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Photoconvertible Pigment States and Excitation in *Calliphora*; the Induction and Properties of the Prolonged Depolarising Afterpotential*

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Abstract. 1. The proposed models of two independent groups, which relate the different states of the visual pigment to the excitation of the membrane in invertebrate photoreceptors (with particular reference to the prolonged depolarising afterpotential, the PDA) are compared and evaluated.

2. The validity of the late receptor potential (the "normal" receptor response) as an index of photoreceptor sensitivity, i.e., an index of the number of rhodopsin to metarhodopsin transitions, is verified by concurrent spectrophotometry.

3. Electrophysiological observations alone allow the calculation of 1.3×10^8 photopigment molecules in the rhabdom of an R1–6 photoreceptor of a vitamin A-bred *Calliphora*.

4. The PDA is shown to be quantifiable in terms of the number of rhodopsin to metarhodopsin conversions by the absorption of single light quanta.

5. The comparison of discrete membrane fluctuations (quantum bumps) during the PDA and during exposure to sustained light stimuli that mimic the PDA suggest that, the PDA, similar to the late receptor potential, may be due to the summation of quantum bumps.

Key words: Prolonged depolarising afterpotential – Visual pigments – Photoreceptor model – Photoreceptor sensitivity – Quantum bumps.

Introduction

The properties of the photoconvertible P490-M580 system of the photoreceptors R1–6 in the blowfly are well documented by abundant spectrophotometric and electrophysiological studies (Dörrscheidt-Käfer, 1972; Hamdorf et al., 1973; Hamdorf and Rosner, 1973; Rosner, 1975; Hamdorf and Schwemer, 1975; Stavenga et al., 1973; Razmjoo and Hamdorf, 1976; Boschek and Hamdorf, 1976; Hamdorf and Razmjoo, 1977; Schwemer, unpublished data). Similar systems have been dem-

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onstrated for *Musca* (Kirschfeld et al., 1977); *Drosophila* (Ostroy et al., 1974; Pak and Liddington, 1974); and in the dronefly (Stavenga, 1976).

As in other P-M systems, λ-specific equilibria ($C_{Peq} + C_{Meq} = C_{Po}$, where C is concentration in moles per unit volume of the substance symbolised in the subscript) can be created by monochromatic illumination, the equilibrium depending mainly on the absorption probabilities of the two pigment states (α_P and α_M) at the wavelength of the adapting illumination. The lowest P concentration (i.e., when $C_{Peq} \simeq 0.2\ C_{Po}$) is caused by blue light of 460 nm, where the ratio $\alpha_M \cdot \gamma_M / \alpha_P \cdot \gamma_P$ has its minimal value (γ is the quantum efficiency of isomerisation); red light reconverts M580 to maximum rhodopsin concentration, C_{Po}.

For several reasons, the visual system of the blowfly lends itself particularly well to the analysis of the sequence of events from the absorption of a photon to the excitation of the membrane. For example, it has been demonstrated electrophysiologically that there is a linear relationship between sensitivity and the concentration of rhodopsin (within the permissible ratio of P : M, as it is varied from $\simeq$ 100% : 0% to $\simeq$ 20% : 80%), regardless of the absolute amount of the photopigment present; this being done by breeding flies on vitamin A-rich or vitamin A-deprived diets (Schwemer, unpublished results; Razmjoo and Hamdorf, 1976; Stark and Zitzmann, 1976). This finding indicates a one-to-one relationship between the number of quanta absorbed by P molecules and the excitation of the membrane. The observation that the slopes of intensity-amplitude functions for P-rich and M-rich equilibrium states are identical implies that this relationship is irrespective of quanta absorption by M molecules. However, in membranes which are apparently full of photopigment (where the distance between photopigment molecules becomes small) the loss of sensitivity in the M-rich state is slightly more than the theoretically expected desensitisation (Razmjoo and Hamdorf, 1976).

Models Relating Excitation to the Photopigments

In the pursuit of the mechanism of the sequence of events from the absorption of quanta to membrane excitation, the prolonged depolarising afterpotential (PDA) was seen to provide fresh insight. The PDA occurs when intense stimuli cause large shifts of rhodopsin to metarhodopsin. It was first reported by Nolte and Brown (1972) in the UV receptors of *Limulus* (containing a visual pigment similar to that reported earlier for *Ascalaphus:* Gogala et al., 1970; Hamdorf et al., 1971), and has been observed in all other invertebrates that have been investigated.

An observation which served as a basis for a model (Hochstein et al., 1973), describing this phenomenon is the following. When a rhodopsin regenerating stimulus is presented *during* a PDA, it suppresses the PDA and allows the induction, quite soon after, of another PDA which can last as long as the original one. However, a similar regenerating stimulus presented *after* the complete decay of a PDA is seen to be followed by an interval during which induced PDAs are of shorter duration than before (Minke et al., 1973a; Hochstein et al., 1973; postscript by Hamdorf and Razmjoo in Hillman et al., 1977; Minke, 1979).

The proposed model which, quite adequately, accounted for these events hypothesised that, after the shift of the photopigment from P $\rightarrow$ M, there are excitors

produced which depolarise the membrane, in the form of the PDA. Once released, these excitors are apparently in no further control of the photopigment, in as much as an M → P shifting stimulus during the PDA does not directly remove the excitors. And thus it was necessary to hypothesise that the M → P process produces inhibitors which then neutralise the excitors, this way terminating the PDA. This model, known as the excitor-inhibitor model (E–I), was recently summed up by P. Hillman (in Hillman et al., 1977) as follows: "... (a) Each R → M transition produces one unit of excitor, and each M → R transition one unit of inhibitor. (b) Excitor opens membrane ionic channels, inhibitor does nothing to membrane but excitor and inhibitor mutually annihilate 1 : 1. (c) There is a positive cooperative effect among excitors. (d) If not annihilated, excitor and inhibitor die slow natural deaths ... In addition, if we assume that the excitor-inhibitor annihilation takes a finite time, the normal response to neutral light (one which results in no net pigment transfer) would be predicted, but other properties of this response do not fit the model."

While this model gives an adequate explanation of the PDA, it is its shortcomings in explaining the late receptor potential (LRP) (as is indeed acknowledged at the end of the above quotation) that have made us propose an alternative model which, it is our claim, explains both the PDA and the LRP (Hamdorf and Razmjoo, 1977). As an introduction to our 'photopigment model', we shall start by pointing out some of what appear to us as inadequacies of the E–I model.

The excitor-inhibitor model has been used to account for the LRP of the uv cells in the median eye of *Limulus* in the following manner (Minke et al., 1973a). A UV stimulus to the UV-adapted photoreceptor elicits no PDA because equal P → M and M → P transitions occur, producing equal quantities of excitor and inhibitor which neutralise each other. From its onset to its completion this neutralisation has a finite time, and during this period excitors can lead to membrane depolarisation, which is why an LRP is elicited. Based on our present data (not available at that time), we can interpret the above explanation to mean that during a stimulus which produces mainly rhodopsin (e.g., the red light of Figures 1, 2, and 3 of Hamdorf and Razmjoo, 1977, where the production of inhibitor is greater than that of the excitor), with the onset of the neutralisation process membrane depolarisation would be reduced. If the neutralisation is considered to be a dynamic process, i.e., the excitors which continue to be produced throughout the stimulus continue to take time to be annihilated and therefore continue to be present in some quantity, then during such a sustained regenerating stimulus, the steady-state does not decline to zero, but to an amplitude which should reflect: "... the natural lifetimes of the excitor and inhibitor, ... the annihilation rate constant, ... the relative rate of absorption of light by the two stable states of the pigment, ... excitor concentration ..." (Hillman, personal communication). Now, the absorption spectra of rhodopsin and metarhodopsin in *Calliphora* reveal that the regenerating stimulus (used by us, Hamdorf and Razmjoo, 1977) is absorbed about 100–1000 fold more by metarhodopsin than by rhodopsin. From the foregoing concept of a dynamic annihilation process, one would expect this great concentration difference of a thousand times more inhibitors than excitors to be reflected in the amplitude of the steady-state, i.e., if the amplitude is not actually zero, then it should be smaller than when no inhibitors are present. Yet as it can be seen from the same figures it is hardly lower than when there are almost no inhibitors produced, i.e., the plateau of the steady-state response to the

blue PDA-inducing stimulus when starting from a state of $C_P \simeq 100\%$, $C_M \simeq 0\%$.

The shortcomings of this model in explaining the LRP is further exemplified by its attempt to account for *differences* in the amplitude of the LRP. The following explanation of the events observed in *Balanus* is quoted from Hochstein et al. (1973): "Neutral stimuli produce equal quantities of excitor and inhibitor. These rapidly netralize each other, ... During and shortly after the stimulus, however, because of the finite neutralization time, a certain quantity of excitor (and of inhibitor) is always present. Thus all stimuli will induce stimulus-coincident LRP's. Blueing stimuli[1] produce little excitor but much inhibitor and should give smaller stimulus-coincident LRP's than if the same stimulus were neutral (that is, after blue adaptation)".

The above passage indicates that at least the transient phase of the LRP – the "stimulus-coincident LR" – is manifested *before* all the excitors are annihilated. In anticipating the amplitude of the transient phase we may again take note of the foregoing factors which could determine the excitor concentration. Now, since the excitor is assumed to have a much longer life-time than the latency of the neutralisation process, it can be safely assumed that from the time of its release to the manifestation of the LRP, much of the excitor produced is still extant, and thus with insignificant neutralisation during this period, the only remaining factor dictates that the amplitude of the transient phase must depend *only* on the *absolute* amount of the excitor produced, regardless of the amount of inhibitors produced. Yet note that in contradiction to this, the explanation quoted above states that it is the difference in the concentration of excitors and inhibitors that determines the size of the LRP, since by a neutral stimulus is meant a stimulus at a wavelength identical to that of the adapting (photopigment equilibrating) light, which will then produce equal forward and backward transitions, i.e., production of excitors and inhibitors in equal numbers.

