatory interaction among the PDA's excited in different pigment molecules appear in Figs. 4 and 5. Fig. 4 shows the response to two successive equal red pulses (latter part of each trace) in a cell which initially was fully blue adapted (early part of each trace). The PDA after the second of the two equal red pulses is grossly higher than that after the first, even though the potential apparently returns, between the two, to the same level as before the first (a hyperpolarized level, see below). Since the successive equal pulses of course transfer successively smaller amounts of pigment into the 495 meta state, the successive PDA's would be reduced if there were no facilitation; in fact they are considerably enhanced. From ERP observations, as above, we determined that the first red pulse in the top and bottom traces transferred $P(R)/P(R_o) = 0.14$ and $0.22$ of the transferable pigment into the meta state, respectively.

This facilitatory effect thus clearly outlasts the response. Its actual duration was examined by increasing the spacing between the successive red pulses. The degree of facilitation was found to decline with time constants which varied from cell to cell in the range of seconds to minutes. The duration corresponded approximately, in each cell, with that of the full PDA in that cell.

One must consider the possibility that the observed voltage facilitation could arise from an increase in the cell resistance after the red stimuli, and not from a conductance facilitation. Bridge measurements indeed sometimes showed such
an increase, but never more than by a factor of 2, and generally by much less. The observed facilitation was by a factor far larger than this (Fig. 4). Furthermore, C. Shaw has done a few voltage clamp experiments at our request and has observed large conductance facilitations. These facilitations could not be due to residual cell resistance changes.

In order to confirm that the large PIH's observed in this preparation do not substantially affect the results, the experiment was performed as follows (Fig. 4): The blue adaptation was presented immediately after a full red stimulus which induced a PDA. In this way the blue stimulus caused the complete suppression of the PDA, left no anti-PDA effect, but the red-blue succession induced a maximal pump PIH. Thus, the following red stimuli, presented before the PIH had declined more than minimally, could not increase the PIH substantially and the facilitation is not due to pump saturation.

If this facilitation has a rapid onset, it should also manifest itself in a nonlinear dependence of PDA amplitude on stimulus amount. We examined this dependence using stimulus durations which were short compared with that of the full PDA in the same cell. Some sample recordings, in which the nonlinearity of the PDA dependence on pigment transfer is clearly visible, are shown in Fig. 5. Extraction of the quantitative dependence from such observations, however, is complicated by the fact that not only the PDA amplitude changes, but also its shape, so that different possible measures of the strength of the PDA (amplitude at various times, width at various heights, step decrease upon cessation of stimulus) give different dependences. These differences are seen in Fig. 6, where various measures of the PDA in one cell are plotted against net pigment...
transferred, as a fraction of the total transferable, that is, $P(R)/P(R_\infty)$, which was derived from ERP measurements as above. One sees that, while they do differ quantitatively, all the measures of the PDA exhibit an initial nonlinear, concave-up dependence.\(^3\) Conversion of the membrane potential to conductance would have the effect of raising the higher points on the graph, and so emphasizing further the nonlinearity. The upward concavity indicates that there is a facilitatory interaction among the PDA's or the excitor processes responsible for them,

\[\text{Intensity} \quad (-\log) \quad \text{Pigment fraction transferred} \]

\[0.09 \quad 0.30 \quad 0.37 \quad 0.48 \quad 0.57 \quad 0.80 \]

**FIGURE 5.** The nonlinear dependence of the PDA on pigment transfer. *B. eburneus*. The traces are the LRP responses of a blue-adapted cell to red light transferring from one state to the other the indicated proportions of the transferable pigment as measured by the ERP. By any measure the PDA dependence on pigment transferred appears to be nonlinear. The calibration bar represents 20 mV, and the light durations were 10 s.

induced in different pigment molecules. No quantitative analysis is attempted here.

**DISCUSSION**

(a) We have shown that the previously demonstrated interaction between the excitor and inhibitor components of the transduction process in the barnacle photoreceptor is nonlocal: the excitor and inhibitor neutralize each other even when induced in different pigment molecules. In every trial of the "test"

\[\text{The contrary thesis (linearity) was tentatively accepted by Hochstein et al., 1973, based on a wide scatter of data consistent with both a linear and a square law.}\]
paradigm (center column of Fig. 2) the PDA induced in one fraction of the receptor pigment is suppressed by a following stimulus which induces inhibition in a statistically different set of pigment molecules. The remaining PDA is clearly smaller than that of the response to a “control” stimulus, which we showed induces excitor in a fraction of the pigment which is smaller than the nonoverlapping fraction in the test paradigm. (Compare middle and right columns of Fig. 2.) In fact, for small amounts of light, \( B \), and hence pigment fractions, \( P(B) \), we have \[ P(B) \approx P(B) \times [1 - P(B)] \] so that we would expect almost no overlap in the “test” paradigm and almost no effect of the blue inhibitor-inducing stimulus if the inhibition were entirely local. Instead a marked difference is seen (compare middle and left columns of Fig. 2).

This excitor-inhibitor interaction encompasses at least 50 of the nearest neighbors. If the distribution of the pigment molecules is similar to that seen by Blasie et al. (1969) in frog photoreceptor membranes, this implies a range of at least a few hundred angstroms. We conclude that either the excitor process, the inhibitor process, or both, or possibly the pigment molecules themselves, must travel distances of at least hundreds of angstroms in less than perhaps half a second (the PDA suppression in Fig. 2 is complete within that time).

