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THE DISTRIBUTION OF BUMPS IN THE TAIL OF THE LOCUST PHOTORECEPTOR AFTERPOTENTIAL

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SUMMARY

An extended tail or prolonged depolarizing afterpotential (PDA) follows the receptor potential of a locust retinula cell when the stimulating light is in the intensity range that saturates the receptor potential. The amplitude and duration of this afterpotential depend on the intensity and duration of the stimulus. As the afterpotential decays, apparently exponentially, it becomes resolved into bumps, which we call light-induced dark bumps (LID bumps).

The intervals between light-induced dark bumps are distributed in a way that is indistinguishable from a random (Poisson) distribution. As previously demonstrated, LID bumps are indistinguishable from bumps directly induced by low intensity light in light-adapted cells, which in turn grade into the slightly larger bumps produced, each by a single photon, in dark-adapted cells.

The light-induced dark bumps continue for up to an hour in darkness, slowly becoming like dark-adapted bumps in amplitude and shape. To account for the random occurrence and discrete features of bumps after so long a latency, we propose that intense light generates a significant amount of an intermediate molecule or packet which decays slowly to start the same process that normally generates bumps with a short delay.

INTRODUCTION

Dim light acting on dark-adapted visual cells of some arthropods causes a stream of discrete depolarizing potentials which are here called bumps. These were first found in *Limulus* by Yeandle (1958) and in the locust by Scholes (1964). Recently Lillywhite (1977) has shown that in the locust about 60% of the incident peak photons from a point source on the axis of the ommatidium are effectively caught by the rhabdom as a whole, and each photon gives rise to one bump and no more. Peak photons are at the peak wavelength of the spectral sensitivity curve. In these dark-adapted cells the statistical properties of the bumps are consistent with the theory that one photon excites one photopigment molecule which by a process of amplifi-

cation indirectly causes one bump. Lillywhite also found that fully dark-adapted locust retinula cells have no spontaneous bumps, and that after a brief weak flash all bumps have a latency of less than 0·5 s. These new findings simplify the consideration of the results presented below.

When the stimulus intensity is increased, bumps of dark-adapted cells add together non-linearly to form an average depolarization called the receptor potential (RP). The present theory is that each bump is the electrical sign of the simultaneous opening of a variable number of sodium channels, so that each photon adds an increment of conductance increase (which is not necessarily constant) at the retinula cell membrane. This theory explains the distribution of bump durations and amplitudes and the familiar sigmoid curve obtained by plotting the receptor potential against the log of the intensity. The maximum bump latency of 0·5 s agrees with the rapid decay of the receptor potential at 'off'.

With a high intensity stimulus the response does not fall at once to the base line, but continues as a *prolonged depolarizing afterpotential* (PDA). The PDA begins to appear at intensities near those which saturate the receptor potential. In flies, the curve of PDA amplitude plotted against log intensity is steeper than the corresponding curve for the RP, and most of the curve lies within a tenfold intensity range (fig. 2 in Tsukahara, Horridge & Stavenga, 1977). Therefore the PDA in flies differs from the RP in the kinetics of the reaction which captures the light. In other respects, namely in spectral sensitivity and ionic basis, the PDA has many features in common with the RP: both appear to be caused by the same process following photoactivation of rhodopsin (Tsukahara *et al.* 1977) and to be brought about by increase in sodium conductance (Baumann & Hadjilazaro, 1972). The PDA is seen, however, when the stimulus causes a nett formation of metarhodopsin, and it is reduced or swamped by a hyperpolarization of quite a different nature which also follows strong stimuli.

That the PDA is composed of bumps in the same way as in the RP was first suggested by observations on the noise in the PDA of a *Drosophila* mutant (trp) in which the receptor potential decays rapidly (Minke, Wu & Pak, 1975). The records are clearer in *Locusta*, where we have distinguished three occasions when bumps are seen (Tsukahara & Horridge, 1977). *Dark-adapted* (DA) bumps are initiated at very low intensities from dark-adapted cells. *Light-adapted* (LA) bumps are similarly initiated with short latency from light-adapted cells: in the extreme light-adapted state they are smaller and briefer than dark-adapted bumps, but all intermediate states occur. We found that even though the cell may have been in darkness for half an hour after the stimulus, the tail of the afterpotential is composed entirely of bumps which we called light-induced dark bumps (LID bumps) or PDA bumps. The LID bumps are not distinguishable in shapes or amplitude from short-latency (LA) bumps that are caused by an intermittent light stimulus during the afterpotential tail. We now present further data from which we can set up a hypothesis to explain how DA, LA and LID bumps are all basically the same, and also to explain the origin of the long latency of the LID bumps.

METHODS

Measurements were made from the same series of bumps used for a previous publication (Tsukahara & Horridge, 1977). The bumps were recorded from a *Locusta* retinula cell which was selected for the large size and clarity of the bumps. An improved signal-to-noise ratio was obtained by use of bevelled microelectrodes (Brown & Fleming, 1975). The bumps used for the measurements are in the final stages of the tail of an afterdepolarization which is produced by a 900 W unfiltered Xenon arc giving 3×10^{13} photons $cm^{-2} s^{-1}$ at 393 nm at the cornea, and applied for 270 s. The afterdepolarization returns to the resting potential only after about 15 min. As the afterdepolarization declines it becomes more noisy and eventually the noise separates itself into a series of bumps which can be recorded continuously for many minutes.