Another objection to the E–I model concerns the state of the membrane during the decay of the PDA. What follows is an attempt on our part to apply the E–I model to one of our findings. The P → M transitions, and thus the release of excitors necessary for the production of a PDA occur in a very short time (less than the 0.5 ms limit of the transmission change measurements, see Figure 5 and the text pertaining to it). Thus the long duration of the PDA could be due to the fact that the number of excitors exceeds the number of closed channels (as suggested by Stark and Zitzmann, 1976). The bottleneck of the above reaction would then appear to be the rate at which the membrane can process the awaiting pool of excitors. If the latter interpretation is valid, the model fails to explain why during the course of the PDA the membrane can still respond to relatively weak test stimuli (Figs. 2, 7, and 9), i.e., how small additions to the pool of excitors (which already exceed the capacity of the membrane) can be manifested in the form of the superimposed LRPs, unless it is further postulated that these newly created excitors are different from the rest and have different affinities for the membrane and for the inhibitor.

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[1] In *Balanus* the position of the spectral absorption curves of P and M are interchanged with respect to those for *Calliphora*, so that red light causes P → M whereas regeneration needs blue light

But this is *our* interpretation of the consequences of this model. The E–I model says nothing about the relative numbers of excitors and closed channels, and ascribes the long duration of the PDA to the excitors' dying a "slow natural death". Nor does the model predict the fate of the excitors after opening membrane channels. But a consideration of the excitor-channel interactions can suggest augmentations which if made, *do* enable the E–I model to account for the manifestation of the responses to test stimuli during the decline of the PDA. Consider the following.

Two models for such interactions are readilly conceivable.

1. $$\underset{k_d \downarrow}{E} + \bullet \underset{k_c}{\overset{k_o}{\rightleftharpoons}} \circ \,.$$
e

Here an equilibrium process can be envisaged, where the coupling of the excitor (E) and a closed channel (●) produce an open channel (○), which in closing does not destroy the excitor but releases it for 'endless' further couplings. However, the duration of the PDA is limitted by the decay rate constant of the excitor (k_d), as it breaks down to the inactive form (e).

2. $$\underset{k_d \downarrow}{E} + \bullet \underset{\downarrow k_c}{\overset{k_o}{\rightleftharpoons}} \circ \,.$$
e e

In this visualisation the open channel, in closing, *does* break down the excitor.

In both cases one initial condition is that the long duration of the PDA is because the natural decay rate constant of the excitor (k_d) is much smaller than both the rate constants of channel opening (k_o) and closing (k_c), i.e., $k_o, k_c \gg k_d$. The initial condition $E < \bullet$ is a trivial case, and we are interested in situations when the excitors initially produced are greater in number than the available closed channels, i.e., at the beginning of the PDA, but soon decreasing to $E < \bullet$, for only then will the excitors produced by the test stimulus find closed channels for coupling. Thus we find that this condition can be met in both cases when $k_c > k_o$.

Another objection to the E–I model is the following. Since the amplitude of the PDA at any time must be *some* expression of the fraction of open channels and thus the number of excitors active at that time, the manifestation of the test response which is due to a relatively weak stimulus (the production of a small number of additional excitors) indicates the smallness, in comparison, of the number of the excitors producing the PDA. And as the PDA progressively declines, the ratio of the PDA-inducing excitors to the test stimulus-produced excitors becomes even smaller. Now, since the test stimulus is not a "neutral" stimulus (the isosbestic test stimulus of Figs. 2, 7, and 9) it should produce, in this blue-adapted equilibrium ($C_P \simeq 20\%$, $C_M \simeq 80\%$), about four times more inhibitors than excitors. Given the finite time of the annihilation process, after the manifestation of the test response due to the excitors the greater number of inhibitors should not only neutralise the excitors produced by the test stimulus but also the smaller number of the PDA-inducing excitors, and thus either completely or partially depressing the PDA. But as can be seen in these figures, after each test response the rate of the decline of the PDA appears uninfluenced.

From *these* considerations we must then conclude that either there is no such thing as an inhibitor in this system, or that the inhibitor exists, but the premises of the two equations for the excitor-channel interactions are false. Therefore, to maintain the E–I model it shold be supplemented further by the assumptions that, either a) the inhibitor has a smaller affinity for the PDA-inducing excitor than for the test stimulus-produced excitor, or b) the affinity of the PDA-inducing excitors for membrane channels is much less than the affinity of the stimulus-produced excitors, which would then mean that the number of PDA-inducing excitors which produce the amplitude of the PDA are much greater than the number of the test stimulus-produced excitors needed to produce the same amplitude. Only then could the inhibitors produced by the test stimulus neutralise some of the PDA-inducing excitors without affecting the rate of decline of the PDA. And thus to keep the concept of the inhibitor it becomes necessary to further postulate the existence of two excitors.

In avoiding such a complication, we find it necessary only to postulate that the excitor (or the "energy" for membrane depolarisation) is released at different rates from two sites.

In this alternative model, schematised in Figure 1 (from Hamdorf and Razmjoo, 1977), the main assumption is that each P molecule excites the membrane only once, after having been converted by absorption of a single quantum to one of the membrane-excitatory states of metarhodopsin, namely [M] or [M]. The absorption of two or any even number of quanta which could reconvert metarhodopsin to rhodopsin *before* it has lost its potential for membrane excitation does not contribute to excitation. The PDA and the LRP, therefore, last as long as there are active ("energy-rich") [M] and [M] molecules, i.e., they are proportional to the *net* production of *active* metarhodopsin molecules. Membrane excitation, therefore, is due to the "release of energy" by active metarhodopsin, which concurrently decays to its inactive and *thermostable* form (Ⓜ), contrary to the suggestion that the life-time of the PDA corresponds to the duration of the dark conversion of metarhodopsin to rhodopsin (Wright and Cosens, 1977). Were another short stimulus to cause a further [P] → [M] shift by single quantum absorptions, simultaneously converting some inactive Ⓜ to Ⓟ, this loss of Ⓜ would not subtract from the effect of the newly produced [M], i.e., the degree of membrane excitation would no longer be due to the *net* production of metarhodopsin[2]. But in the excitor-inhibitor model, where *every* M → P transition creates an inhibitor that neutralises an excitor, the extent of the PDA is assumed *always* to be due to a *net* production of excitors, i.e., *net* P → M transitions.

In order to allow the membrane, during the PDA, to still respond to the excitors produced by the test stimuli, this 'photopigment model' envisages the following. P → M transitions produce "active" metarhodopsins which release their energy ('excitors') for excitation at rates determined either by the membrane (t_1), or slower (t_3). This slower rate of energy release could be due to the sharing of energy with other structures. This sharing may be brought about when active metarhodopsins ([M])

[2] An exception to this is the already mentioned extra loss of sensitivity in vitamin A-rich flies after adapting stimuli that leave the pigment maximally in the Ⓜ state. In this case the proximity of the adjacent photopigment molecules may facilitate the uptake, by inactive Ⓟ molecules, of the energy released when test stimuli produce active [M] molecules. However, we have already stated that "the probability of neighbouring [P] and [M] molecules capturing quanta almost simultaneously must be *extremely low*" (Hamdorf and Razmjoo, 1977)

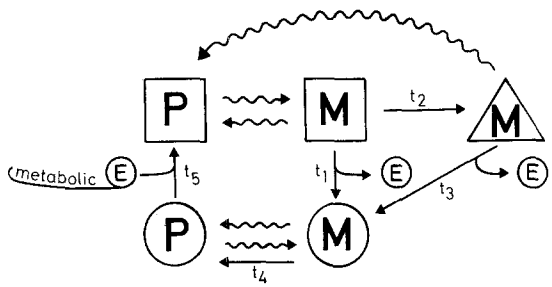

Fig. 1. A schematic model of the coupling of photopigment states to excitation in invertebrate photoreceptors (from Hamdorf and Razmjoo, 1977). [P], [M], and [M*] are symbols for the "energised", active states of rhodopsin and metarhodopsin, whereas (P) and (M) are the corresponding "de-energised" or inactive forms. These are not necessarily new states of the pigment, but represent different functions, possibly due to association with other neighbouring structures. Thus the release and uptake of energy (E) is only schematic; a direct transfer of energy from the active pigment states to the membrane is not implied, and therefore does not preclude the possible existence of intermediate transmitters as in the case of rod outer segments (Farber et al., 1978). After red and subsequent dark adaptation ($\simeq$ 3 min) all the pigment molecules are in the [P] state. Quantum absorption (i.e., blue light) will convert [P] to [M], and the latter by the release of energy for membrane excitation will be converted to an inactive form (M). The absorption of quanta by this (M) will not excite the membrane, but will yield (P) which will need metabolic energy to assume the active form [P]. With intense illumination (e.g., the PDA-inducing stimulus), the number of [P]→[M] transitions exceed a certain value and the remaining [M] will enter a stored form [M*] in greater amounts than may normally occur. This [M*] releases its membrane-excitatory energy much more slowly ($t_3 < t_1$), thus producing the PDA. Red adaptation when quickly following blue (e.g., the absorption of even number of quanta) will photoreconvert both active forms [M] and [M*] back to [P], where upon further blue illumination (i.e., the absorption of a single or odd number of quanta) can cause a PDA. However, *after* the exhaustion of the PDA, when (M) is the main existing form, further illumination will elicit a much smaller PDA. Under such conditions, and only in densely packed membranes, the release of energy by [M] *may* occur simultaneously with the uptake of energy by (P), and thus the transfer of some (E) between neighbouring M and P could explain reduction in the degree of excitation. Wavy arrows denote photic, and straight arrows denote thermal reactions. The thermal reaction t_4 is based on the data from Stavenga et al. (1973). However, it has been found that for up to an hour after the production of maximum metarhodopsin, there is no measurable dark regeneration of rhodopsin (Schwemer, personal communication). Thus, in comparison with the other time constants in the model, t_4 can be considered to be either extremely long or actually non-existent, as has been found in some other invertebrates, e.g., in the larval mosquito (Brown and White, 1972) and in the barnacle (Minke et al., 1973b)

are not able to deactivate through the t_1 path within a certain time. The ensuing rate of deactivation from this shared state ([M*]) is slower than the capacity of the membrane for excitation, and hence the reason for the long duration of the PDA in comparison with that of the LRP. Thus during the PDA, a regenerating stimulus which returns metarhodopsins back to rhodopsin removes the *source* of excitation, without the need for inhibitors.

