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Dependence of Bump Rate and Bump Size in *Limulus* Ventral Nerve Photoreceptor on Light Adaptation and Calcium Concentration*

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Abstract. Bumps were recorded in *Limulus* ventral nerve photoreceptor as deflections in membrane voltage during 10 s illuminations by dim light which were repeated every 20 s. The bump amplitude vs frequency distribution and its dependence on the intensity of a preadapting light flash are described. Light adaptation which diminishes the average bump amplitude alters the character of the bump amplitude distribution from a curve with a convex region to a continuously falling concave curve. Weak light adaptation can increase frequency (and height) of the bumps elicited by constant stimuli. Raising the external Ca^{2+}-concentration from 10 to 40 mmol/l augments the effect of a preadapting light flash in diminishing the bump amplitudes and also increases the bump frequency.

The results are consistent with the assumptions
– that light adaptation is based on a Ca^{2+}-dependent reduction of the amplification factor which determines the bump size and
– that the coupling between light induced rhodopsin reactions and bump generation is Ca^{2+}-dependent.

Key words: *Limulus* photoreceptor – Bump – Light adaptation – Extracellular calcium.

Introduction

Bumps which can be observed individually at very low light intensities are elementary excitatory events. In invertebrate photoreceptors they consist in a transient sodium ion specific increase of the membrane conductance of the photoreceptor cell (Millecchia and Mauro 1969), thus causing a transient deflection of the membrane potential in the unclamped cell (Yeandle 1958; Fuortes and Yeandle 1964). A light evoked bump is caused by the absorption of

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a single photon (Fuortes and Yeandle 1964; Fuortes and O'Bryan 1972). It is generally assumed that one photo-stereoisomerisation causes the generation of at most one bump. However, it is not ruled out by experimental data that a single photo-stereoisomerisation may (under certain circumstances) cause more than one bump.

In *Limulus* ventral nerve photoreceptor the light induced conversion of rhodopsin to metarhodopsin (measured spectroscopically or by the early receptor potential) takes place within ca. 5 ms (Fein and Cone 1973; Lisman and Sheline 1976; see also Ostroy (1977). This can be considerably shorter than the latency of a bump, which is 10 up to more than 300 ms. During this long delay unknown reactions take place coupling the rhodopsin reactions with the transient conductance change of the visual cell membrane. The photostereo-isomerisation of one rhodopsin molecule affects many molecules either by an avalanche-type of multiplication or by an amplification. In the dark adapted state a bump probably represents the opening of 10^3-10^4 light activated Na-channels (Cone 1973; Stieve 1977; Wong 1978; Brown and Coles 1979). This amplification factor is diminished by light adaptation.

In the *Limulus* photoreceptor bumps can occur even after a prolonged stay in total darkness (e.g., 10 min). It is generally assumed that these "dark-bumps" originate spontaneously (Yeandle 1958; Adolph 1964; Yeandle and Spiegler 1973), although it is conceivable that they may be caused by photon absorption, being generated with great delay after photo-stereoisomerisation of rhodop-sin.

At higher light intensities the fluctuation in membrane potential or membrane current caused by individual bumps add up and fuse to form the smooth wave shape of the light response. According to the "adapting bump model" of Dodge et al. (1968) light adaptation causes diminution of the size of the bumps without affecting the number of bumps evoked; that is, without affecting the probability of bump generation due to light absorption. This hypothesis has been confirmed by Dodge et al. (1968) and by Wong (1978), using noise analysis. Since however the interpretation of noise analysis data requires certain assumptions we thought it desirable to test this hypothesis additionally directly under conditions where the individual bumps can be observed.

Material and Methods

Bumps were recorded as deflections in the membrane voltage, measured with one intracellular electrode in a ventral nerve photoreceptor cell of *Limulus* in a standard way (Stieve and Bruns 1978).