On the record every bump which reached the criterion of 0·5 mV was included in the statistics. When two bumps overlap there are all possible signs of their summation in the record. In general, two bumps were counted as separate when there were two clear rising phases and all times of occurrence were taken from the start of the rising phase. On account of the problem of distinguishing bumps which overlap there is an inevitable difficulty in ruling out a possible dead-time, but it would have to be less than 20 ms if present. In the statistical work the durations of intervals have been grouped into periods of 40 ms (1 mm on the record). The record used for analysis had average intervals of about 500 ms so that indecipherable overlaps of bumps must be relatively infrequent.

RESULTS

Relative intensities for bumps, receptor potential and afterdepolarization

When the DA bump frequency, the heights of the receptor potential (RP) and the PDA are plotted against stimulus intensity on the same graph, the total response spreads over 5 orders of magnitude of intensity (Fig. 1). The frequency of dark-adapted bumps at low intensity is proportional to the light intensity, and Lillywhite (1977) has calculated that 60% of the peak photons falling on the cornea on the optical axis are effectively absorbed. There is therefore no lower threshold, but the statistical expectation of seeing a bump falls linearly with intensity.

The height of the resting potentials at the lowest intensities cannot be measured because they consist of bumps. At an intensity which would induce a bump frequency of 100 s^{-1} the mean resting potential has reached 10% of saturation, near 5 mV. In our example (Fig. 1) the height of the peak receptor potential as a function of intensity fits the relation $V/V_{max} = I^{0.9}/(I^{0.9}+I_{\frac{1}{2}})$ where $I_{\frac{1}{2}}$ is the intensity that produces a 50% response. That the response heights fit this curve, rather than $V/V_{max} = I/(I+I_{\frac{1}{2}})$ emphasizes that as the stimulus intensity increases there are additional processes, called light adaptation, which have the effect that each additional photon causes less than the conductance component that would be expected for the dark-adapted case. A part of this fall-off is attributable to the smaller amplitude of LA bumps.

In our locust retinula cells, the height of the afterpotential plotted as $V_{PDA}/V_{max\ PDA}$,

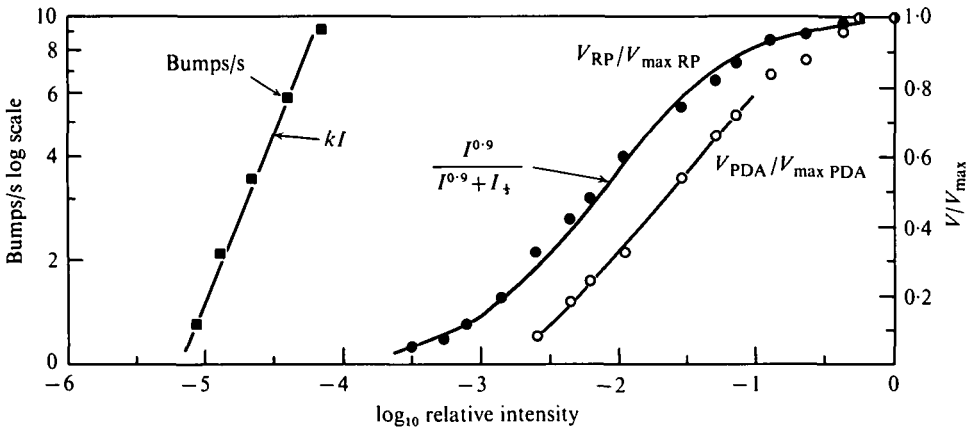

Fig. 1. The relative positions of the three types of locust retinula cell response on the same intensity scale. The DA bump frequency is proportional to intensity. The smooth line through the receptor potential (RP) values is the function $V_{RP}/V_{max\ RP} = I^{0.9}/(I^{0.9}+I_{\frac{1}{2}})$, where $I_{\frac{1}{2}}$ is the intensity which gives a 50% response. The afterpotential (PDA) is plotted as $V_{PDA}/V_{max\ PDA}$. At log I = 0 there were 10^{13} photons cm^{-2} s^{-1} at peak wavelength (which was near 445 nm).

where $V_{max\ PDA}$ is the maximum observed voltage of the PDA, gives a curve which is parallel to the corresponding curve for the height of the receptor potential, but shifted half a log unit to the right. This parallel relation suggests that in the locust the PDA has the same mechanism of non-linear summation of amplitudes which gives rise to the receptor potential. The non-linearity of the receptor potential is in turn considered to lie in the summation of bumps, each of which contributes, but not necessarily equally, to an increase in membrane conductance.

When the PDA is plotted as a fraction of the maximum *peak receptor potential* (Fig. 2), we see two additional factors. First, the PDA never exceeds the saturating amplitude of the plateau of the receptor potential, if the stimulus is long enough to induce the plateau. In Fig. 2 the PDA saturates near 50% of the RP with a 1 s stimulus. Secondly, the amplitude of the PDA depends on the *duration* of the stimulus, as if the PDA were caused by a persistent substance which is formed slowly under the action of intense light. Many hypotheses about the nature of this substance can be advanced but two simple ones are set out in the discussion.

Decay of the receptor potential and afterpotential

The elementary observation that the PDA decays much more slowly than the RP can be related directly to the difference in latency distribution of DA or LA bumps in contrast to light-induced dark (LID) bumps. With 900 repeated stimuli, Lillywhite (1977) found most DA bumps within 100 ms latency, all in 500 ms, and no spontaneous bumps. This brief duration of the excitation process and its rapid decay, also observed by Scholes (1965), agrees with the theory that the receptor potential is formed by the summation of bumps.

