

Statistical Analysis of Quantum Bump Parameters in *Limulus* Ventral Photoreceptors*

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A statistical analysis of current quantum responses ("bumps") to the absorption of single photons in the ventral photoreceptor of *Limulus* is presented. The analysis is performed in terms of bump parameters among which two groups are distinguished: "time-like parameters" with distributions independent of individual cells and "current parameters" the distribution of which differ for different cells. The analysis yields a set of "primary" parameters with mutually uncorrelated fluctuations. The results are interpreted by a new model formulation on the basis of a transmitter hypothesis for the transduction. The model separates latency from amplification and postulates the existence of different sources of fluctuations among which one includes individual cell properties.

1. Introduction

The electric response of the ventral nerve photoreceptor of *Limulus* stimulated by light steps or light flashes of decreasing intensities dissolves into single transient depolarising voltage changes of up to 15 mV maximum amplitudes, the so-called "voltage bumps". Under voltage clamp conditions, the corresponding responses of the photoreceptor are transient inward currents with maximum amplitudes of up to 3 nA, the so-called "current bumps". The experimental technique of the measurement of these bumps discussed here has been described in detail by Stieve [1, 2].

A typical record of a current bump is shown in Fig. 1. The measured bumps of the same photoreceptor cell vary with respect to their size, duration and form. The analysis of this variation is appropriately performed by evaluating bump parameters from the records as there are the maximum amplitude A , the rise time t_r (time interval between the onset of the response and its maximum amplitude A), the total area F , the slope m of a distinct

linear part of the rising phase and the time constant λ of an exponential decay phase (c.f. section 2 of this paper and Fig. 2).

At low intensities, the frequency of the bump events is strictly proportional to the applied stimulus intensity (Yeandle and Spiegler [3]). The light-induced receptor current is a linear function of the stimulus energy even for several decades of the applied light intensity (Lisman and Brown [4]; Brown and Coles [5]). Under dim illumination, equal numbers of photons produce identical average response curves, independent of the light

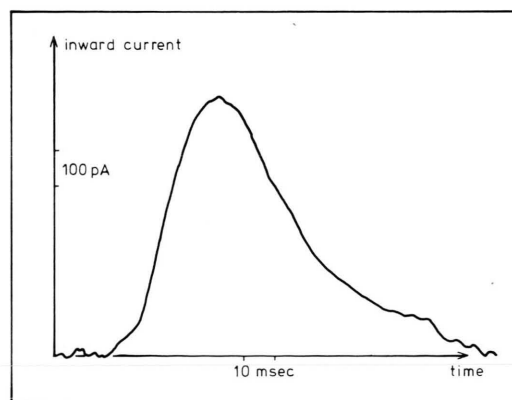


Fig. 1. Typical record of current bump.

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intensity applied (Borsellino and Fuortes [6]), and the waiting time between two bumps is distributed exponentially (Yeandle and Spiegler [3]; Keiper [7]), as in a Poisson process. Hence it is generally accepted that bumps are responses of the photoreceptor to the absorption of single and independent photons.

The total net charge of bumps as to be determined by their area is of the order of up to 200 pC corresponding to a number of more than 10^9 elementary charges. In section 4 of this paper, we shall argue that this amount of charge is transferred across the membrane through a number of a few hundred up to a thousand of light-activated channels. Beyond this phenomenon of amplification the photoreceptor shows another striking property, namely a latency period between the absorption of the photon and the onset of the current response. The bump latency time t_{lat} is measured in flash experiments and is of the order of 200 ms in dark-adapted cells. The value of t_{lat} varies from bump to bump and is thus considered as a further bump parameter.

The phenomena of amplification and latency led Fuortes and Hodgkin [8] to propose a model for the bump mechanism which consists of a chain of a number of ten coupled enzymatic amplifiers, all with identical rate constants. The chain is initiated by the absorption of a photon, its links are intermediate molecules and it ends with the generation of open light-activated channels. The non-stochastic or "deterministic" integration of the model yields a t^9 power law for the onset of the response due to the number of ten chain links where t is the time elapsed since the absorption of the photon. In the stochastic evaluation of the model as performed by Borsellino and Fuortes [6] the t^9 -behaviour is equivalent to a latency behaviour with statistically fluctuating values of the duration of the latency period – as intended and expected. As a consequence of the coupled mechanism of amplification and latency in this model, one expects some relationship between the averages of latency period and duration of bumps. In fact we find that the Fuortes-Hodgkin model with 10 equal amplification steps predicts an average bump duration at least twice as long as the average t_{lat} . For experimentally observed bumps, however, the inverse relationship holds: bump durations are on the average shorter than 100 ms, *i.e.*, shorter than half the average value