The ventral nerve was situated in an experimental chamber which was continuously perfused with salines of 15° C which could differ in calcium ion concentration. The cell was illuminated by constant dim 10 s "bump evoking stimuli" which were repeated every 20 s. The light intensity was adjusted to evoke bumps of a frequency of approximately 1 bump per second (Fig. 1). The 10 s bump evoking stimulus was repeated 50 times while the responses were recorded by a pen writer (Helcoscriptor HE 17, bandwidth DC up to 75 Hz, 3 dB).

In the following period a 20 ms "preadapting light flash" of constant weak intensity (2^4 fold-higher than the intensity of the bump evoking stimulus) was applied 2 or 3 s before each bump

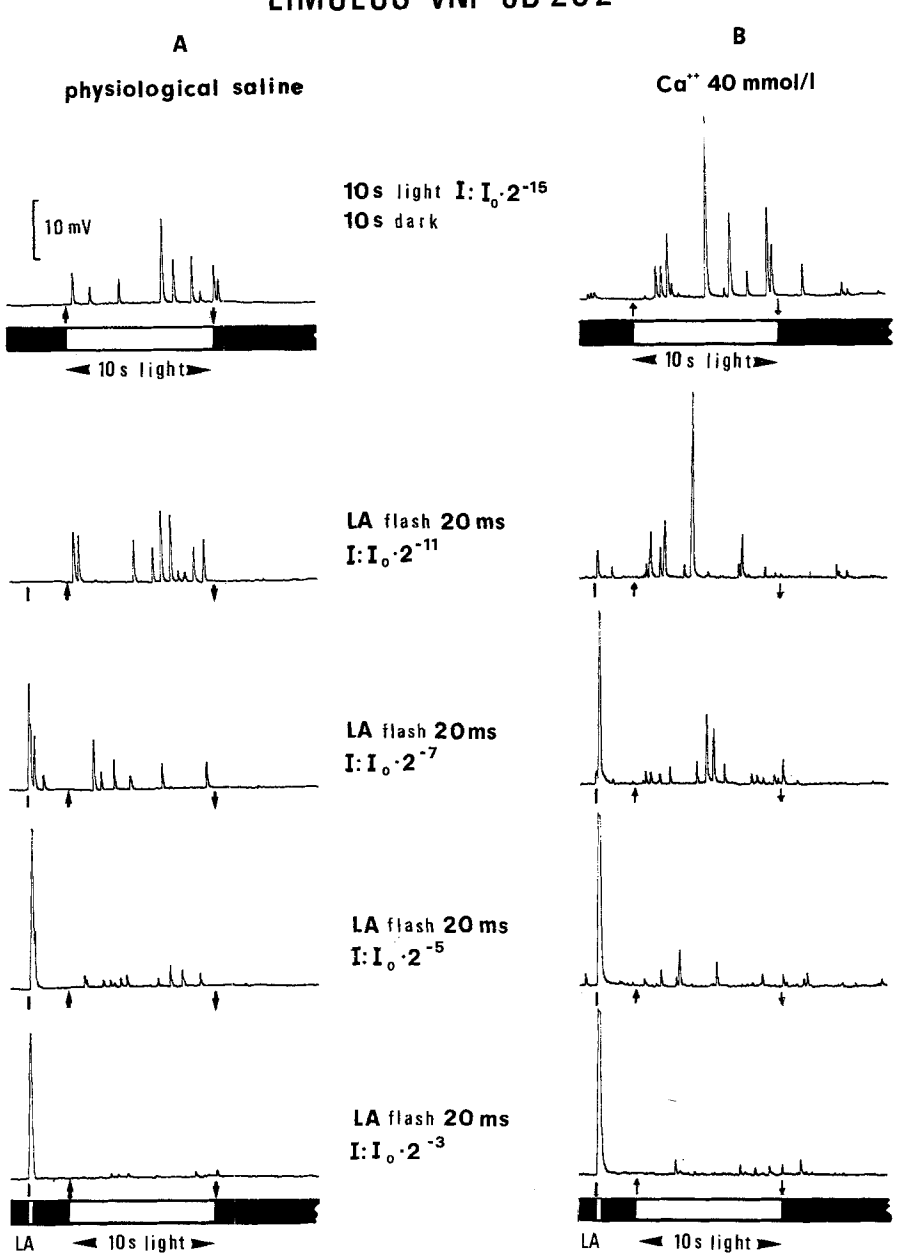
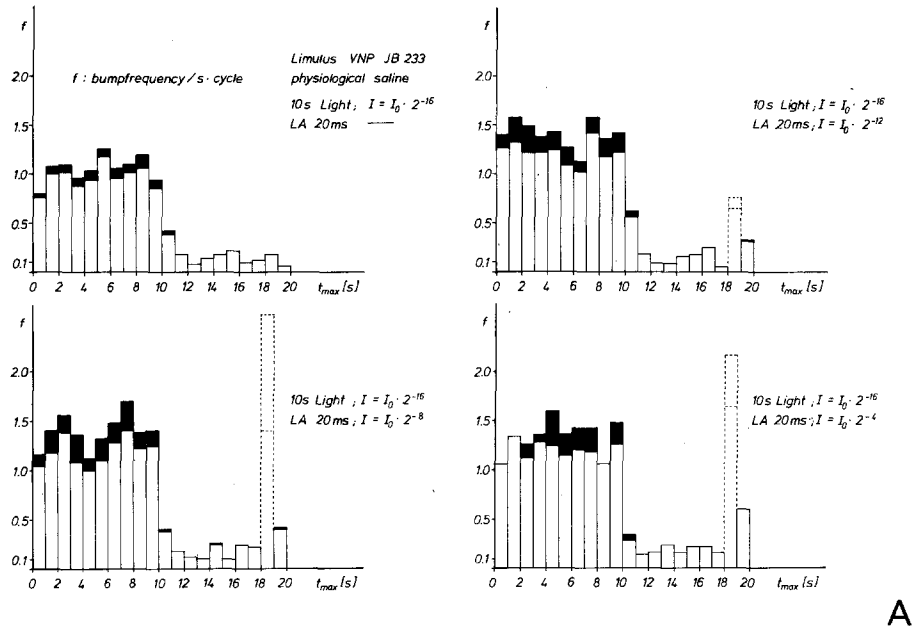