In contrast, the afterpotential and the light-induced dark (LID) bumps decay extremely slowly in frequency so that little trend is observed over several minutes (Figs 3A and 4). The frequency decays to half in periods of over 500 s. For this

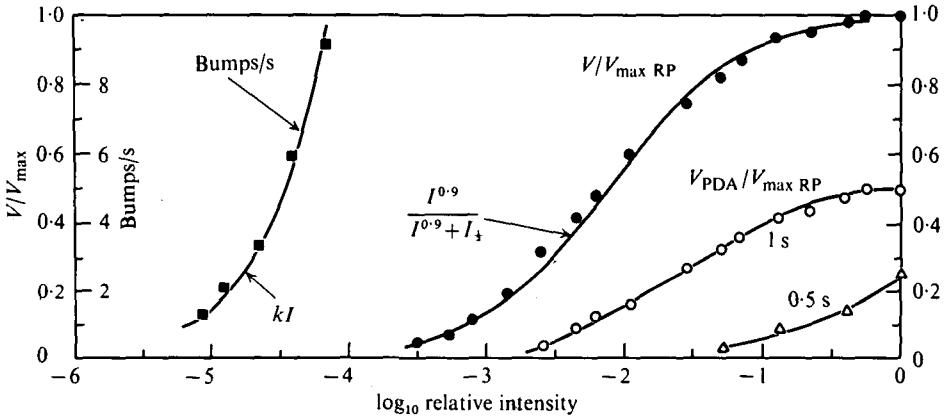

Fig. 2. The data of Fig. 1 plotted to show amplitudes of the PDA in relation to the amplitude of the RP. The amplitude of the PDA depends on the duration of the stimulus as well as on intensity, and never reaches the amplitude of the RP.

reason we propose that LID bumps owe their long latency to a stable photopigment or transmitter packet which lies on a side-branch of the path leading directly from a photon capture to a DA bump.

Statistical analysis of light-induced dark bumps

Trends

Because they form the tail of a decay process, the bumps described here inevitably have a trend. Often in our records of bumps, however, many minutes after a bright flash we find that the progressive decline in frequency is interrupted by temporary stabilization or even increase in frequency for no apparent cause. Other workers have noted a similar slow fluctuation in average frequency of bumps in the direct response to constant low light levels in *Limulus* (Fuortes & Yeandle, 1964). In selecting a length of record for statistical analysis, the intervals themselves were not considered but only the number of bumps in every 13 s length of record, as in Fig. 3A. Two continuous lengths where the trend is minimal were selected; the trends are shown in Fig. 4. This method avoids the laborious alternative of taking out the trend by calculation. Nevertheless, the long-term fluctuations in bump frequency, which certainly occur in all preparations, indicate that unknown factors outside our control influence the frequency of bumps. In this case the migration of screening pigment and other optical effects are ruled out because the record is made in total darkness.

Test for serial correlation

A series of 349 successive intervals was measured and plotted as a frequency distribution. Values greater than the median interval were marked A and those less than the median were marked B. Then the series of intervals was examined with reference to whether an interval A (less than the median) was followed by another interval A, or by an interval B (greater than the median). Similarly, intervals B followed by intervals A and intervals B followed by B were counted. If there is no tendency for an interval to be followed consistently by either a longer or shorter