of t_{lat} (*c.f.* section 2). The theoretical prediction of a ratio of bump duration and t_{lat} greater than 2 is not a consequence of a particular choice of parameters in the Fuortes-Hodgkin model and not even a peculiar property of this model but applies to all models which involve amplification already in the first step. This finding will be presented in a subsequent theoretical paper [Schnakenberg *et al.*, in preparation]. It led us to consider models whose mechanism of amplification is preceded by a gain-free latency process. Detailed calculations by one of us (W.K.) showed that *e.g.* a series of conformational changes of a molecule with subsequent amplification gives satisfying agreement between model and experiment as far as average and distribution of the bump's latency is concerned – independent of the other bump parameters which depend solely on the steps after the latency process. It should be noted that because of the stochastic nature of the reaction chain kinetics, neither our models nor the Fuortes-Hodgkin model predict any correlation of t_{lat} with the other parameters of individual bumps. Indeed, the experimental findings as shown in section 2 prove that t_{lat} is uncorrelated to all other bump parameters which we have evaluated. It is thus the ratio of the parameter averages that speaks against the Fuortes-Hodgkin model as mentioned above, but not their correlations.

Recently, Goldring and Lisman [9] have reported computer simulations of the Fuortes-Hodgkin model. Their findings are in full agreement with ours: bump shapes and latency periods fit the experimental data only if it is assumed that the chain of reactions leading to a bump contains a number of initial no-gain steps, so that latency and amplification arise mostly from separate parts of the chain of events.

A further motive for considering alternative possibilities for a model is another result of our analysis of the experimental data which will be presented in section 2: the experimental distributions of maximum amplitude A , slope m , and area F vary from cell to cell to such an extent that a realistic model has to account for individual properties of a cell. We shall give arguments in section 3 that this variation between different cells can be associated with individual properties of the membrane. On the basis of this hypothesis, we shall propose an alternative model in section 4 of this paper. This model takes into account individual

properties of the cell membrane and separates them from a “bump generating process” which is assumed to be essentially independent of individual cell properties. The bump generating part of our model also includes a suggestion for the mechanism of the transduction of the information that a photon has been absorbed to the positions of the light channels to be opened. We will give arguments that this transduction is very likely realized by a hypothetical “transmitter” molecule which is released or activated in the region where the photon has been absorbed, then diffuses along the inner side of the membrane to the sites of the channels and eventually opens them by binding to the channel molecules.

2. Distributions and Correlations of Bump Parameters

The experimental results to be reported in this section are based upon voltage-clamp bump measurements at the ventral photoreceptor of *Limulus* performed by Stieve and coworkers in the Institut für Neurobiologie of the Kernforschungsanlage Jülich. Current bumps were recorded from single dark-adapted photoreceptor cells superfused in saline at 15 °C. For details of the preparation, see Stieve and Bruns [1]. A total of 10 cells have been investigated by us, to date. Bumps were evoked either by 50 μ s-flashes of green light (photon number $3 \cdot 10^{11}/\text{m}^2$) every 10 s, yielding in average 0.5 to 1 bumps per flash, or by 10 s continuous dim illumination (intensity of photons $10^{12}/\text{m}^2 \text{ s}$) every 20 s, resulting in about one bump per second.

The membrane current data, under voltage-clamp conditions, were digitized in pulse-code modulation technique (bandwidth 0–300 Hz) and recorded on magnetic tape at a rate of one current value per ms. Further details of the experimental setup will be published elsewhere by Stieve and coworkers.

The original recordings were then transferred to a mainframe computer (Control Data Cyber 175, RWTH Aachen), decoded, and stored as sequential data files. Our evaluation of the experiment is based upon the choice of a number of bump parameters as illustrated in Fig. 2.

For reasons to be presented in section 3 we distinguish two groups of parameters:

- a. *time-like parameters*: latency time t_{lat} , rise time t_r , rise time $t_{1/2}$ to half the maximum amplitude,

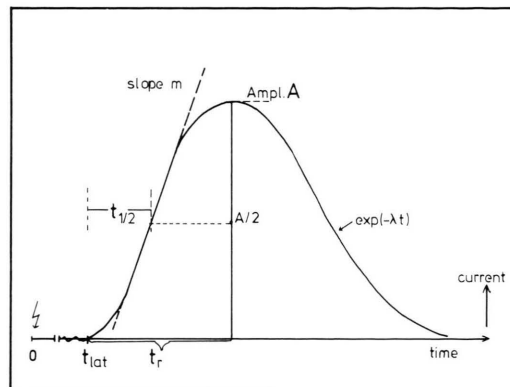


Fig. 2. Definitions of bump parameters.

decay rate λ , expressed by units of time or inverse time;

- b. *current parameters*: maximum amplitude A , total bump area F , slope m of the linear portion of the rising phase, involving the unit of a current.