Fig. 1 A and B. Bump registrations at different levels of light adaptation of a *Limulus* ventral nerve photoreceptor (pen recording of membrane voltage). Each record is one of the last registrations of 50, repeated every 20 s with identical stimulation. The 10 s "bump evoking stimulus" is identical in all records whereas the intensity I_p of the "preadapting flash" varies from line to line. **A** The ventral nerve was superfused by physiological saline of 15° C (see Stieve and Bruns 1978) containing 10 mmol/l Ca^{2+}. **B** Superfusion of the same photoreceptor by saline containing 40 mmol/l Ca^{2+}. Upper record: no preadapting flash; lower records: Intensity I_p of the preadapting flashes is indicated in each of the following records. $I_0 = 3 \times 10^{13}$ 550 nm photons $cm^{-2}\,s^{-1}$



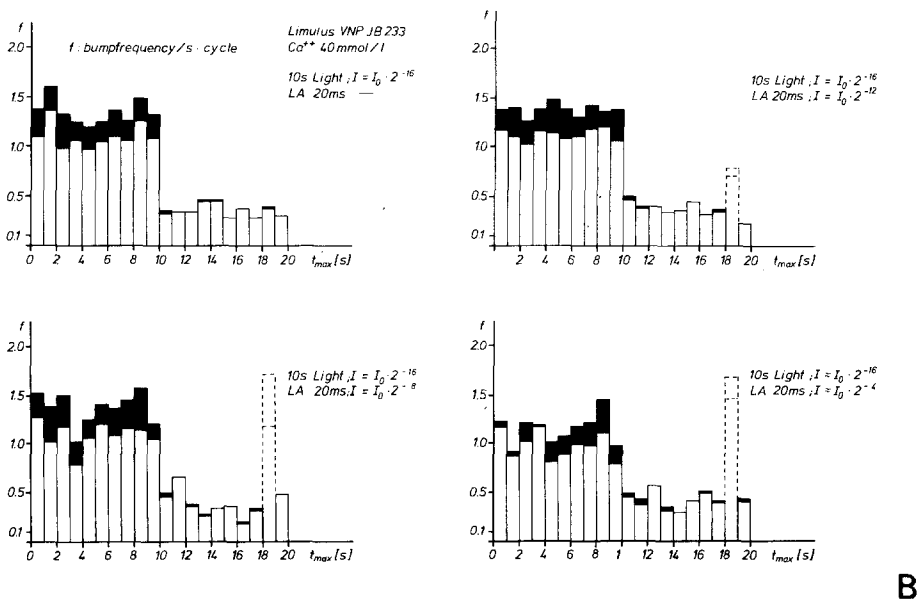


Fig. 2 A and B. Average bump frequency distribution in time during the stimulating cycle at different levels of adaptation. **A** Physiological saline containing 10 mmol/l Ca^{2+}. **B** Ca^{2+}-concentration raised to 40 mmol/l. The height of each column represents the number of bumps observed during an interval of 1 s. Empty sections of columns: single (or first) bumps. Filled sections of columns: "multiple bumps" (riding on first bumps). The broken columns contain the response to the preadapting flash. (Same experiment as Table 1 and Figs. 3 and 4)

evoking light stimulus and this stimulus regime was again repeated 50 times. The responses were recorded during the 50 periods. After that the intensity of the preadapting light flash was raised by a factor of 2^4 (the bump evoking stimulus being unchanged), and the whole procedure was repeated and so forth. The preadapting light flash caused a deflection in membrane potential (bumps or a receptor potential) which had entirely faded away before the 10 s bump evoking stimulus began (that is, the membrane potential had already returned to its original value) (Fig. 1). The lowest intensity used for the preadapting light flash was very low; so the preadapting flashes failed occasionally to evoke even bumps in some of the 50 stimulation cycles of the period with the weakest preadapting flashes (see e.g., Fig. 1A second line).

Thus the bump responses to identical bump evoking stimuli of 10 s duration were recorded at at least four different levels of adaptation while the ventral nerve was superfused by physiological saline containing 10 mmol/l Ca^{2+} (Stieve and Bruns 1978). Following this the superfusion was switched to a saline containing 40 mmol/l Ca^{2+} with all the other ion concentrations unchanged (the osmotic pressure of the physiological saline used as reference saline for these experiments contained an added amount of 90 mmol/l sucrose which was omitted when the calcium concentration was raised to 40 mmol/l by adding $CaCl_2$). In the new saline, after 20–30 min to allow the photoreceptor to become accustomed to the new environment, the whole stimulation procedure was repeated as in physiological saline. Finally the response reversibility was tested by applying a shortened program in physiological saline. Each experiment lasted at least 4–5 h, and here we report five experiments in which this whole program was applied.

For the following considerations we were concerned mainly with the amplitudes and frequencies of the "light"-bumps occuring during the 10 s of the bump evoking illumination (Figs. 1 and 2). The bump amplitudes A were measured by hand with an accuracy of 1 mV, and the bump amplitude vs frequency distribution was plotted (Fig. 3).

The frequency of the "dark" bumps was measured by counting the bumps occuring in the dark in the 13th–17th s of the stimulus cycle (Fig. 2 and Table 1). Among the light bumps there may have been a number of spontaneous bumps, the frequency of which may with certain assumptions be estimated from Table 1 to be ca. 15% of the total number of light bumps in 10 mmol/l Ca^{2+}-conditions. When bump superposition was observed the number of bumps were counted as the number of recognizable maxima, but only the undisturbed amplitudes of the first bumps were measured and plotted in the amplitude vs frequency histogram. Bumps riding on top of a foregoing bump were called "multiple bumps".

Results and Discussion

Figure 1 shows individual recordings from one experiment, Fig. 2 the average bump frequency as observed in the time course of the stimulating cycle. Figure 3 shows the amplitude vs frequency distribution of the light bumps of another experiment.