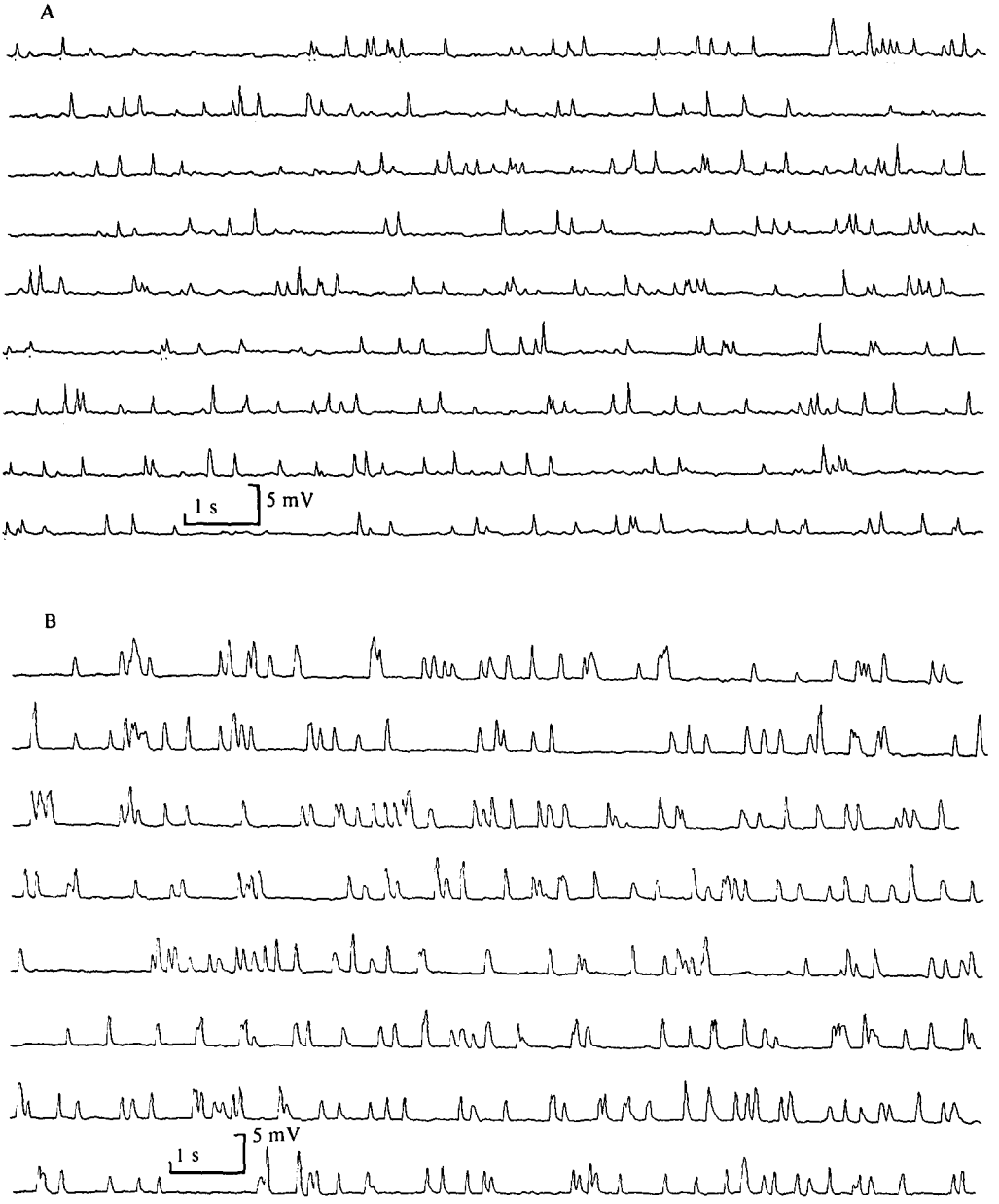

Fig. 3. A, Bumps in the final stages of the tail of the afterpotential, after 20 min have elapsed since the stimulus. This is a section from one of the records that were analysed. B, Dark-adapted bumps recorded $\frac{1}{2}$ h previously from the same cell.

interval then the number of counts in the four possible sequences AA, BB, AB, BA, will be the same.

For the first 195 intervals the values were AA 47, AB 48, BA 48 and BB 52. For the first 349 intervals the counts were AA 94, AB 80, BA 83, BB 92. For a different set of data, with a total of 311 intervals, the values were AA 80, AB 77, BA 77 and BB 77.

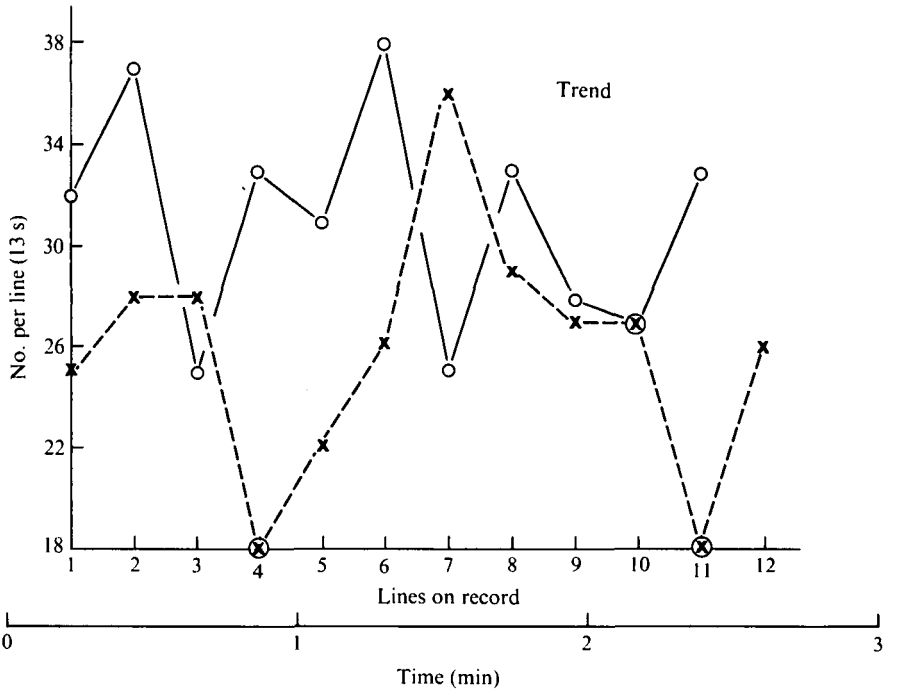

Fig. 4. Test for trend. The number of bumps in each line of the record, as in Fig. 1, was plotted against the line number (and also time). For the selected sections of record there was no obvious trend.

There is therefore no significant tendency for an interval shorter than the mean to be followed by an interval consistently either longer or shorter than the mean. Similarly, when the same distributions were broken down into quartiles, there was no significant pair of values falling into different or the same quartiles which consistently followed each other more frequently than any other pair.

Distribution of runs

To test for further periodicity in the series of bumps the distribution of runs was examined in the following way. All intervals between bumps were marked as greater or less than the median interval. A run is defined as a sequence of successive intervals which are all either less or greater than the median. If there is no periodicity in the distribution then for any interval there should be an equal chance that the next interval is greater or less than the median interval. This means that a run of $x = 1$ has a probability of 0·5. If the intervals are all independent of each other, a run of $x+1$ intervals has a probability of 0·5 of a run of x intervals. Failure to fit this theory therefore reveals a tendency for long intervals to be followed by either long or short intervals, or for there to be regular or irregular oscillatory changes in interval durations.

The tabulated results (Table 1) show that there is no tendency for more runs than would be expected from a random series of events. The observed frequencies of runs of different length are not significantly different from the expected frequencies.

Table 1. *Distributions of runs of intervals that are greater or less than the median interval*

1	Number in run	1	2	3	4	5	6	7	8	9	10+
	First series										
2	Number of runs less than median	37	22	9	7	2	3	2	0	0	0
3	Number of runs greater than median	38	24	7	9	2	0	1	2	0	0
4	Total, lines 2, 3	75	46	16	16	3	3	3	2	0	0
	Second series										
5	Number of runs less than median	39	18	11	4	1	1	0	1	2	0
6	Number of runs greater than median	37	24	8	5	3	0	0	0	0	0
7	Total, lines 5, 6	76	42	19	9	4	1	0	1	2	0
8	Total, lines 4, 7	151	88	35	25	7	4	3	3	2	0
9	Expected value of line 8	159·5	79·7	40	20	10	5	2·5	1·25	0·6	0·3

Distribution of numbers of LID bumps in equal time intervals

When a series of events are Poisson-distributed in time, the series can be divided into equal time intervals and the probability of seeing η events in an interval is

$$P(\eta) = e^{-\mu}.\mu^{\eta}/\eta!$$

When $\eta = 0, 1, 2, 3, 4$ etc. and μ is the mean number of events per interval.