We have developed a FORTRAN-program which determines the numerical values of the parameters for each bump from the experimental data. First of all, the program identifies a current fluctuation as a bump by applying the following criteria:

1. The fluctuation's maximum amplitude must be at least 100 pA above the local baseline, as determined in the direct vicinity of each potential bump.
2. The time integral of the fluctuation's deviation from the baseline, *i.e.* the potential bumps' area, must exceed a limit of 2 pC. The criterion values are conservative estimates dictated by experience. In all experiments the computer's findings were checked by eye for reliable discrimination between random noise or occasional brief erratic signals, and real bumps. Some cells with particularly low noise levels gave fully satisfactory results with criterion values less than half of those given above. The area criterion is similar to that applied by Goldring and Lisman [10]. After safe recognition of a bump, its time of onset was determined by extrapolating the first significantly rising signal points back to the local baseline.

The estimated error of this method is less than or equal to 2 ms for the onset of medium-size bumps. The computer program checked every bump curve

for a regular shape with only one maximum. If more than one bump maximum occurred, the event was earmarked as “multiple bump”.

a. Time-like parameters

Latency times t_{lat} for bumps in the ventral photoreceptor of *Limulus* have been reported by Fuortes and Yeandle [1], Wong *et al.* [12], Stieve [2] and others.

Our results qualitatively coincide with the earlier measurements. Fig. 3a shows distributions of t_{lat} for two different cells and displays some of the variation that occurs in individual latency distributions.

Rise times t_r for voltage bumps in *Limulus* have been reported by Borselino and Fuortes [6]. Our results for t_r -distributions of current bumps are shown in Fig. 3b for two different cells. t_r was determined as the time between onset and maximum of the current of a single bump.

As for the rise time $t_{1/2}$ to half the maximum amplitude of the bump, we prefer to discuss the ratio $q = t_{1/2}/t_r$ rather than $t_{1/2}$ itself. The value of q serves us as a dimensionless formparameter: a long initial super-linear rise phase following the onset of the bump will tend to increase q whereas an extended sub-linear phase of slow rise preceding the peak of the bump amplitude will tend to decrease q . Our results of q -distributions for two different cells are shown in Fig. 3c.

We have observed, by eye, that the descent of almost every bump (if not too small and noisy) can be approximated by an exponential $e^{-\lambda t}$ for times t sufficiently larger than t_r . This observation has been reported already by Wong [13] who found λ -values of about 70 1/s.

Our program determined λ -values by a simple numerical routine: starting from the time where the bump has decayed to about half the maximum current amplitude, the time integral (=area) of the sample of the following 20 bump points (one recorded point per ms) is compared with the integral of an exponential function with the same starting point, and a temporary λ -value is assigned to the sample. The same procedure is then applied to other samples of 20 bump points each, taken from the further decay phase of the bump, and each time a temporary λ -value is determined. Bumps with small variations of their temporary λ -values (less than 10 percent deviation) are considered “stable”