In the experiment mentioned on page 2, the regenerating stimulus presented after the decline of the PDA was an attempt to *isolate* the 'inhibitory' period (or the inhibitor), i.e., to observe its life-time unchallenged by the neutralisation effect of the excitors. It is this same observation that leads to the hypothesis of the two active and inactive subsystems in our photopigment model. For, after the complete decline of the PDA, i.e., the depletion of the active metarhodopsin (which in *Calliphora* is

maximally 5—10 min), photic regeneration causes only the formation of an 'inactive' rhodopsin, and it is the metabolically determined rate of its activation, we hypothesise, that causes the delay in the possibility to induce as long a PDA as before (in *Calliphora* no longer than $\simeq$ 10 s, unpublished results, and also Hamdorf and Razmjoo in postscript to Hillman et al., 1977).

The evidence that follows in this presentation seeks to demonstrate that the PDA is proportional to the number of single (or odd number of) quanta absorptions by rhodopsin molecules. We shall also demonstrate that the membrane is not "overburdened" during the PDA, i.e., that the excess metarhodopsin produced continues to excite the membrane at its own limitted rate, possibly through a different path, leaving a margin for the membrane to respond to further instantaneous productions of metarhodopsin. We also show that the PDA, manifesting itself in the dark, is no different from the normal plateau of the receptor response to continuous light, and therefore could also be due to the summation of quantum bumps.

Results and Discussion

As stated in the Introduction, the extent of the PDA is dependent not only on the number of photo-induced transitions P $\rightarrow$ M (for a study of the spectral efficiency of PDA induction and depression see Tsukahara et al., 1977), but also on the total number of photopigment molecules in the photoreceptor membrane. To quantify this relationship further it would help to know the total number of photopigment molecules in the photoreceptor, as well as the number of absorbed quanta of light which lead to the production of the PDA, and we shall show how both these values can be obtained from electrophysiological observations alone.

The Number of Photopigment Molecules in a Rhabdom

The relative photopigment content of the membrane can be calculated on the basis of its direct proportionality to sensitivity (Hamdorf and Schwemer, 1975), a relationship further corroborated in this presentation by simultaneous electrophysiology and spectrophotometry (Figs. 5 and 6). Figure 2 shows the photoreceptor response to

Fig. 2. Intracellular recordings of receptor responses during PDA and during stepwise-increasing stimuli. **a:** The cumulative effect of PDA-inducing stimuli on the receptor response to constant test pulses. After the 4 s stimulus (a total of 8 s), it can be seen that responses to test stimuli are reduced no further with additional PDA-inducing stimuli, i.e., the equilibrium is almost reached. **b** and **c:** Steady-state levels (on the right hand side of each trace) induced by light adaptation to increasing intensities (log I_A = −6, −5, −4, −3, −2, −1, and 0) in comparison with the levels of depolarisation during the PDA. After photoregeneration (30 s red), a stimulus of $I_A \cdot 8$ s converts P almost maximally to M, eliciting the PDA. Note that the amplitude of the noise after 30 s of red adaptation is markedly smaller than during the PDA. At the beginning of trace b, after blue adaptation, the responses to test stimuli of reduced intensity are shown (I_T = 0.5, 0.25, 0.13, 0.06, and 0.03). Responses of maximally red adapted states are shown in traces a and c, prior to the first PDA in each trace. Traces a and b are from the same cell. The transient response to I_{A_0} (maximum intensity blue light causing the PDA) is 56 mV. *Red stimulus:* edge filter transmitting up to 645 nm. *Blue stimulus:* band-pass filter having ½ transmission points at 430 and 470 nm. *Test stimulus:* 20 ms pulses of isosbestic wavelength (506 nm) given every 5 s

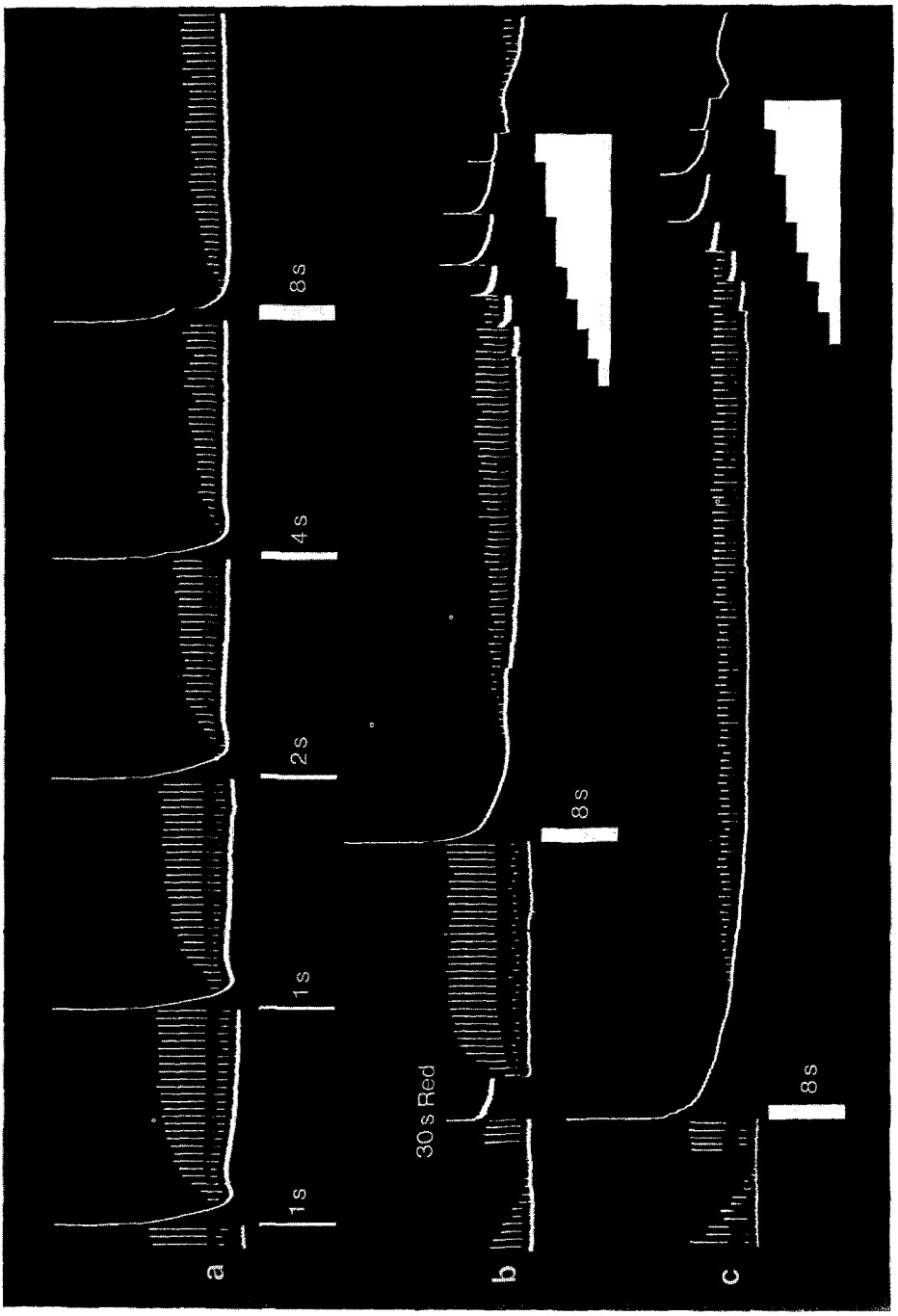

Fig. 2. (Legend see page 144)

PDA-inducing stimuli. Each successive stimulus of the same intensity is seen to further decrease the sensitivity (measured after the decay of the PDAs as the amplitudes of the LRP to constant test stimuli, when the responses increase no further). It is seen that after the 4 s exposure (a total cumulative exposure of 8 s), further exposures no longer reduce the sensitivity. Thus the first 8 s of cumulative exposures have reduced the sensitivity to the minimum. This is again shown in rows b and c, where after the complete regeneration of rhodopsin by red light, a single 8 s exposure leads to the same decrease in sensitivity. Figure 3 (curve b) shows amplitudes of these successively smaller responses after each exposure (measured when responses increase no further), versus the time of blue adaptation. Also presented in this figure (curve a), is the lower portion of the intensity-amplitude function for this photoreceptor. When each point of the post-PDA responses is moved horizontally to meet the intensity-amplitude function, it relates the response to an intensity. This intensity, when compared to the intensity of the maximum response, gives a measure of sensitivity, and in this way we obtain a sensitivity scale (for this relationship see Hamdorf and Schwemer, 1975). This allows the description of the decrease of the responses in terms of sensitivity and time. In view of the demonstrated relationship between sensitivity and the concentration of rhodopsin, these post-PDA responses can finally