(b) Our results suggest a small influence on the PDA of the molecules not undergoing a net change in conformation such that molecules remaining in the meta state facilitate and/or molecules remaining in the rhodopsin state inhibit

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4 These authors found a square array with a center-to-center pigment molecule spacing of 70 Å in frog photoreceptor membranes.
the PDA. In attempting to decide whether this effect is significant or not, one must consider alternate sources of distortion of the PDA-ERP correlation. These include: (i) possible residual effects of preceding PDA's or anti-PDA's. However, additional dark times of several minutes before or between the red stimuli had no effect on the results. In every case, both the potential and the conductance of the cell had returned to their resting values long before a further stimulus was presented; (ii) the effects of the electrogenic ion pump in this preparation (Koike et al., 1971). However, the activity of this pump is usually considered to depend on the change in the ionic composition of the intracellular medium due to the preceding stimulus, which change should be nearly the same for the nearly equal PDA's in this experiment; (iii) the effect of any systematic variation through the cell of the ratio of the contributions of the pigment molecules to the ERP and to the PDA. If the visual pigment were sufficiently absorbing that the initial and saturating red stimuli effectively activated different regions of the cell, an artifactual distortion would appear. However, the cell is so transparent that, even if an inhomogeneity existed (for which there is no evidence), the effect would be very small.

One should note that the state of the nonparticipating molecules is not the only difference between the experiments of Fig. 3A, traces 1 and 2. The stimulus in the second experiment was of larger amount than in the first and so resulted in a much larger number of pigment molecules making the "round trip," that is, returning to their initial state. These round trips, or the stimulus-coincident response resulting from them, might influence the PDA. However, such round trips do not induce PDA's or anti-PDA's (these are not observed after neutral stimuli; Hochstein et al., 1973) so we consider the effect unlikely.

We thus conclude that the most probable explanation for the observed result is, in fact, the presence of a small influence of the nonparticipating molecules on the PDA. The interaction (if it exists) is by definition nonlocal, but present data do not require a range beyond nearest-neighbors, and this is in a time of several seconds. (This is the duration of the saturating red stimulus [Fig. 3A, trace 2] after which the PDA's of traces 1 and 2 have nearly the same time courses.)

(c) We have found a strong facilitatory excitor-excitator interaction manifested in the PDA. This facilitatory effect outlasts the response (Fig. 4) but has a rapid onset leading to a nonlinear dependence of the PDA amplitude on fraction of pigment transferred (Figs. 5-6). A similar effect has been seen by Hanani and Hillman, 1976, in the stimulus-coincident late receptor potential. This latter effect is seen in the first traces of our Fig. 5 but not in the remainder of our responses because of voltage saturation. With respect to the range and velocity of the excitor-excitator interaction, the argument is similar to that for the excitor-inhibitor interaction. The fact that the nonlinearity in Fig. 6 appears clearly by about 0.3 pigment transfer, however, does not require an interaction beyond nearest-neighbors. Again this interaction must take place within a few seconds.

About the functional dependence of the various interactions we can say the following: (a) The fact that a neutral stimulus induces no PDA or anti-PDA suggests that the excitor and inhibitor neutralize each other in the same ratio in which they are produced by equal (and opposite) pigment transfers, but tells us nothing directly about their rates of production. By far the simplest hypothesis
would be that their production is linear with amount of light and that they neutralize each other in fixed ratio, independent of concentration. (b) Since the dependence of the excitor process on the nonparticipating molecules is so weak, we have not attempted to determine the functional dependence on nonparticipating population distribution. (c) The nonlinear dependence of the PDA on pigment transfer could arise either from a nonlinear dependence of excitor strength on pigment transfer or from an excitor-excitator interaction. The linear production hypothesis mentioned above is only compatible with the latter.

Possible mechanisms which may be suggested to explain the observations of nonlocality presented here are: electrical spread, translation of the pigment molecules themselves, or some internal transmitter related to either the excitor or the inhibitor process discussed above. Of these, electrical spread cannot explain the PDA facilitation (excitor-excitator interaction) as the effect occurs under voltage clamp. Translation of transmitter related to the inhibitor process cannot explain the excitor-excitator interaction nor the interaction of the excitor process with the nonparticipating molecules as inhibitor is involved in neither of these. Translation of pigment molecules or of an excitor-related transmitter remain as possibilities.

Cone, 1073, cited evidence for the existence of an internal transmitter in photoreceptors. In invertebrates, he noted that the maximum current response per photon absorbed in the ventral photoreceptors of Limulus appears to be too large to arise from a single-ion channel, or even a single microvillus. This could arise from either of the mechanisms found acceptable above. However, this observation requires a diffusion speed (t < 100 ms) and distance (>1 µm) greater than those arising from our own results and less compatible with the diffusion of a large pigment molecule in membrane. Furthermore according to Wehner and Goldsmith (1975) there appears to be no pigment diffusion in the photoreceptors of another invertebrate (crayfish).

The substantial reduction of sensitivity observed in many photoreceptors after absorption of relatively few photons (Borsellino and Fuortes, 1968; Behbehani and Srebro, 1974; Dowling, 1963) implies a spread of adaptation. Hamdorf, 1970, directly demonstrated this spread in the fly. However, none of the above mechanisms is excluded by these observations, although pigment diffusion seems implausible for the times and distances involved.

In the vertebrate photoreceptor light reduces cell conductance, so an “excitor” cannot be directly involved. Cone, 1973, noted that a spreading process is needed to explain the facts that (a) in some cases absorption of one photon can reduce the cell conductance by as much as 1%; and (b) the sacs in vertebrate rods which contain the bulk of the visual pigment are isolated from the plasma membrane. Translation of pigment or excitor molecules cannot be responsible for these phenomena, so an alternate or additional process must be present in vertebrates.

CONCLUSION

We have demonstrated some nonlocalities in the barnacle photoreceptor transduction process. We suggest that the most likely explanation involves translation of an internal “excitor.”
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