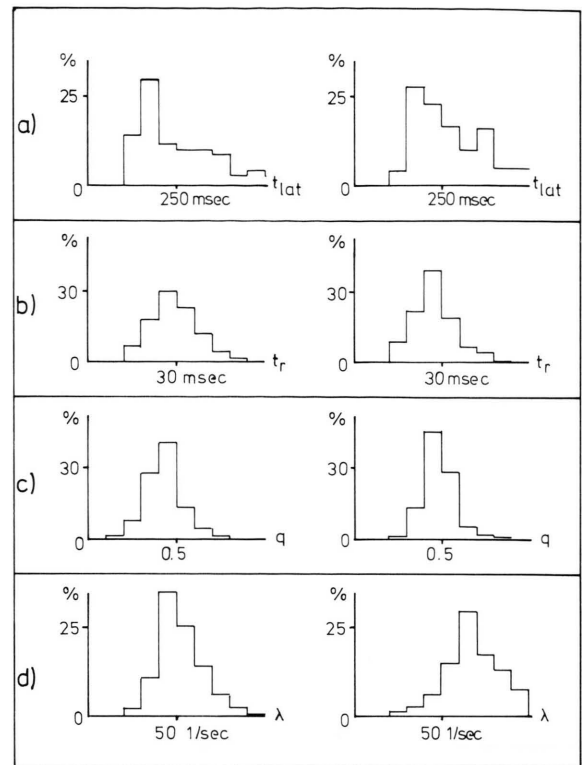


Fig. 3a–d. Distributions of time-like parameters for different cells (left and right column): a. latency time t_{lat} in two flash experiments with 122 and 59 bumps; b. rise time t_r , c. dimensionless form factor $q = \frac{t_{1/2}}{t_r}$, $t_{1/2}$ = rise time to half the maximum amplitude, d. decay rate λ . Fig. 3b–d, and Fig. 4 and 5, represent two cells under continuous illumination. Number of bumps: 576 (left column) and 414 (right column). Exp. K160, K163.

and their best λ -value is considered a reliable representative of the whole decay phase with an uncertainty of less than 10 percent. Other bumps could still be assigned a best λ -value, but less reliably and more error-prone due to fluctuations in their time course, and were earmarked as such. The program also investigates the bump curve between maximum and time of half-maximum decay in order to find out how far upwards the exponential fit is valid. The results confirm that the bump's descent is well approximated by an exponential function from about 15–30 ms after the bump's peak.

This method proved to be more appropriate and more reliable than various regression methods

(linear or logarithmic) investigated. Our results for λ -distributions of current bumps of two different cells are shown in Fig. 3 d.

b. Current parameters

Distributions of amplitudes A and total areas F of bumps of *Limulus* ventral photoreceptors have been published by Stieve and Bruns [1]. Recently, Goldring and Lisman [10] have reported on area distributions of voltage clamp bumps.

Our evaluation program determines the total area F of single bumps by integrating, *i.e.* summing up, the deviation of the current signal from the baseline during the course of the bump. The determination of A is unambiguous. Figs. 4a and 4b shows our results for the distributions of A and F for the same two different cells as for the time-like parameters in Fig. 3b–3d.

The slope m of the linear portion of the rise phase of a bump has been included in our evaluation in order to have a further current parameter which does not involve the total bump course like the total area F . The reason for having such a parameter will be given in our discussion in section 3. The qualitative inspection of the time courses of the majority of bumps indicates the existence of such a linear portion. In fact, since the bumps start as a super-linear function of time and eventually take a maximum value of their amplitudes, one expects a turning point in between and a region around it where the time course can be approximated by a linear function of time. Our evaluation program determines the m -values by the following method. At first, a linear regression subroutine finds a preliminary value of the bump slope m in the vicinity of $t_{1/2}$. The program then stepwise extends this number of sample points both toward the bump maximum and down back to its onset, every time computing the standard deviation of the bump points from a straight line with the preliminary slope m . When the extended interval begins to include the superlinear and sublinear portions of the bump ascent, the standard deviation value increases drastically. The program then determines the final value of the slope m of the merely linear rising phase by a second linear regression. The beginning and the end of the linear portion are also stored. In bumps of reasonable size, the linear part of the bump's ascent usually constitutes no less than

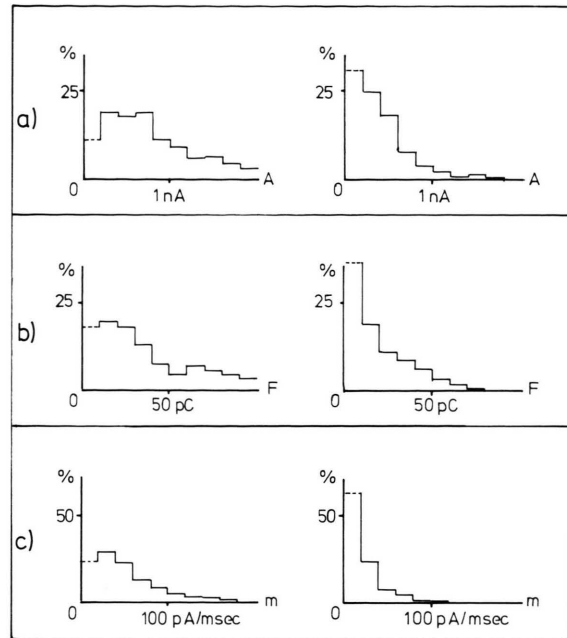


Fig. 4a–c. Distributions of current parameters for the same two cells (left and right column) as in Fig. 3b–d: a. maximum amplitude A , b. total area F , c. slope m of the linear portion of the rising phase.