Figure 3A is the amplitude vs frequency distribution of the bumps recorded without a preadapting flash, that is, the bumps are recorded in a fairly dark adapted state of the photoreceptor cell. The amplitude distribution, which shows similarities to those observed by Srebro and Behbehani (1971) and even more with Bayer and Barlow (1978), suggests a frequency minimum for bump amplitudes around 3 mV separating a maximum around 10 mV from the small bumps. The bump frequency rises from the mimimum with decreasing bump heights until the bumps vanish in the noise (0.5 mV). In 10 of 13 evaluated experiments the bump amplitude histogram of dark adapted cells in physiological saline containing 10 mmol/l Ca^{2+}, has its highest frequencies at the smallest bump heights. The frequency decreases in 11 cells in the first part of the curves from the smallest to greater bump heights. In all 13 cases the curves show

Table 1. Bump frequencies at different levels of light adaptation and at two different external Ca^{2+}-concentrations

Preadapting light flash I_p	Bumps in	Single bumps $n \times s^{-1}$		Multiple bumps $n_M \times s^{-1}$		All bumps $(n + n_M) \times s^{-1}$	
		10 mmol Ca^{2+}/l	40 mmol Ca^{2+}/l	10 mmol Ca^{2+}/l	40 mmol Ca^{2+}/l	10 mmol Ca^{2+}/l	40 mmol Ca^{2+}/l
	Light	0.97 ± 0.04 (n = 484)	1.10 ± 0.03 (550)	0.09 ± 0.01 (43)	0.23** ± 0.02 (116)	1.05 ± 0.04 (527)	1.33 ± 0.03 (666)
	Dark	0.16 ± 0.03 (40)	0.39 ± 0.03 (97)	– (0)	– (0)	0.16 ± 0.03 (40)	0.40 ± 0.03 (99)
$I_0 \times 2^{-12}$	Light	1.22 ± 0.04 (607)	1.12 ± 0.03 (561)	0.19* ± 0.02 (93)	0.26 ± 0.03 (129)	1.41 ± 0.05 (700)	1.39 ± 0.04 (690)
	Dark	0.15 ± 0.02 (37)	0.37 ± 0.03 (92)	– (0)	– (0)	0.15 ± 0.02 (37)	0.37 ± 0.03 (92)
$I_0 \times 2^{-8}$	Light	1.19 ± 0.04 (596)	1.10 ± 0.03 (548)	0.20 ± 0.02 (98)	0.26 ± 0.02 (133)	1.39 ± 0.04 (694)	1.36 ± 0.03 (681)
	Dark	0.15 ± 0.03 (38)	0.33 ± 0.03 (82)	0.004 ± 0.004 (1)	0.012 ± 0.006 (3)	0.16 ± 0.03 (39)	0.34 ± 0.03 (85)
$I_0 \times 2^{-4}$	Light	1.13 ± 0.03 (563)	0.97 ± 0.03 (483)	0.21 ± 0.02 (105)	0.16 ± 0.03 (82)	1.34 ± 0.04 (668)	1.13 ± 0.04 (565)
	Dark	0.20 ± 0.02 (50)	0.44 ± 0.04 (109)	– (0)	– (2)	0.20 ± 0.02 (50)	0.44 ± 0.04 (111)

Each bump frequency listed as mean ± SE per 20 s cycle recorded in 50 repititions. Each cycle consists of a 10 s bump evoking stimulus of constant light intensity ($I_e = I_0 \times 2^{-16}$) followed by a 10 s dark period. The preadapting light flash, if applied, is administered each time 2 s prior to the bump evoking illumination (i.e., at the start of the 19th second)

I_p: Intensity of preadapting flash

The corresponding number of bumps is listed in parentheses

Light bumps: bumps recorded during the 10 s of the bump evoking illumination

Dark bumps: bumps recorded in the dark during the 13th–17th s of the cycle (i.e., over 5 s)

n: Total number of recognized single (or first) bumps

n_M: Number of recognized multiple bumps riding on top of others (expected number for a Poisson process: * 0.16 s^{-1}; ** 0.14 s^{-1})

Experiment JB 233

a maximum or at least a shoulder at medium bump amplitudes. In two cells a more pronounced minimum was observed as compared to Fig. 3A. In two experiments a histogram with only one broad maximum and no minimum was observed similar to that described by Lillywhite and Laughlin (1979). Whether this lack of small bumps is due to a significant difference between different recordings, or to the fact that in the latter cases the higher frequencies of bumps with small amplitudes are hidden in the noise, cannot be decided at present.