Two series of bumps were analysed by this method. For the first, with 349 bumps in 182 equal intervals $\mu = 1{\cdot}917$, and for the second, with 324 bumps in 211 intervals $\mu = 1{\cdot}535$. The experimental and the expected frequencies of occurrence of 0, 1, 2, 3 etc. bumps per interval are plotted in Fig. 5. By inspection and by chi-square test, there is no significant difference between the measured and the theoretical distributions.

Distributions of intervals between bumps

In a Poisson-distributed series of bumps the relation between the frequency of occurrence and the duration of intervals is given by

$$N_t = N_0 n e^{-nt}.\Delta t,$$

where N_0 is the total number of intervals, n is the average number of bumps per second and Δt is the width of the columns in the grouping of intervals.

Plotting the frequencies of intervals of different durations against the interval should therefore yield an exponential curve which is predictable from the mean if the bumps are randomly distributed. Plots for two sets of data are given in Fig 6. The theoretical values fit remarkably well to the observed values. On this criterion, therefore, the series of bumps is indistinguishable from a Poisson distribution. In particular, there is not an excess of very long or very short intervals over that predicted from theory. This in turn means that we can discount the possibility that packets of long-lived transmitter generate groups of bumps close together. It does not eliminate the possibility, however, that many long-lived molecules each cause repeated bump initiation in such a way that the result resembles a Poisson process. Such a process is described quantitatively by Weiss & Yeandle (1975).

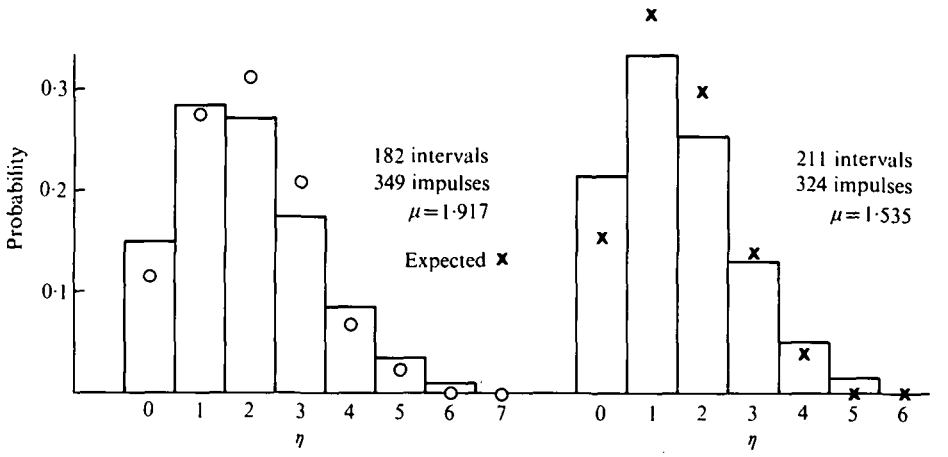

Fig. 5. Actual and expected frequencies of occurrence of 0, 1, 2, 3 etc. bumps in equal time intervals. The record was divided into intervals of 0·8 s and μ is the mean number of bumps per interval.

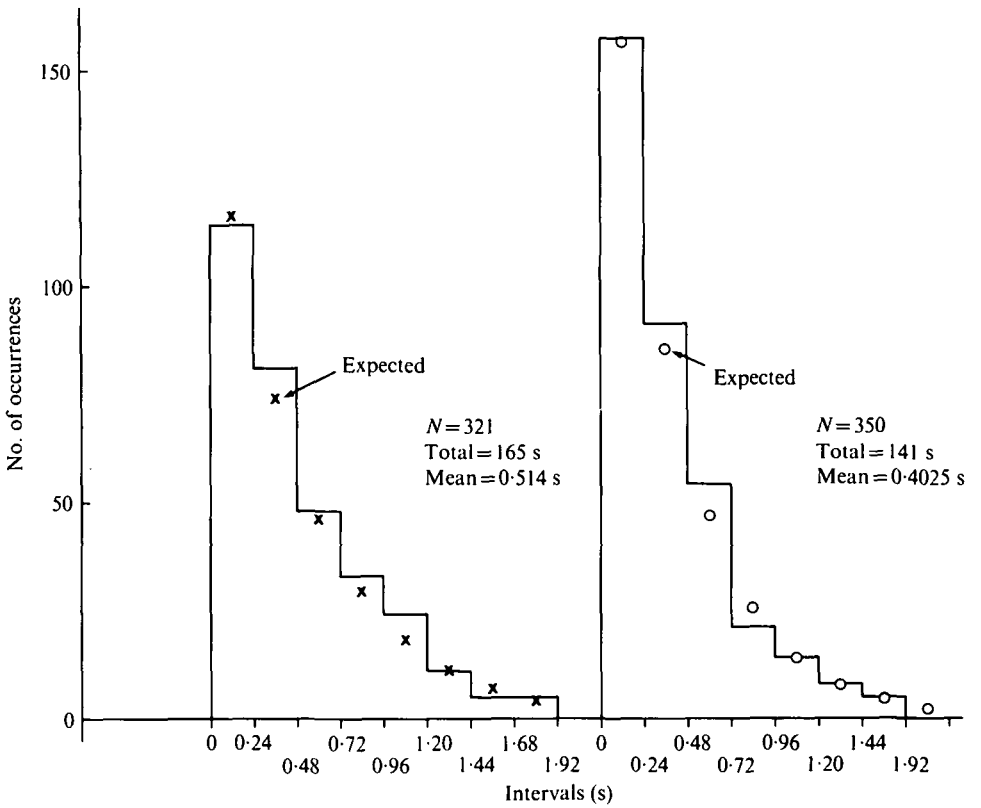

Fig. 6. Distribution of intervals between bumps for two sets of data, showing experimental values as columns and the values (○) and (×) expected from an exponential distribution of similar mean.