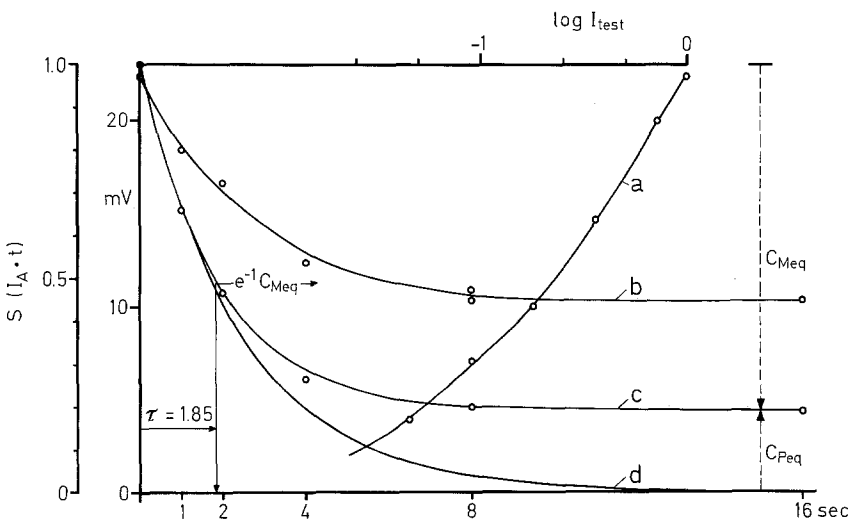

Fig. 3. Response amplitudes to test stimuli measured about 2 min after the decay of cumulative PDAs, and their interpretation in terms of photopigment interconversions. Curve b shows the responses to successive post-PDA test stimuli (from Fig. 2), plotted as function of time of PDA induction. This, when correlated with the intensity-amplitude function (curve a) after red adaptation (when C_P = max), translates into relative sensitivity as a function of time and the intensity of adaptation ($I_A \cdot t$). Because of direct proportionality between sensitivity and C_P (see Fig. 6), the responses are finally interpreted in terms of changes in C_M (or C_P) as a function of blue adaptation. When plotted in this form they are seen to coincide with the theoretical equilibration function for forward and backward reactions (curve c) described by Eq. (1). This function has a τ of 1.85 s, during which time it overlaps the plot (curve d) of C_P decay due only to single quantum absorptions, i.e., only the forward transitions, described by Eq. (3). This indicates that equilibration reactions that take place during this period of overlap are due only to forward transitions as the result of the capture of single or odd numbers of photons

be described as decreases in the concentration of rhodopsin with increasing times of blue adaptation (points along curve c). These points are seen to coincide well with the continuous curve which is plotted from the following theoretical equation:

$$C_P(I_A, \lambda, t) = C_{Po} \frac{\alpha_M + \alpha_P \cdot e^{-(\alpha_M + \alpha_P)I_A \cdot t}}{\alpha_M + \alpha_P} \tag{1}$$

or, when re-arranged to give the concentration of metarhodopsin,

$$C_M(I_A, \lambda, t) = C_{Po} \frac{\alpha_P(1 - e^{-(\alpha_M + \alpha_P)I_A \cdot t})}{\alpha_M + \alpha_P}, \tag{2}$$

where the equilibrium between C_P and C_M is seen to be a function of the absorption probabilities (α) of the two pigment states (Hamdorf and Schwemer, 1975). The time constant, τ, of this equation is defined by the value $(\alpha_M + \alpha_P)\, I_A \cdot t$ where the concentration of metarhodopsin becomes $(1 - e^{-1})\, C_{Meq}$. However, Eq. (2) yields only relative concentrations of P and M and not the absolute number of photopigment molecules in the photoreceptor, nor does it reveal anything about the manner of arriving at any instantaneous value of C_M, whether by single (designated by the subscript 1, e.g., C_{M1}) or by multiple absorptions. However, consider the following. The theoretical function

$$C_{M1}(I_A, \lambda, t) = C_{Po}(1 - e^{-\alpha_P \cdot I_A \cdot t}) \tag{3}$$

yields instantaneous values of metarhodopsin build-up (C_{M1}) produced *only* from the absorption by P molecules of a *single* quantum and involves only forward transitions. A plot of this function (curve d in Fig. 3) shows that the upper part of this curve agrees closely with the equilibration function (curve c), diverging from it only for values greater than τ. Therefore, in this region of overlap (low $I_A \cdot t$ values), the equilibration equation, once the terms for the backward transitions are deleted from it, becomes equal to the equation for forward transitions only.

Since spectrophotometry provides only the ratio of the absorption probabilities of P and M (α_M/α_P) and not α_P alone (Hamdorf and Schwemer, 1975; for a comprehensive treatment see Hamdorf, 1979), we can substitute for α_P, starting with the original form of the exponent in Eq. (2). When we use our adapting light of maximum intensity ($I_A = I_{Ao}$) and define every equilibration reaction by its time constant τ, where $e^{-t/\tau}$ defines τ as:

$$\tau = \frac{1}{(\alpha_M + \alpha_P)I_{Ao}}$$

then, at $t = \tau$, the exponent becomes

$$-(\alpha_M + \alpha_P)I_{Ao} \cdot \tau = -\tau/\tau = -1\,.$$

Substitution of $\alpha_M/\alpha_P = f$, now yields

$$\alpha_P = \frac{1}{I_{Ao} \cdot \tau(1 + f)}$$

and the replacement of α_P in Eq. (3) [or the reduced form of Eq. (2)] gives

$$C_{M1}(I_A, \lambda, t) = C_{Po}\left(1 - \exp\left[-\frac{I_A \cdot t}{I_{Ao} \cdot \tau(1+f)}\right]\right). \quad (4)$$

This equation is applicable for any value of I_A and t, and the determined value of 'f' at the wavelength of adaptation. Values for 'f' have been determined at several wavelengths by spectrophotometry of extracts as well as of intact eyes (Hamdorf et al., 1973).

We further wish to find a value of $I_A \cdot t$ which produces a single metarhodopsin molecule. If at very low $I_A \cdot t$ values we are able to detect distinct and countable fluctuations in the membrane voltage, the so-called quantum bumps, and if we assume that each quantum bump is due to the absorption of a single photon (Fuortes and Yeandle, 1964; Scholes, 1965; Borsellino and Fuortes, 1968), leading to a single P → M transition (the latter assumption is also made by Lisman and Bering, 1977), we then have the value of $I_A \cdot t$ for the production of a single molecule of metarhodopsin as a result of the absorption of a single quantum [designated as $(I_A \cdot t)_1$]. Eq. (4) will then relate the production of one molecule of metarhodopsin to the maximum number of photopigment molecules that can, limited by the system's α_M/α_P, exist in the rhodopsin state:

$$\frac{1 \text{ molecule of M}}{\text{max. number of P molecules}} = \frac{C_{M1}(I_A \cdot t)_1}{C_{Po}} = 1 - \exp\left[-\frac{(I_A \cdot t)_1}{I_{Ao} \cdot \tau(1+f)}\right]. \quad (5)$$

Figure 4 shows quantum bumps at three different *relative* intensities (at the same wavelength as the PDA-inducing stimulus, for later we wish to show that the PDA may be the sum of quantum bumps), where direct and linear proportionality was found to exist between bump frequency and the intensity, as has also been found in *Limulus* (Adolph, 1964) and in *Drosophila* (Wu and Pak, 1978). A count of these bumps over a long period at a particular intensity affords a value of $(I_A \cdot t)_1$ for a quantum bump, that is, for the production of one molecule of metarhodopsin. Over a period of 30 s, the number of bumps were as follows:

rel. $I = I_A/I_{Ao}$	Bumps/30 s	Duration of illumination for production of 1 bump	Number of photopigment molecules
1×10^{-8}	15	2 s	1.14×10^8
2.5×10^{-8}	44	0.682 s	1.33×10^8
5×10^{-8}	87	0.345 s	1.32×10^8
			Mean $\simeq 1.3 \times 10^8$

The insertion of these values, and the value of 0.3 for 'f' at the PDA-inducing wavelength used, gives the mean value of 1.3×10^8 photopigment molecules in this particular rhabdom. As was mentioned earlier, this is the maximum number of photopigment molecules that can exist in the rhodopsin state. The regenerating light is chosen at a wavelength at which α_P is *almost* zero. Since $C_{Peq(\lambda)} = \alpha_M/(\alpha_M + \alpha_P)$ determines the total number of P molecules, as $\alpha_P \to 0$, P approaches the total number of photopigment molecules in the rhabdom. It is not possible to accurately