The average bump amplitude

$$\bar{A} = \frac{\Sigma A}{n} \text{ mV}$$

can be determined by summation of bump amplitudes A over all classes of the amplitude distribution of single (or first) bumps as those in Fig. 3, and division by n, the number of single (or first) bumps (Tables 2 and 3).

Light adaptation changes the amplitude vs frequency distribution in two respects:

1) On the average the bumps become smaller (Fig. 3, Table 2). Weak light adaptation however can cause an increase in average bump amplitude $\bar{A}$. This is not shown in the experiment from which Tables 1 and 2 and Fig. 3 are taken, but it can be seen in Table 3 which shows the average of all five experiments.

2) The shape of the amplitude vs frequency distribution changes: Minimum and maximum or shoulder disappear and the distribution in all 13 cells mentioned above becomes strongly monotonic decreasing and concave.

Since we recorded the amplitudes of the bumps as displacement of membrane voltage, the diminution in bump height caused by light adaptation may appear different than under voltage clamp

Table 2. Average bump amplitude $\bar{A}$ and sensitivity E at different levels of light adaptation and at two different external Ca^{2+}-concentrations of experiment JB 233

I_p	$\bar{A}$ mV		E mV × s^{-1}	
	10 mmol Ca^{2+}/l	40 mmol Ca^{2+}/l	10 mmol Ca^{2+}/l	40 mmol Ca^{2+}/l
–	7.9	9.8	8.3	13.1
$I_0 \times 2^{-12}$	7.5	8.9	10.5	12.3
$I_0 \times 2^{-8}$	6.8	6.7	9.5	9.1
$I_0 \times 2^{-4}$	2.5	1.6	3.4	1.8

Each $\bar{A} = \frac{\Sigma A}{n}$ mV value listed is determined by summation of bump amplitudes over all classes of the distributions of Figs. 3 and 4, and division by n, the number of single (or first) bumps

Each $E = \bar{A} \frac{n + n_M}{50 \cdot 10}$ mV s^{-1} value listed is the average bump amplitude multiplied by the number of all "light" bumps observed during one second of the bump evoking illumination. Line 1, 2, 3, and 4 different levels of adaptation

I_p: Intensity of preadapting flash

$I_e = I_0 \times 2^{-16}$: Intensity of bump evoking illumination

Rest as in Fig. 2 and Table 1

Table 3. Bump frequency $n + n_M$, average bump amplitude $\bar{A}$ and sensitivity E at different levels of light adaptation and at two different external Ca^{2+}-concentrations. Averages of normalized values of five experiments (as in Tables 1 and 2)

I_p	All bumps $(n + n_M)$		$\bar{A}$ mV		E mV × s^{-1}	
	10 mmol Ca^{2+}/l	40 mmol Ca^{2+}/l	10 mmol Ca^{2+}/l	40 mmol Ca^{2+}/l	10 mmol Ca^{2+}/l	40 mmol Ca^{2+}/l
–	**0.68 ± 0.11 s^{-1}**	164 ± 19%	**4.18 ± 0.94 mV**	124 ± 23%	**3.21 ± 1.30 mV s^{-1}**	193 ± 28%
$I_e \times 2^4$	117 ± 13%	168 ± 22%	137 ± 23%	130 ± 20%	157 ± 26%	205 ± 21%
$I_e \times 2^8$	135 ± 18%	162 ± 15%	134 ± 32%	113 ± 20%	177 ± 38%	176 ± 23%
$I_e \times 2^{12}$	135 ± 21%	134 ± 17%	70 ± 18%	48 ± 11%	88 ± 18%	64 ± 14%

Mean ± standard error of mean
I_e: Intensity of bump evoking illumination
I_p: Intensity of preadapting flash
Fatt: average of reference values
Experiments JB 232, 2333, 236, 237, 250

conditions due to the voltage dependence of membrane conductances. The prestimulus membrane potential (measured in the last second before the bump evoking stimulus) is not significantly changed by the light adaptation applied in the experiments described.