DISCUSSION

Twenty minutes after a bright flash the PDA resolves itself into individual light-induced dark (LID) bumps as it falls towards the resting potential. The implications of these long-latency bumps depend first on whether the PDA is considered to be caused by the same process as the receptor potential (RP). Evidence that this is so comes from several preparations. In the fly *Eristalis* the spectral sensitivity of the PDA shows that it is caused by photoactivation of one rhodopsin system (Tsukahara *et al.* 1977) but only under conditions when intense light can act on the photoproducts of rhodopsin activation. Secondly, the ionic mechanisms of the PDA are similar to those causing the RP in the barnacle (Brown & Cornwall, 1975) and in the bee (Baumann & Hadjilazaro, 1972). Similar data for the locust is desirable but we have shown that the LID bumps in the tail of the afterpotential are indistinguishable from LA bumps that are directly induced by light in the same cell at that time (Tsukahara & Horridge, 1977). Therefore LID bumps and RP bumps have a common origin which must explain why they are single events, and a common ionic mechanism which explains why they are indistinguishable. The problem is to explain the extraordinary latency of the LID bumps.

The statistical distribution of latencies of DA bumps is predicted by two models (Borsellino & Fuortes, 1968; Srebro & Behbehani, 1971) which have important differences, but both introduce a stochastic process between the activation of the rhodopsin and the generation of the bump in order to describe the latency and the amplitude distribution as a result of the physically constant process of activation of a rhodopsin molecule. The time constants of the hypothetical stages preceding the bump are comparable to the maximum latency of 0·5 s for DA and LA bumps: an additional and much longer arbitrary term would have to be added to describe LID bump latency.

Conditions for formation of LID bumps

Any theory of afterpotential bumps must take into account the following:

(*a*) The PDA is seen only after a stimulus of a wavelength which moves the wavelength-dependent photo-equilibrium reaction R → M to the right, at which time the intense light could also act on the photoproducts of rhodopsin. Activation of metarhodopsin (M) alone does not cause a PDA, RP or negative-going potentials in a cell which restores its resting membrane potential (Tsukahara *et al.* 1977).

(*b*) The bumps of the PDA are unitary events in every way similar to LA bumps (Tsukahara & Horridge, 1977). Noise levels in the fly indicate that much and perhaps all of the PDA is composed of summed bumps (Minke *et al.* 1975) as confirmed by our own qualitative observations in the locust.

(*c*) PDA bumps in the fly differ from ordinary bumps in that the PDA is susceptible to the knock-down effect, as demonstrated by others and discussed in our previous paper (Tsukahara *et al.* 1977). The knock-down effect is a suppression of the afterpotential by a stimulus that converts M → R, and it shows that a persistent pigment state or unspecified internal transmitter is inactivated by light which photoregenerates R from M.

(*d*) In the locust one activated rhodopsin molecule gives rise to one DA bump

(Lillywhite, 1977) and by some unknown process the discrete nature of the bump is preserved through a variable latency period, lasting for a maximum of 0·5 s.

(*e*) That the actual bump is caused when a molecule of transmitter packet reacts with not one but with a group of conductance sites of the membrane, has been inferred by several authors, as discussed and elaborated in the previous paper (Tsukahara & Horridge, 1977).

(*f*) At the production of a DA bump the activated molecule or transmitter packet is inactivated after initiation of one bump, and no further bump is released.

(*g*) As implied by (*f*) a definite and additional process is required to account for LID bumps and the PDA, and *this process also preserves the quantized nature of the bump*.

(*h*) LID bumps are Poisson-distributed, as is consistent with any process of random decay of molecules or unitary packets of transmitter.

The quantal nature of afterpotential bumps

One of our previous findings is that the LID bumps in the tail of the PDA are indistinguishable from bumps of short latency which are directly caused in the same cell by flashes of dim light during the tail of the PDA (Tsukahara & Horridge, 1977). From this we infer that LID bumps and LA bumps are *generated* by the same mechanism. Secondly, as the cell dark-adapts, LA bumps grade into DA bumps, and we make the hypothesis that there is only one amplification and ionic mechanism for the generation of a bump, whether it is a DA, LA or LID bump.

The DA bumps are *initiated* by the transformation of a single rhodopsin molecule which absorbs a photon, but the energy in a bump is much greater than can be provided from the activation of the single molecule. It has been suggested that the first intervening stage of amplification is the release of an internal transmitter (Baylor & Fuortes, 1970; Yoshikami & Hagins, 1971) which then causes the opening of many sodium channels. Whether one bump is caused by one molecule of transmitter which opens many sodium channels, or by many molecules of transmitter released by the activation of one rhodopsin molecule, is undecided, but is crucial for the choice of model to explain LID bumps.