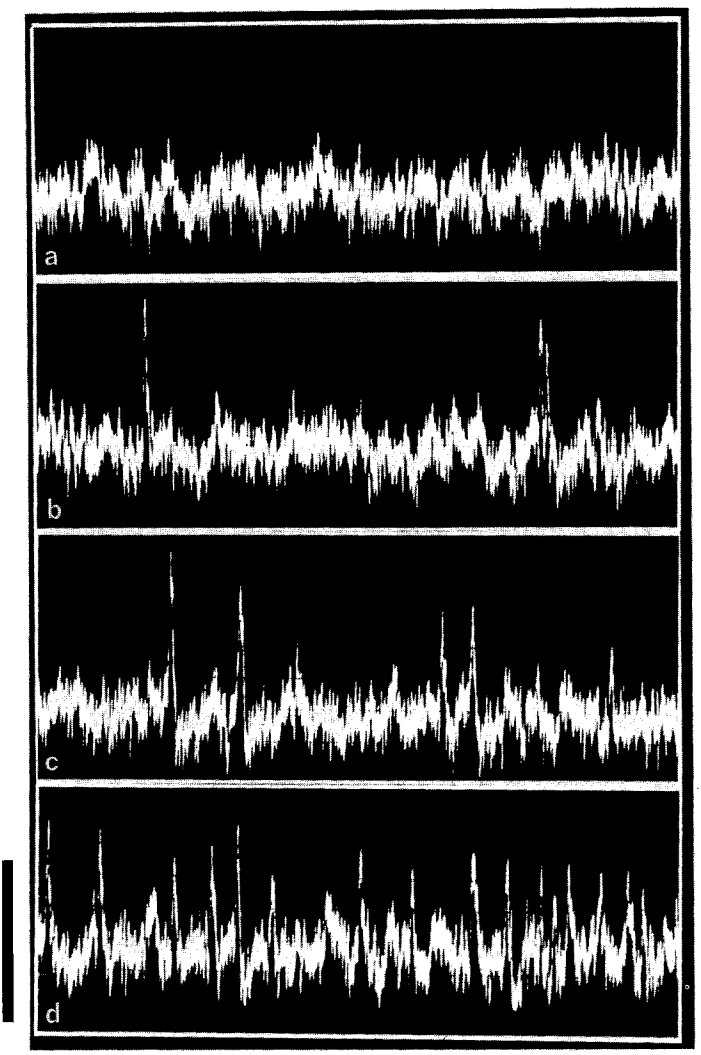

Fig. 4. Quantum bumps in a red and dark adapted photoreceptor, evoked by exposure to continuous low intensity light of the same wavelength as that used for the induction of PDAs (see Fig. 2). Trace a: photoreceptor noise in the dark; traces b, c, and d: exposures to the relative intensities (I_A/I_{Ao}, where I_{Ao} is the maximum intensity of the adapting light) of 1×10^{-8}, 2.5×10^{-8}, and 5×10^{-8}, respectively. Vertical bar represents 1 mV

determine α_p at such low values, but it can be said that the value of 1.3×10^8 is 99% of the total photopigment molecules. We can estimate the total surface area of the rhabdom from micrographs, which, with the approximate length of the rhabdom and the number of photopigment molecules we have just calculated, gives the concentration of photopigment in the rhabdom. Using Lambert-Beer's law and the approximate extinction coefficient of 4×10^4 (Hamdorf and Schwemer, 1975) we arrive at an extinction value of 0.87, which is in rather good agreement with the directly measured value of 0.7 (Schwemer, unpublished results).

It is important to point out here that this method of relating the intensity of light to the excitation of the membrane is a direct one, using the photoreceptor itself as the counter in calibrating the light intensity. This overcomes not only errors incurred when, because of dispersions in all parts of the optical system, estimations must be made for the flux, but also errors due to further deviations from direct proportionality between the light incident on the photoreceptor and the excitation, due to the quantum yield of isomerisation. For example Lisman and Bering recently (1977) estimated, in two different ways, the total number of photopigment molecules in a ventral photoreceptor of *Limulus* by the purely electrophysiological means of measuring quantum bumps and the early receptor potential (ERP). One method suffers from the *assumption* that the white flash they employed induced only about 50% isomerisations. This assumption is questionable since it has been shown (Hamdorf et al., 1968) that the photoequilibrium due to a bright white light depends on the integral of the products of a) the absorption probabilities of all the photopigment intermediates at all wavelengths of the light source, b) the mean life-time of each intermediate, and c) the intensities of the wavelengths, i.e., the spectral distribution of the light source.

In this same approach, the saturating amplitude of the negative component of the ERP has been *assumed* (not demonstrated by, for example, parallel or concurrent spectrophotometry) to correspond to this 50% isomerisation. Although in this approach they avoid errors due to measurements of the absolute value of the incident light, the relative intensities used are derived from the ERP amplitudes, and therefore depend on the validity of the assumption that the ERP *is* an accurate measure of the rhodopsin concentration.

In their second method they require absolute values of light intensities. Here they assume that of the measured incident light about 50% reaches the photoreceptor after being attenuated and scattered by the intervening olfactory nerve. They further have to assume values for the molar extinction and quantum efficiency of isomerisation. However, if their estimate is verified in future by other means this will attest to the correctness of their assumptions.

Fig. 5. Simultaneous transmission and ERG recordings after blue and red adaptation. **a:** Original red adapted ERG responses to the test stimuli. **b:** The elevation of the ERG baseline and the reduced amplitude of the responses to test stimuli is due to the incidence of the measuring beam of the spectrophotometer. The upper trace is the transmission level at minimum C_M. **c:** With the onset of a strong flash the transmission level falls very rapidly, indicating rapid conversion to metarhodopsin in less than 0.5 ms, after which the measuring beam is discontinued and the ensuing PDA allowed to decay to the baseline. Superimposed responses to the test stimuli show reduction in C_P (compared to **a** and **e**). **d:** With the resumption of spectrophotometric measurement (measuring beam 'on') there is a slow exponential return of transmission to the original level of minimum C_M. The elevated ERG baseline and the reduced size of the responses to test stimuli are simply the steady-state response to the measuring beam. **e:** Transmission measurement is discontinued, showing gradual return of the ERG to the baseline, together with the return of sensitivity. **f:** Response amplitudes to reduced test stimuli for the determination of the intensity-amplitude function. Each reduction in amplitude corresponds to an intensity reduction of 0.25 log units. The ERG recordings were made after the elimination of ganglionic activity by procain. *Test stimuli:* isosbestic wavelength (506 nm) given every 5 s. *Measuring beam:* band-pass filter at 605 nm. *Flash:* 1.5 ms duration, for wavelength see Figure 2. Horizontal black bars represent the 'off' periods of the measuring beam

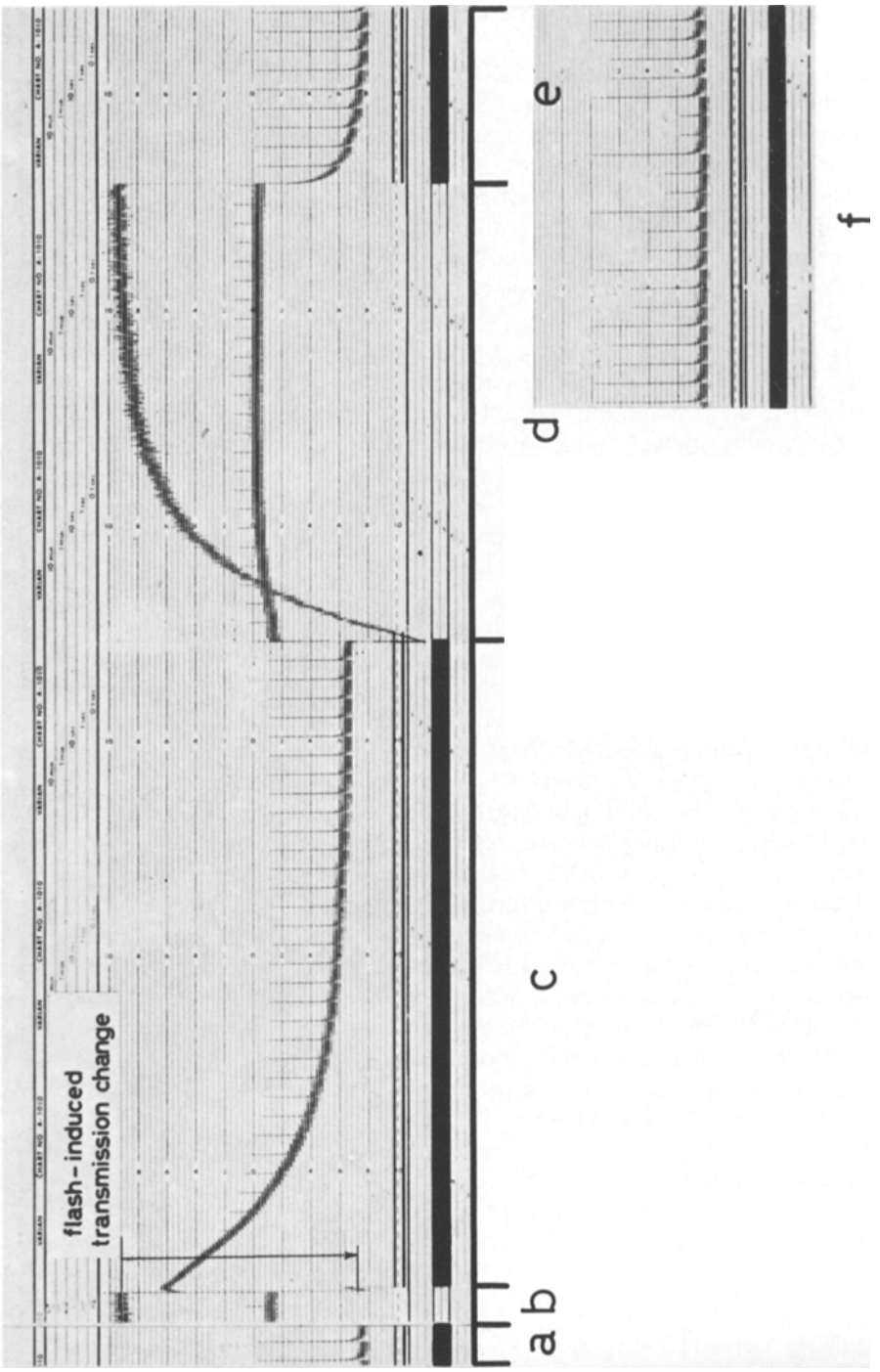

Fig. 5. (Legend see page 150)

Photoreceptor Sensitivity and the LRP

Earlier attempts to characterise the PDA, employing long lasting illumination could not relate its extent to the number of M molecules produced, as the recycling of the pigment during illumination complicated the relationship. The use of blue flashes lasting about 1.5 ms, which ensures that the final equilibrium is reached before the manifestation of the LRP, prevents recycling back to active rhodopsin and thus recontribution to the PDA. This, with reference to our model, would mean the uncoupling of $\boxed{P} \rightleftarrows \boxed{M}$ from the rest of the system. Figure 5 shows the effect of such a flash on simultaneous transmission change and the extracellular receptor response (after the elimination of ganglionic responses by the application of procain).