50% of the total ascent. We have compared the findings of the computer with approximations by eye and found satisfactory agreement. The estimated errors of the computed m -values are a few percent for bumps of not too small a size, slopes below 10 pA/ms being less reliable. The resulting m -distributions for the two different cells are shown in Fig. 4c.

c. Correlation of parameters

Beyond the determination of distributions of bump parameters we have also determined the correlations between them. Table I shows the correlation coefficients. Since the ten cells did not show significant differences of the correlation coefficients, Table I only gives an interval for each of the possible correlation coefficients.

Correlation coefficients including t_{lat} can only be evaluated from flash experiments, whereas those not including t_{lat} were computed both for bumps from flash experiments and under continuous light stimulation.

Table I. Correlation coefficients of the parameters t_{lat} , t_r , λ , m and A (as defined in Fig. 2). The numbers in the first and second lines are the minimum and maximum values of the correlation coefficients, respectively, as obtained from various experiments at 10 different cells. The number of bumps in the experiments vary from 52 to 576.

	t_{lat}	t_r	λ	m	A
t_{lat}	1				
t_r	-0.08 +0.09	1			
λ	-0.26 +0.23	-0.11 -0.28	1		
m	+0.06 +0.12	-0.18 +0.22	-0.10 -0.36	1	
A	+0.13 +0.22	+0.15 +0.41	-0.03 -0.26	+0.83 +0.95	1

3. Discussion

First of all, we would like to emphasize that the results of the earlier investigations of voltage bump parameters as cited above and ours of current bumps under voltage clamp cannot be expected to agree. The time course of voltage bumps will at least be co-determined by the dark conductance of the cell, in particular by voltage-dependent channels (Lisman, Fain, O'Day [14]), and by the membrane capacity. In contrast, current bumps under voltage clamp (clamp voltage = resting potential in the dark) are not influenced by these co-factors but are expected to give a direct signal of the light-evoked processes.

a. Individual cell properties enclosed in the current parameters

The distributions of the time-like parameters t_{lat} , t_r and q as given in Figs. 3 a, b, c do not vary significantly for the two different cells, nor for the other 8 cells which we have investigated so far. The differences are within the range of the numerical statistical fluctuations due to the finite number of bumps evaluated. The λ -distributions of the two cells in Fig. 3d are similar in their shape and spread, but show a difference of their maxima which may be slightly beyond the range of numerical statistical fluctuations. The λ -distributions of other cells lie between or near those of Fig. 3d. We shall come back to this point in subsection 3c.

The difference between the distributions of the current parameters A , F and m for the two different cells as given in Fig. 4, however, unambiguously exceeds the ranges of statistical and experimental errors. Even the qualitative types of these distributions are significantly different for the two cells: one is rather strictly exponential, the other shape contains a plateau-like flat part. Apparently, individual cell properties enter into the parameters a , F and m .

In contrast to the time-like parameters the maximum amplitude A , the total area F and the slope m directly contain the bump current in a linear way. We suspect that the nonuniform behaviour of the current parameters is caused by two separate sources of variations: within each cell, amplitude, area, and slope vary from bump to bump due to the fluctuations in the molecular amplification mechanism. These fluctuations may be expected to be similar in all cells, like those of the time-like parameters t_{lat} , t_r and q . A second, cell-specific factor, however, may well mask the "pure" distributions differently in different cells. The histograms of the experimentally accessible current parameters a , F , and m would then be compound distributions which vary widely from cell to cell, even in type.

In order to examine this hypothesis we have evaluated the distributions of the "scaled" parameters A/m , F/m and F/A . We expected that the influence of the cell-specific factor would cancel if the quotient of two current parameters was formed and that the resulting distributions of the scaled parameters were again very similar in different cells. Note that the units of the scaled parameters are again composed of time units only like that of the time-like parameters. The resulting distributions of the scaled parameters for the two different cells are shown in Fig. 5.