The primary observation is that after 20 min of darkness we see what look like ordinary bumps. We infer that each is initiated by a unitary process such as the spontaneous breakdown of a single molecule or of a single packet of transmitter. If many molecules of transmitter are necessary to cause a bump then it follows from the unitary nature of the LID bumps that the transmitter must be in packets. The question is whether the latency of half an hour or so depends on the persistence of activated pigment or of packets of transmitter. Therefore we have one model set out with persistent transmitter packets (Fig. 7A), and one with persistent visual pigment molecules (Fig. 7B).

Models for LID bump initiation

In the transmitter type of model (Fig. 7A) the photo-activation of rhodopsin forms a transmitter packet, T_{active}, which generates a bump and reverts to its resting state, T_{rest}. With an intense stimulus, T_{active} can generate a stable transmitter packet

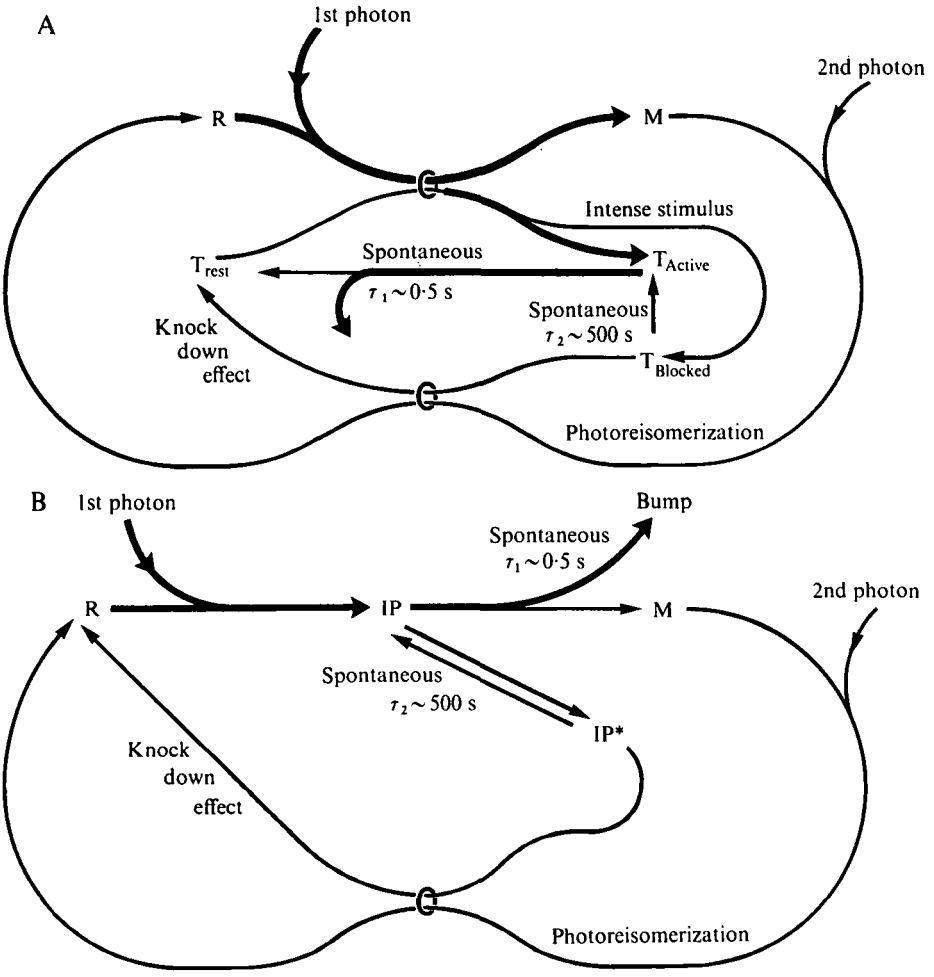

Fig. 7. Alternative models of the activation of either molecules or transmitters by photons. A, In this model an active transmitter packet T_{active} is produced by excitation of rhodopsin (R). By intense light some of this transmitter can become blocked in its decay and cause a PDA. The bumps of the PDA must then be attributed to the spontaneous decay of blocked transmitter to active transmitter packets which in turn generate bumps. B, A model in terms of visual pigment molecules. The 1st photon produces an intermediate pigment, IP, each molecule of which generates a bump as it changes to metarhodopsin. In intense light, a small part of IP is activated by a 2nd photon to IP* which slowly decays back to IP, so ultimately causing the bumps of the PDA. In both A and B, the conversion of M to R causes an elimination of the source of the PDA in the fly, as on the right-hand side, but this effect (the so-called knock-down effect) is not observed in the locust.

$T_{blocked}$ which reverts slowly to T_{active} over periods up to about an hour. To account for the knock-down phenomenon in the fly, we could add the deactivation of $T_{blocked}$ by the photon-induced reaction $M \rightarrow R$, as on the right in Fig. 7A. Unanswered questions are whether there is an alternative cause of afterdepolarizations, and how $T_{blocked}$ is formed.

An alternative model, based on visual pigment molecules, places an intermediate pigment IP in the $R \rightarrow M$ reaction (Fig. 7B). We make the hypothesis that a second photon activates IP to give a relatively stable form IP* which reverts slowly to IP.

To explain the knock-down effect in the fly we could add the deactivation of IP* by the M → R reaction, in Fig. 7B.

In both models, all three kinds of bump (DA, LA and LID bumps) arise from one source, which generates only one bump as it reverts to its resting state. This final process is the one which causes the mysterious statistical distribution of bump amplitudes and latencies up to 0·5 s. The models in Fig. 7 are the simplest we can think of which bring together the observations.