In a number of experiments a significant change in the average bump frequency is also observed (Fig. 2, Tables 1–3, and Fig. 3). In our example light adaptation by very weak flash intensities increases the total number of bumps evoked during identical bump evoking 10 s stimuli by 20–40% (Tables 1 and 2). Stronger light adaptation in the adaptation range applied in our experiments does not cause a further significant change in bump frequency. (A slight reduction in bump number at higher intensities of the preadapting light flash may be due to the fact that the smaller bumps are lost in the noise). The increase in bump frequency caused by moderate light adaptation is more pronounced in some experiments and less pronounced in others than in the example shown in Fig. 3 and Tables 1 and 2; Table 3 shows the average value of the five experiments.

Srebro and Behbehani (1972) report a decrease of bump probability in *Limulus* photoreceptor due to light adaptation and in the mutant trp of *Drosophila;* Minke et al. (1975) observed a light adaptation induced strong reduction in quantum efficiency for bump generation. An increase of dark bump frequency caused by preillumination was observed by Adolph (1964) in *Limulus* lateral eye, Tsukahara and Horridge (1977) in locust eye and by Fein and Hanani (1978) in *Limulus* ventral nerve photoreceptor. Minke et al. (1975) interprete an elevated noise level, which follows illumination in the trp mutant of *Drosophila,* as a light induced increase in dark bump frequency.

The observed increase in light bump frequency is probably not caused by an increase in spontaneous bump activity: The number of dark bumps recorded in our experiments during the 13th–17th s of the cycle does not appear to be significantly increased by light adaptation (Fig. 2 and Table 1). If the same 20 ms "preadapting light flashes" used in our experiments are administered without the 10 s "bump evoking illumination" they do not cause a significant increase in the number of dark bumps occuring in the time span otherwise occupied by the bump evoking illumination.

Adaptation is defined as change in sensitivity. From our results we can derive a measure of sensitivity

$$E = \bar{A}\,\frac{n + n_M}{50 \cdot 10}\ \mathrm{mV\,s^{-1}}$$

i.e. The average bump amplitude $\bar{A}$ multiplied by the number of all bumps $(n + n_M)$ observed during one second of the bump evoking illumination, where n_M is the number of multiple bumps (riding on top of others), 50 the number of stimulation cycles, 10 s the duration of the bump evoking illumination. For example shows Table 2 for one experiment (the same experiment of which Table 1 and Figs. 2, 3, and 4 are taken), that a weak preadapting light flash causes an increase in sensitivity E from 8.3 mV s^{-1} to 10.5 mV s^{-1} (in the same experiment the average bump amplitude $\bar{A}$ does not increase concomitantly). A stronger preadapting light flash causes a decrease in E to 3.4 mV s^{-1}.

Table 3 lists the averaged normalized values of the sensitivity E for all five experiments. It shows generally the same results: low intensity of the preadapting light flash causes a significant increase, higher flash intensities a significant decrease in sensitivity E. The increase in sensitivity E observed with weak light adaptation is larger than the increase in average bump amplitude $\bar{A}$ (Table 3). This shows that weak light adaptation can cause an increase in sensitivity both by enlarging the average bump size and by raising the bump frequency.