Fig. 5 shows that the distributions of A/m and F/m of the two different cells are indeed very similar to each other like those of the time-like parameters. The differences lie again within the range of the statistical fluctuations due to the finite number of bumps evaluated. Our hypothesis for an individually fluctuating factor enclosed in the current parameters is that it may be due to individual properties of the membrane of the rhabdomeral lobes of different cells. As argued already by Cone [15] and Hillmann [16], a bump is expected to be a

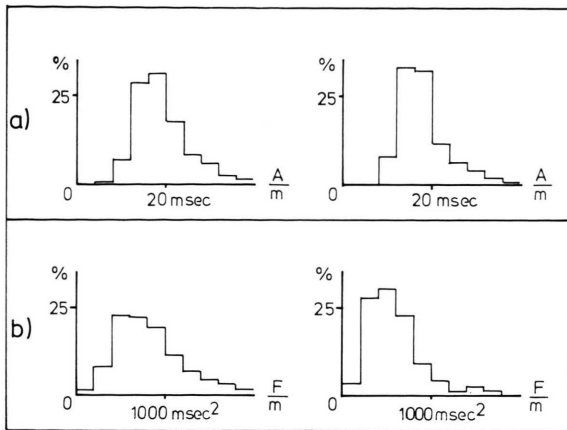


Fig. 5a, b. Distribution of the scaled parameters for the same two cells (left and right column) as in Fig. 3b–d and 4: a. A/m , b. F/m . (A = maximum amplitude, F = total area, m = slope of the linear portion of the rising phase).

local event in the rhabdomeral membrane and includes the opening of a few hundred light channels within a membrane region of a few μm in diameter. The bump current will then be proportional to the product of the density c of available channels and their conductances in that area and may moreover depend on the local physico-chemical and structural properties of the membranes. The investigations of Calman and Chamberlain [17] have shown that the membrane of the rhabdomeral part of the ventral photoreceptor of *Limulus* appears to be rather inhomogeneous including folds and locally fluctuating directions of the microvilli. From studies of pores in artificial membranes (*e.g.* by Lauser *et al.* [18]; Kolb and Bamberg [19]) it is also known that for instance the conductances of pores considerably depend on the structure of the surrounding lipids.

Our result that the distributions of the time-like parameters and that of the scaled parameters A/m and F/m are essentially independent of individual cell properties may be interpreted by assuming that there exists a bump-generating process including transduction which depends much less on local and individual cell and membrane properties. The latter are then reflected by the distributions of the unscaled current parameters.

b. Latency period

The bump latency is not correlated to the other bump parameters, as Table I shows. It is obvious,

therefore, that *e.g.* the bump amplitude A is not determined by the waiting time that expires between rhodospin activation and channel opening. The standard deterministic treatment of linear amplifiers fails to explain these results: a probabilistic, stochastic description of the processes leading to a bump is required.

As mentioned in the introduction, the experimental value 1:2 for the ratio of bump duration ($\approx t_r + 1/\lambda$) and t_{lat} suggests to consider models with no-gain (or low-gain) latency steps preceding the amplification steps in the chain of events. Such models are well compatible with the experimental distributions of t_{lat} and with the actual bump shapes (Tiedge [20], Keiper [7]). This suggestion is confirmed by the absence of any correlation of t_{lat} with the other bump parameters. With this hypothesis, fluctuations among a number of only a few molecules (*e.g.* 1–3) in the latency steps are responsible for large variations of t_{lat} . Similarly, the fluctuations of few molecules in the input stage of the amplification step may lead to large variations in bump amplitudes (see below). In comparison with that, fluctuations among larger numbers of molecules in the amplification and transduction steps including the opening of the light-activated channels cause only relatively small variations and leave the time course of the bump rather smooth.

c. “Primary” parameters: t_{lat} , t_r , m , λ

On the basis of our results for the various correlation coefficients as shown in Table I we propose to treat the rise time t_r , the slope m , and the decay rate λ as “primary” parameters in addition to t_{lat} since t_r , m and λ are also largely mutually uncorrelated. This is obvious for t_r and m (correlation coefficient between -0.18 and $+0.22$) whereas the correlation coefficients of λ and t_r (between -0.11 and -0.28) and of λ and m (between -0.10 and -0.36) may indicate a slight trend towards an anti-correlation. Our models to be described in section 4 indeed produce a very weak anti-correlation of t_r and m with λ even if the bump decay is assumed to be caused by a spontaneous decay or inactivation of the channels or of some other species of molecules within the amplification or transduction steps. The relatively wide spread of the t_r - and m -distribution, however, is expected to have reasons which are independent of λ . Following a transmitter

hypothesis for the transduction between the end of the latency period and the opening of channels (Cone [15], Hillman [16]) we propose the variations of t_r to be caused by fluctuating release or activation durations of the transmitter molecules and of m by fluctuating values of the transmitter release or activation rates per time besides the locally varying channel densities and conductances as mentioned in subsection 3 a.