Given the limited bandwidth of the recording equipment (3 kHz), the change in transmission recorded at the fastest speed possible (not shown here) was seen to occur in less than 0.5 ms, followed about 4 ms later by the LRP and the PDA[3]. The effect of the measuring beam (of low intensity) on the regeneration of rhodopsin is seen by the subsequent return of transmission to the original level, with a time constant of about 23 s. Also to be seen in Figure 5 is the usual decrease in the membrane response after the PDA as compared to the red-adapted state. The relationship between the intensity of the adapting flash and the change in extinction is plotted as points along the theoretical curve shown in Figure 6a. The characterisation of the PDA by its time constant is sketched in Figure 6b, and using this criterion the relationship of the PDA to the Δ-extiction and hence the concentration change can be seen in Figure 6c. From Figure 6a it is clear that the values found empirically agree quite well with the theoretical curve of Eq. (2), permitting one to equate the extinction scale with a scale for the concentration of metarhodopsin produced, and hence the relative concentration of M at the equilibrium value.

In Figure 6c, the losses in sensitivity observed after the production of PDAs are plotted against a Δ-extinction scale, revealing a very close agreement between sensitivity and the production of metarhodopsin. The lowest point shown ($6 \times I_{max}$) represents the maximum concentration of metarhodopsin that the particular equilibrium of the system permits. By extrapolating to zero sensitivity, corresponding to a theoretical zero rhodopsin concentration ($C_P = 0$) and maximum metarhodopsin concentration (a situation never permitted by the absorption characteristics of the system), we thus define the limits for a metarhodopsin concentration axis. It can be seen that the 0.5 concentration point corresponds exactly with the sensitivity loss of 50%, which argues strongly for the correctness of the direct relationship between C_P and receptor sensitivity as measured by the transient phase of the LRP. Similarly, the axis for the C_M provides a value of 79% for C_{Meq}, which agrees well with the value independently arrived at in Figure 6a. The C_M axis in Figure 6c now provides an almost linear correlation between the concentration of metarhodopsin produced and the extent of the PDA, as characterised by its time constant τ, which is consistent with the hypothesis that it is the capture of single or odd numbers of quanta

[3] It can be seen that during the course of the PDA and thereafter, the new transmission level shows a small drift towards higher metarhodopsin levels. During this period there could have been no shift in the equilibrium through regeneration of rhodopsin by the red measuring beam as this was turned off.

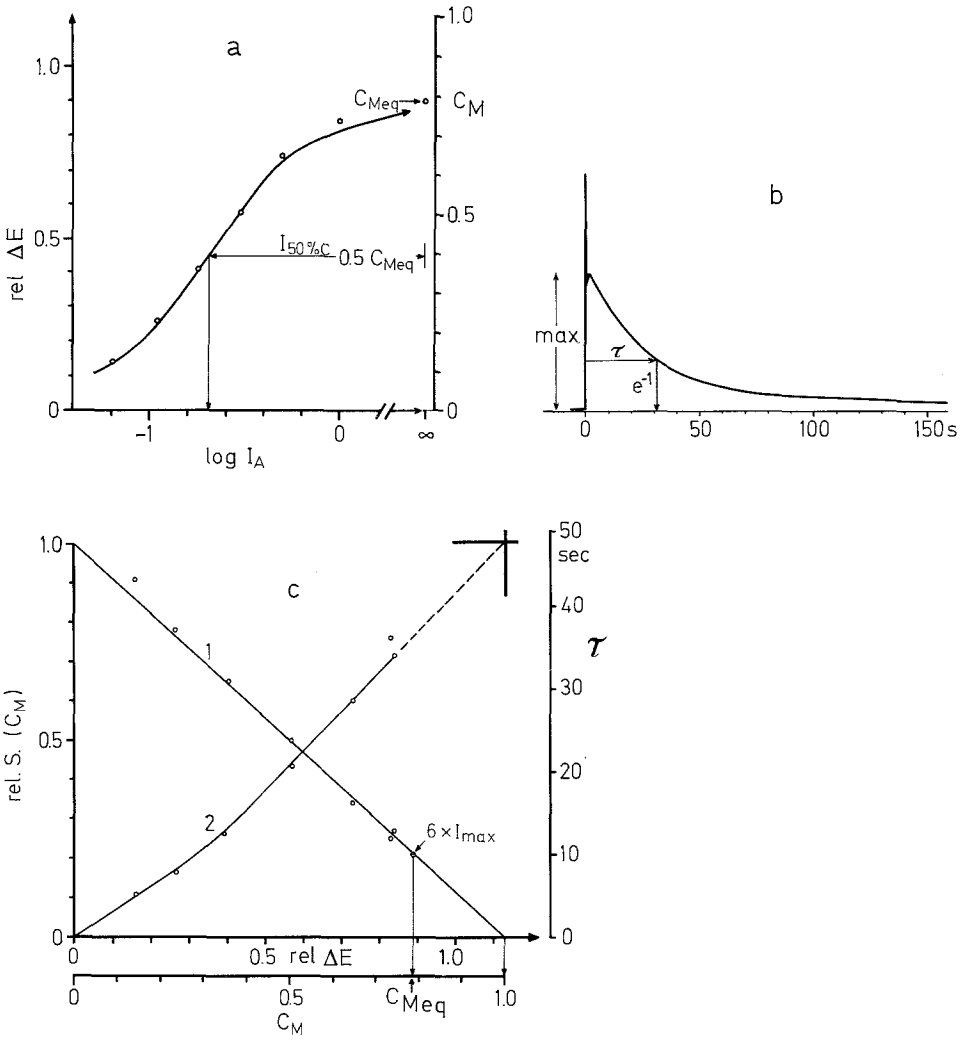

Fig. 6. Correlations between receptor sensitivity, time constants of PDAs (τ), and the production of metarhodopsin by PDA-inducing flashes of 1.5 ms duration. The data are from simultaneous spectrophotometric and electrophysiological measurements similar to those shown in Figure 5. **a:** The dependence of the relative change in extinction (ΔE) on the intensity (log I_A) of the flash. The experimental data (O) are seen to agree well with the curve plotted from the theoretical function of Eq. (2). **b:** PDA induced by an adapting stimulus ($I_A \cdot t$) of a flash of maximum intensity ($I_{max} \cdot t$). The maximum amplitude of the PDA (max) was used in the determination of τ for all PDAs plotted in **c** (curve 2). **c:** The relative sensitivity of the receptor as a function of C_M (curve 1, determined after the return of the PDA to the resting potential), and the PDA parameter τ (curve 2), both plotted as functions of the relative change in extinction and the concentration of metarhodopsin. C_{Meq} was determined after adapting with six flashes of I_{max}. The *rel.* $\Delta E = 1$ corresponds to ΔE of 0.05

which lead to the transformation of active rhodopsin molecules that contribute to the PDA.

Quantification of the PDA

We have proposed (Hamdorf and Razmjoo, 1977) that the PDA is similar to a continuous stimulus of declining intensity. To test this hypothesis, after the production of C_{Meq} and the subsequent completion of the PDA, the photoreceptor was subjected to stepwise increasing blue stimuli, as well as to superimposed test stimuli (Fig. 2, rows b and c). This figure shows stepwise simulation of depolarisation levels that can occur during the course of the PDA. In the mid-intensity ranges it can be seen that the transient response to these stepwise stimuli settles to a steady-state phase, with time courses and steady-state levels similar to those of the PDAs. These responses illustrate the effect of increasing intensity by: 1) the size of the transient phase, 2) the steady-state level, 3) the reduction in response amplitudes to superimposed test stimuli with increasing intensities of the sustained light, and 4) the increase and decrease of the membrane noise up to the 5th step. (Further increases in intensity beyond the saturation level at the 5th step produce a decrease in the size of the transients and of the steady-state levels.)

Such comparisons between the PDA and the effect of a sustained light suggest that the PDA may be quantified in terms of the number of absorbed quanta. Figure 7 shows results of an experiment using long lasting stimuli. This particular experiment is presented because it shows one of the longest PDAs ever observed by us in *Calliphora*. The test stimuli are about ten times more intense than those used in the experiment just described, so as to produce a) responses which show earlier during the course of the PDA, and b) so that the membrane still responds to the test stimulus when more intense sustained stimuli are used. It can be seen that with increasingly longer times of stimulation the PDAs (after cessation of the inducing stimulus), have: increasingly higher initial amplitudes; are longer lasting; and exhibit the expected loss in sensitivity due to the successive decrease in the concentration of rhodopsin (measured from the responses to test stimuli).