Modern theories of membranes, however, suggest that ideas of a completely different kind may be more useful in explaining the origin of the bumps. We refer to work showing that the lipid arrangement is modified by the changes in the associated lipophylic proteins (Israelachvili, Michel & Ninham, 1976). After the activation of the protein (rhodopsin) molecule, the cause of the conductance change may be a shift in the surrounding lipid molecules. The conductance change seen as a bump may be caused by the healing process which is a redistribution of strain energy. This type of explanation can be extended to LID bumps which would then be the sign of similar but delayed lipid rearrangements.

Continuous or once-only formation of afterpotential bumps

In the locust receptor, one photon gives rise to one DA bump and no more (Lillywhite, 1977). This is the *single bump hypothesis*. Alternatives are the *multiple bump hypothesis*, in which one photon produces several transmitter packets, each of which causes a bump, and the *continuous bump hypothesis*, in which a cyclical production of bumps follows the photoactivation of a single rhodopsin molecule. Although multiple and continuous bump hypotheses have been ruled out for locust DA bumps, they may still apply to LID bumps in that the slow spontaneous stage of the breakdown of $T_{blocked}$ in Fig. 7A or of IP* in Fig. 7B, is a new hypothesis which has not been examined from this point of view.

The next step will be to compare the calculated number of bumps in the largest and longest PDA with the number of available rhodopsin molecules, to see if the continuous bump hypothesis is necessary. From what is known of several species of Diptera, it appears likely that the single bump hypothesis will not explain the duration of the PDA; for example, the PDA of *Drosophila* can persist for more than 7h (Tsukahara & Merriam, unpublished observations) and in the blowfly one finds a very long period of pupil closure which is related to the PDA (Stavenga, Flokstra & Kuiper, 1975).

Support for the continuous bump hypothesis comes from the spontaneous bumps recorded in *Limulus* in the dark, but these preparations have previously been exposed to bright light and it is impossible from the published accounts to say whether the spontaneous bumps are LID bumps or represent a lowered threshold of another process. One can in fact by definition consider the insect PDA as a temporary period of spontaneous bumps.

The composition of the afterpotential

We await a theory of the non-linear summation of bumps before we can show that the RP and PDA have the noise levels expected at each potential level. Unfortunately

the size and shape of the bumps change with the state of light adaptation (Tsukahara & Horridge, 1977), so exact calculation is not yet possible. Qualitatively we have two lines of evidence that the PDA consists wholly of bumps. First, however long the PDA lasts, its tail always consists of LID bumps. Secondly, the noise on the PDA record is less for larger deflexions, as in the case of the RP, suggesting that the PDA is formed by non-linear summation of bumps. In conclusion there may be non-bump components of the RP and of the PDA but none has been proposed.

REFERENCES

BAUMANN, F. & HADJILAZARO, B. (1972). A depolarizing after-effect of light in the drone visual receptor. *Vision Res.* **12**, 17–32.

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

BORSELLINO, A. & FUORTES, M. G. F. (1968). Responses to single photons in visual cells of *Limulus*. *J. Physiol., Lond.* **196**, 507–539.

BROWN, H. M. & CORNWALL, M. C. (1975). Ionic mechanism of a quasi-stable depolarisation in barnacle photoreceptor following red light. *J. Physiol., Lond.* **248**, 555–578.

BROWN, K. T. & FLEMING, D. G. (1975). Instrumentation and technique for beveling fine micropipette electrodes. *Brain Res.* **86**, 172–180.

FUORTES, M. G. M. & YEANDLE, S. (1964). Probability of occurrence of discrete potential waves in the eye of *Limulus*. *J. gen. Physiol.* **47**, 443–463.

ISRAELACHVILI, J. N., MITCHELL, D. J. & NINHAM, B. W. (1976). Theory of self-assembly of hydrocarbon amphiphites into micelles and bilayers. *J. Chem. Soc.* (*Faraday Transactions II*) **72**, 1525–1568.

LILLYWHITE, P. G. (1977). Single photon signals and transduction in an insect eye. *J. comp. Physiol.* (in press).

MINKE, W., WU, C. F. & PAK, W. L. (1975). Induction of photoreceptor voltage noise in the dark in *Drosophila* mutant. *Nature, Lond.* **258**, 84–86.

SCHOLES, J. H. (1964). Discrete subthreshold potentials from the dimly lit insect eye. *Nature, Lond.* **202**, 572–573.

SCHOLES, J. H. (1965). Discontinuity of the excitation process in locust retinula cells. *Cold Spring Harb. Symp. quant. Biol.* **30**, 517–527.

SREBRO, R. & BEHBEHANI, M. (1971). A stochastic model for discrete waves in the *Limulus* photoreceptor. *J. gen. Physiol.* **58**, 267–286.

STAVENGA, D. G., FLOKSTRA, J. H. & KUIPER, J. W. (1975). Photopigment conversions expressed in pupil mechanism of blowfly visual sense cells. *Nature, Lond.* **253**, 740–742.

TSUKAHARA, Y. & HORRIDGE, G. A. (1977). Miniature potentials, light adaptation and afterpotentials in locust retinula cells. *J. exp. Biol.* **68**, 137–149.

TSUKAHARA, Y., HORRIDGE, G. A. & STAVENGA, D. G. (1977). Afterpotentials in dronefly retinula cells. *J. comp. Physiol.* **114**, 253–266.

WEISS, G. W. & YEANDLE, S. (1975). Distribution of response times in visual sense cells after weak stimuli. *J. theor. Biol.* **55**, 519–528.

YEANDLE, S. (1958). Electrophysiology of the visual system (Discussion). *Amer. J. Ophthal.* **46**, 82–87.

YOSHIMAMI, S. & HAGINS, W. A. (1971). Ionic basis of dark current and photocurrent of retinal rods. *Biophys. Soc. Ann. Meet. Abstr.* **11**, 47a.