As regarding the molecular nature of the decay rate λ , there are at least two possible interpretations:

1. λ is the rate of a spontaneous inactivation of the transmitter.
2. λ is the rate of the spontaneous closure of opened light channels.

Bacigalupo and Lisman [21] have reported on life times of light-activated channels in the rhabdomeral membrane of the ventral photoreceptor of *Limulus* measured by patch clamp recordings of single channels. They found mean open times in the region between 1 ms and 4 ms. The mean life time of an open channel is the reciprocal of its inactivation rate. On assumption 2, the experimental λ -values would correspond to mean channel life times in the region of 20 ms. Bacigalupo and Lisman concluded that λ is therefore determined by the transmitter rather than by the channels. This conclusion, however, is not rigorous since the membrane had to be treated rather rudely in order to obtain patch clamps of single light channels and hence the observed channels may have been largely altered. Moreover, the mean open times of less than 4 ms have been observed under a strong steady illumination of about 3.5×10^{16} photons/m². It has been reported recently (Dirnberger *et al.* [22]) that stimulation of such intensities causes light adaptation effects, in particular a pronounced increase of the λ -values such that $1/\lambda$ may take values of a few ms. This means that the above mentioned patch clamp experiment leaves the interpretation of λ undecided.

If λ is the rate of the spontaneous closure of opened light channels, *i.e.*, if the second of the above interpretations holds, the λ -values could at least be modified by local membrane properties like the channel conductance σ . Moreover, the λ -values may be influenced by the local concentration of an agent for which Ca^{2+} might possibly be a candidate. Both these two possibilities may be explanations for

the facts that the λ -values vary from bump to bump and that their distributions show slight differences for different cells like that of Fig. 3d and as mentioned already in subsection 3 a.

d. Amplitude A , bump area F , and form parameters

The choice of t_{lat} , t_r , m and λ as primary bump parameters leads us to ask how the maximum amplitude A , the total area F and the form parameter q can be interpreted as secondary or composite parameters. First of all, Table I shows a strong correlation of A with m as to be expected, the correlation coefficient having values between 0.83 and 0.95. Since in our interpretation the maximum amplitude A is expected to be determined in some way by the product $m \cdot t_r$ which has the same physical unit as A , we would also expect then a likewise strong correlation of A with t_r , however, the corresponding correlation coefficient in Table I is only between 0.15 and 0.41. This insignificant value is caused by the large fluctuations of m which mask the a - t_r -correlation: we have evaluated the correlation of the scaled parameter A/m with t_r and obtained values between 0.83 and 0.92 for its correlation coefficient. With other words, $A/(m \cdot t_r)$ can be treated as a dimensionless form parameter like $q = t_{1/2}/t_r$. Its distribution (not shown here) turns out to be relatively narrow like that of q and has a mean value $\langle A/(m \cdot t_r) \rangle \approx 0.70$.

A similar procedure can be applied to the total area F . An appropriate dimensionless form parameter is $F/(A \cdot t_r)$ which again has a relatively narrow distribution (not shown here) with a mean value of $\langle F/(A \cdot t_r) \rangle \approx 1.20$.

In this context, we come back to the question why we have chosen m as the primary current parameter instead of A or F . Firstly, A and F turn out to be largely determined by the form parameters $A/(m \cdot t_r)$ and $F/(A \cdot t_r)$ *i.e.*, they are correlated to other parameters. Secondly, m has the nature of a rate or a “velocity” since it is a quantity per time. The value of m is therefore expected to be more closely related to the underlying mechanisms than amplitudes or areas which have the nature of integrated quantities.

4. A New Model Description

The site of primary excitation in the *Limulus* photoreceptor is a rhodopsin molecule hit by a

photon. Rhodopsin is located mainly in the microvilli of the cell's rhabdomeral lobe, which constitutes its light-sensitive part (Calman and Chamberlain [17]; Stern *et al.* [23]). Visual excitation starts in a microvillus.

From receptor current saturation experiments, Brown and Coles [5] estimated the size of the membrane region which changes its conductance during a bump event to be about $10 (\mu\text{m})^2$. With a value of $0.1 \mu\text{m}$ for the diameter of a single microvillus (Calman and Chamberlain [17]) this means that the primary excitation somehow propagates from one microvillus to the light-activated channels in an area encompassing more than 1000 microvilli. Lateral diffusion in the membrane is far too slow to be considered for this propagation (Hillman [16]). Instead of, an internal transmitter (Cone [15]) is the most reasonable assumption.