The course of the PDAs after the cessation of the inducing stimuli are shown, superimposed, in Figure 8a. Here it can be seen how they increase to a maximum at about 5–10 s, and decline at longer times of stimulation. The decay in these latter cases is because most of the course of the PDA coincides with, and becomes masked by, the steady-state response. Information from the sustained stimuli is presented in Figure 8c. When the longest PDA (10 s) of Figure 8a is matched at several points along its course with the stepwise responses (in terms of both the steady-state level and the amplitude of the superimposed test responses; b and c of Fig. 8c), it can then be recalibrated in terms of the intensities of the stepwise stimuli. The result, shown in Figure 8a, is a straight line for times between 1.5 and 3 min. We may have suspected by inspection of the shape of a PDA that its decay obeys an exponential function. The straightness of this plot on a linear-logarithmic scale verifies this. The integration of this function in terms of intensity and time can then relate this particular PDA to the number of absorbed quanta which may be responsible for its production.

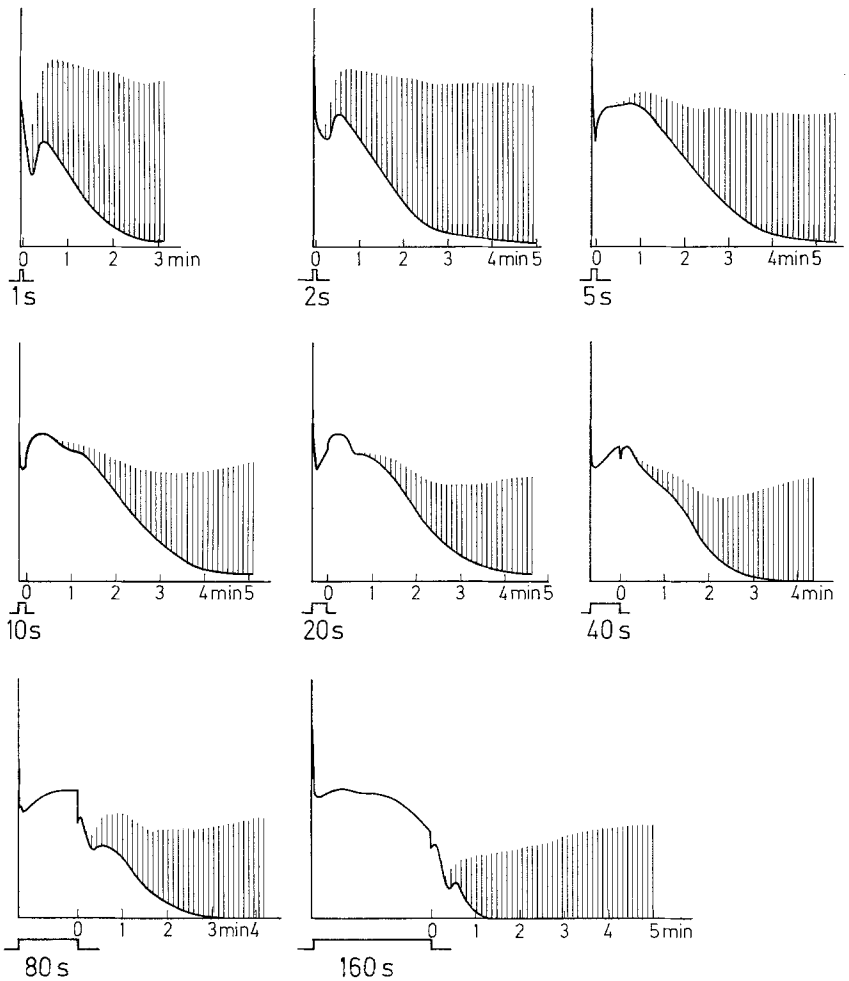

Fig. 7. The induction of PDA depending on the duration (t) of maximum intensity blue light (I_{Ao}). Between each of the eight traces shown the rhodopsin was fully regenerated by exposure to 30 s of red light, restoring sensitivity to the original maximum level. Whereas maximal PDAs seem to be induced after exposure to 5–10 s, their responses to test stimuli measured 4–5 min after the PDA decline are markedly larger than after 80 s or 160 s of stimuli, i.e., when photoequilibrium is reached. For details see Figure 8. Experimental conditions as in Figure 2

Whether or not the decay of a PDA is also exponential during the initial phase is not clear, as it appears to be masked by other phenomena such as: changes in ionic distribution as a result of light adaptation; restitution of the potential due to ionic pump activity; and the rate of reactivation of membrane channels (Hamdorf et al., 1978). However, if we assume that this phase also decays exponentially, we can extrapolate the straight line to zero time. Within the limits of zero and infinity (the latter not shown on the scale of this figure), the integration of this function yields the $I_{Ao} \cdot t$ value of 2.2. The upper curve in Figure 8b shows a plot of responses to test

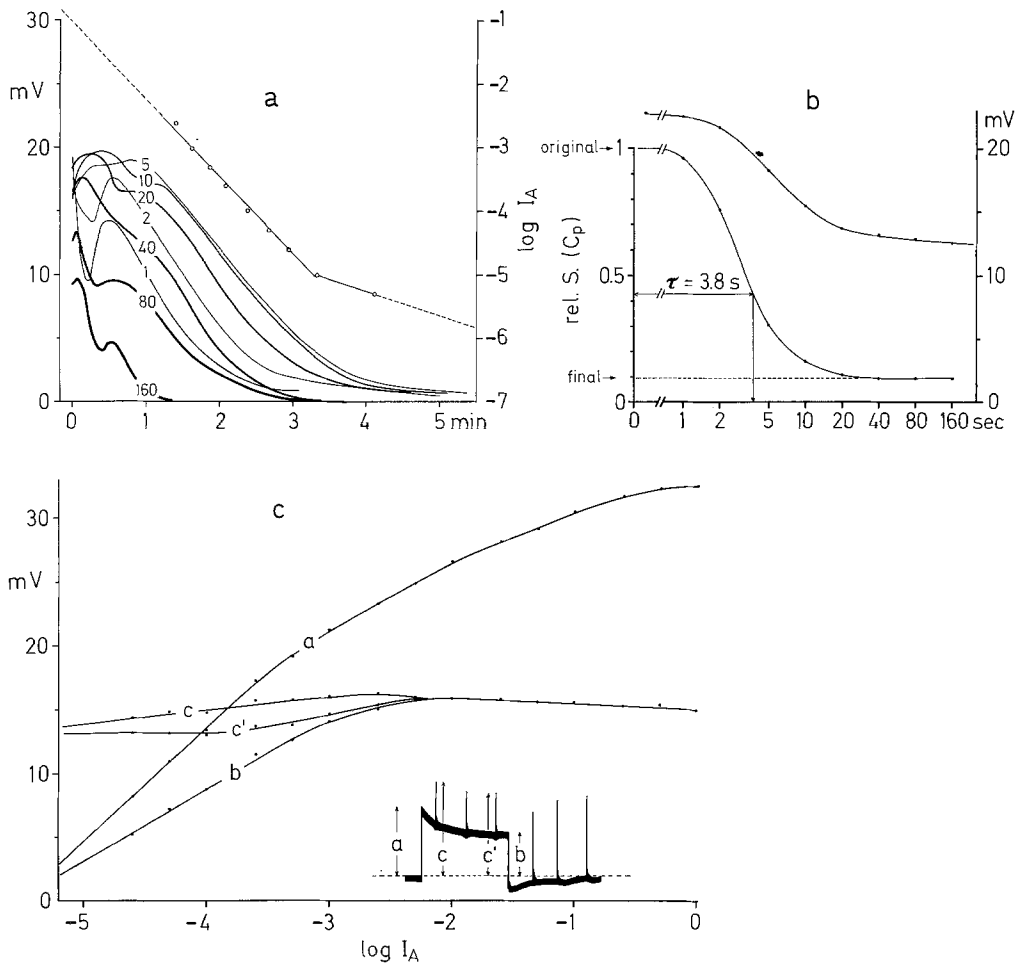

Fig. 8. The analysis of PDA by comparison with the late receptor response in the blue adapted state. **a:** The course of the PDAs of Figure 7 are here shown superimposed (from the time of stimulus cessation). Numbers denote duration of exposure, in seconds, to blue light of maximum intensity (I_{Ao}). The nine points connected by a straight line are obtained when points along the course of the PDA caused by 10 s of exposure are compared in amplitude with the late receptor response to sustained and to superimposed test stimuli (in terms of both the steady-state phase and the amplitudes of responses to test stimuli: curves b and c of Figure 8c, respectively), thus providing calibration in terms of intensity (right hand ordinate). **b:** The upper curve relates the reduction of amplitude to test responses (right hand ordinate) following successive PDAs induced by maximum adapting intensity (I_{Ao}) but increasing induction times (the $I_{Ao} \cdot t$ values along a logarithmic abscissa). This plot, when matched with the intensity-amplitude function (not shown) gives the lower curve, expressing the relative loss in sensitivity (left hand ordinate) as a function of $I_{Ao} \cdot t$ of PDA induction. **c:** A plot of steady-state potentials (after maximum blue adaptation) and those of responses to superimposed test stimuli (constant intensity test stimuli; increasing intensity of sustained stimuli). Notations are, *a:* amplitude of transient response to sustained blue light; *b:* amplitude of its final steady-state; *c:* amplitude of response to superimposed test stimulus; *c':* as in *c* but measured when the steady-state due to the sustained background stimulus is reached. Experimental conditions as in Figure 2

stimuli following the completion of successively larger PDAs, which in terms of the intensity-amplitude function (not shown) and the proportionality with concentration, gives the lower curve. Now, when the calculated $I_{A_0} \cdot t$ of 2.2 for the 10 s PDA is read off on the time scale of Figure 8b (also an $I_{A_0} \cdot t$ scale), it is seen to correspond to a concentration change of about 25%, indicating that the PDA caused by exposure to 10 s of light at intensity I_{A_0} has been due to the conversion of about 25% of the original amount of rhodopsin to metarhodopsin.