A conservative estimate of about 18 pS for the conductance and 18.7 ms for the average open time of a single channel (*e.g.* Wong [14]) puts the number of channel openings in a medium size dark-adapted bump to more than 1000. If Bacigalupo's and Lisman's [21] single channel values of 35 pS for the conductance and 4 ms for the mean open time in sonicated light-adapted membranes are translated to bumps, the number of channel openings is closer to 10,000, all triggered lastly by a single photon.

On the basis of these considerations and taking into account our findings from the experimental data presented in section 2 and the conclusions drawn from them in section 3, we propose a model for the bump event consisting of the following steps:

1. After successful absorption of a photon by a rhodopsin molecule, a series of time-consuming molecular events is initiated which accounts for the latency period. The absence of correlation of t_{lat} with any other bump parameter, the ratio 1:2 of the bump duration ($\approx t_r + 1/\lambda$) and t_{lat} and the large fluctuations of t_{lat} support the hypothesis that the latency period involves no amplification or at most only very low amplification. A possible candidate for the latency period is a series of conformational changes of one or a few species of molecules. Such a mechanism produces t_{lat} -distributions in satisfying agreement with the experimental ones. It may well be that that the processes involved in the latency period represent an optimal solution of the problem to provide information transfer from a few thousands of rhodopsin molecules located in the membrane area of one microvillus to only one amplifier per microvillus located perhaps in its basal region. The latency time would then appear as a consequence of this kind of an information collecting mechanism.
2. The final latency step is linked to one or to a series of a few (*e.g.* 2 or 3) amplification steps. These amplification steps could well be of the same type as in the model of Fuortes and Hodgkin [8] but their number may be much less than 10 since the latency steps are assumed to be separated from the amplification.
3. One of the amplification steps, most likely the final one, is assumed to be the "transmitter source", *i.e.*, the release or the activation of transmitter molecules. The duration of the source and its rate of release or activation are assumed to be quantities with uncorrelated fluctuations. The duration will mainly determine the value t_r of the rise time of the bump, whereas the rate is a first co-factor of the slope m of the linear portion of its rising phase.
4. The transmitter molecules diffuse along the interior side of the membrane and spontaneously decay or are inactivated after a certain life time. The diffusion coefficient of the transmitter will be a second co-factor of the slope m . It may well be that the cisternae of the palisade as observed by Calman and Chamberlain [17] act as a barrier against the interior of the cell. It is well known that the square of the mean diffusion radius and thus its effective area increases linearly with time, at least within the mean life time of the diffusing molecule. It may well be that this linear increase is reflected by the linear portion of the rising time of the bump.
5. The transmitter molecules are assumed to open the light channels. So far we have considered three particularly simple mechanisms:
 - A) the transmitter opens channels "enzymatically", *i.e.* the time the transmitter is bound is assumed to be much shorter than the mean open time of the channels, and the transmitter may open further channels afterwards; the opened channels close at random with a rate constant which is the inverse of their mean open time;

- B1) the transmitter opens channels by being bound, the bound complex represents an open channel and decays with a rate constant as in A; the transmitter is assumed to be inactive afterwards;
- B2) as B1, but the transmitter is assumed not to be inactive after the decay of the complex.

We have calculated the time constants of the decay phase of bumps on the basis of the above described channel mechanisms A, B1, B2. For each version, we obtain a pair of constants involving the decay constants of the transmitter and of the channels and in some cases also the rate constants of transmitter binding by the channels. Only for the version A, the two calculated constants coincide with the decay constants of the transmitter and of the channels. Among the pair of constants, that one with the lower value will control the decay phase of the bump at large times. It may thus be that the observed decay constant λ of bumps cannot simply be identified either with that of the transmitter or with that of the channels.

We have evaluated our model by applying different mathematical methods, namely (a) a stochastic simulation and (b) a combined analytical and numerical calculation. In particular, we have investigated the dependence of bump parameters on

the model parameters. The results of this investigation will be presented in a theoretical paper elsewhere.

5. Conclusion

A new reaction-diffusion model for visual excitation in *Limulus* ventral photoreceptors is presented. It separates the latency steps from the amplification process and proposes an internal transmitter which spreads along the membrane by diffusion. The model explains the experimentally observed bump shapes as well as the distributions and correlations of the primary bump parameters, latency t_{lat} , rise time t_r , slope m of the linear ascent, and decay rate λ . The variability of bumps in each cell, and in different cells, is explained in terms of fluctuations both in the chemical reactions and in local membrane properties.

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