In a previously reported experiment (Hamdorf and Razmjoo, 1977), the percentage conversion for the maximal PDA was about 20%. We wish to emphasize that according to our model a PDA is produced by the absorption of single quanta by active rhodopsin molecules and that the conversion necessary for all large PDAs, and thus the concentration of metarhodopsin in stored form (▲), must be smaller than the maximum number of rhodopsin molecules in the membrane.

PDA as the Summation of Quantum Bumps

Dodge et al. (1968) have presented evidence that in *Limulus* photoreceptors the generator potential is the sum of the quantum bumps. Further support for the suggestion that the PDA, like the LRP, is due to the summation of quantum bumps is provided by the comparison of the noise during a PDA and the noise of the steady-state response to sustained light stimuli. From Figure 2 (row b) it can just be seen that the membrane noise after red adaptation becomes larger during the decline of the PDA (as has also been observed in the dronefly, Tsukahara and Horridge, 1977), and is similar to the noise during sustained light stimuli. This is more clearly demonstrated in Figure 9, where the noise (sampled at different times during the course of the PDA and during exposure to sustained stimuli) is recorded at faster sweep and greater magnification. The first two vertical traces of row a were taken during the 'on' period of the PDA-inducing light. It can be seen that soon after the onset of the stimulus the noise increases to a maximum (comparable to the noise during the stimulus step to $I_A = 10^{-2}$, shown in row b), and decreases soon after. In the middle of the second trace the adapting light is turned off, without any noticeable change in the magnitude of the noise. Shortly before the PDA declines completely to the baseline (at about the 7th vertical trace), the noise again increases and corresponds to that produced by a sustained light of $I_A = 10^{-5}$ (about 100 quantum bumps/sec, when compared to the data of experiments shown in Figure 4). After return to the baseline the noise reduces to the dark level, but still exhibits a greater frequency of spontaneous bumps than that which can be seen from the trace taken during the dark (trace d). The greater noise of the 7th trace can be seen to be due to a higher frequency of bumps (similar to the fusion of bumps that occurs at high intensities, see Scholes, 1965), which gradually decreases with the decline of the PDA. This is an indication that the PDA, like the LRP, is due to the summation of quantum bumps, as has been proposed by Tsukahara and Horridge (1977). Minke et al. (1975) have also suggested that the PDA noise is due to quantum bumps, and that the PDA and the LRP "share some common pathway". This would provide further evidence that the PDA is "a retarded membrane excitation by a substance in stored form" (Hamdorf and Razmjoo, 1977).

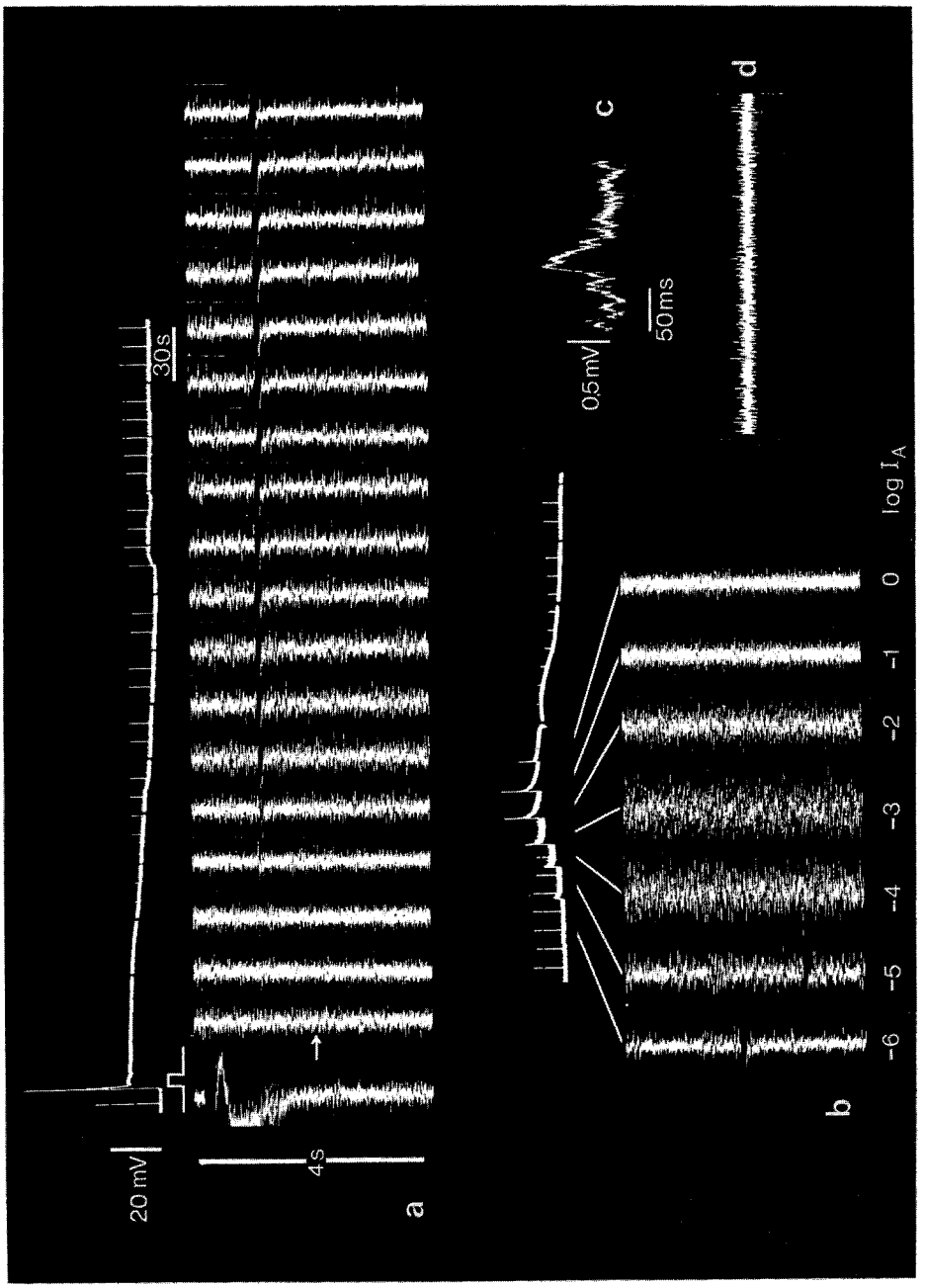

Fig. 9. (Legend see page 159)

The changes in noise during the course of a PDA from the steady-state to the baseline, resemble the changes in noise during the progression of the sustained stimuli from the highest to the lowest intensity (i.e., the vertical traces of row b, viewed from right to left). However, at no point during the decay of the PDA, is the noise as great as that during the sustained intensities of −4 and −3. The reason for this may be that, at these particular intensities, it is the fluctuation in the number of isomerisations (varying from relatively silent periods to periods when many isomerisations superimpose) that causes such large peak-to-peak potential changes, whereas during the PDA, when no isomerisations occur, the rates of other intermediate processes which lead finally to excitation of the membrane are subject to less fluctuation.

In conclusion, the foregoing demonstration of the induction and some properties of the PDA allow us to predict a further property of this phenomenon. When the counts of quantum bumps are related to the levels of membrane depolarisation, rough calculations show that a membrane depolarisation of 10 mV corresponds to the summation of about 1000 bumps/sec. If the possibility of the regeneration, and therefore the recontribution of rhodopsin molecules to the duration of a long depolarisation is excluded, the number of rhodopsin molecules in the membrane (1.3×10^8) allows a 10 mV depolarisation to last for about 10^5 s, i.e., about 30 h! Although due to technical difficulties there have been no direct demonstrations (i.e., intracellular recordings) of the entire duration of very long lasting PDAs believed to exist in some species, it is quite conceivable that the description of the PDA phenomenon by our "photopigment model", namely the variation in the rate of the process $\mathbb{A} \xrightarrow{k} \mathbb{M}$, could account for wide interspecies differences which may occur in the rate of the return of sensitivity to levels commensurate with the remaining rhodopsin concentration after exposures to such PDA-inducing stimuli.

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Fig. 9. Membrane voltage noise during PDA and during steady-state responses to sustained stimuli. **a:** The induction and the time course of a PDA by 8 s of blue light at maximum intensity (I_{A_0}). The vertical traces (each running from top to bottom) are high amplification and fast sweep recordings taken at times shown above each trace as a gap during the course of the PDA. The first vertical trace shows that during PDA induction the noise increases within the first second; decreasing quite soon after. The arrow marks the cessation of the inducing stimulus, but without any noticeable change in the noise. Shortly before the PDA reaches the baseline the noise becomes greater (vertical traces 7–9). About 7–10 min after the induction of the PDA (the last five vertical traces), the frequency of the observable single discrete excitations becomes less, approaching that of the dark-adapted membrane (trace d). Their shapes (not included here) are similar to the "stimulus-incident" bump shown in trace c. The hump as the PDA settles to baseline is a recording artefact. **b:** Transients and steady-states of the late receptor response caused by stepwise increasing blue stimuli after the completion of the PDA shown in trace a. Note that the amplitude of the noise is highest at $\log I_{A_0} = -3$ and −4, and that the noise due to $\log I_{A_0} = -5$ is comparable to the maximal noise during the PDA. The sequence of changes in the noise amplitude when viewed from right to left resembles the sequence of the changes in noise during the course of the PDA shown in row **a.** At no point during the course of the PDA is the noise as great as that during the sustained stimuli of $\log I_{A_0} = -4$ and −3, shown in trace b. For explanation see end of section on Results and Discussion. Blue stimulus as in Figure 2; test stimulus: 20 ms pulses (506 nm) at an intensity corresponding to $\log I_{A_0} = -3.5$, presented every 10 s

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