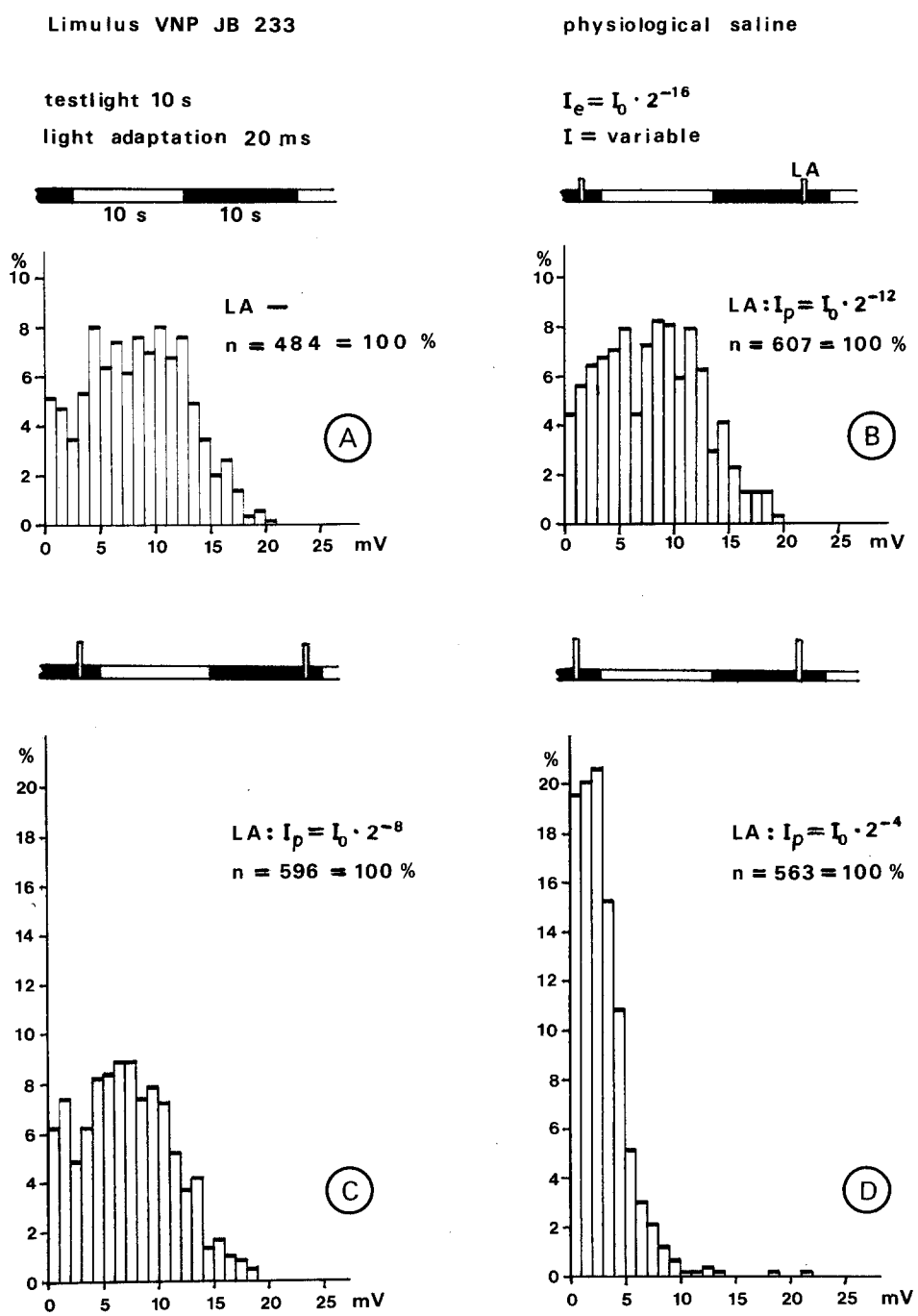


Fig. 3 A–D. Frequency distribution of the amplitudes of the bumps recorded during illumination by the 10 s bump evoking stimuli ("light-bumps") at different levels of adaptation of a *Limulus* ventral nerve photoreceptor superfused by physiological (10 mmol/l) Ca^{2+}-concentration of 15° C. Only the amplitudes of single (or first) bumps are plotted. The number of "multiple bumps" (riding on top of others) were ca. 15% (Table 1)

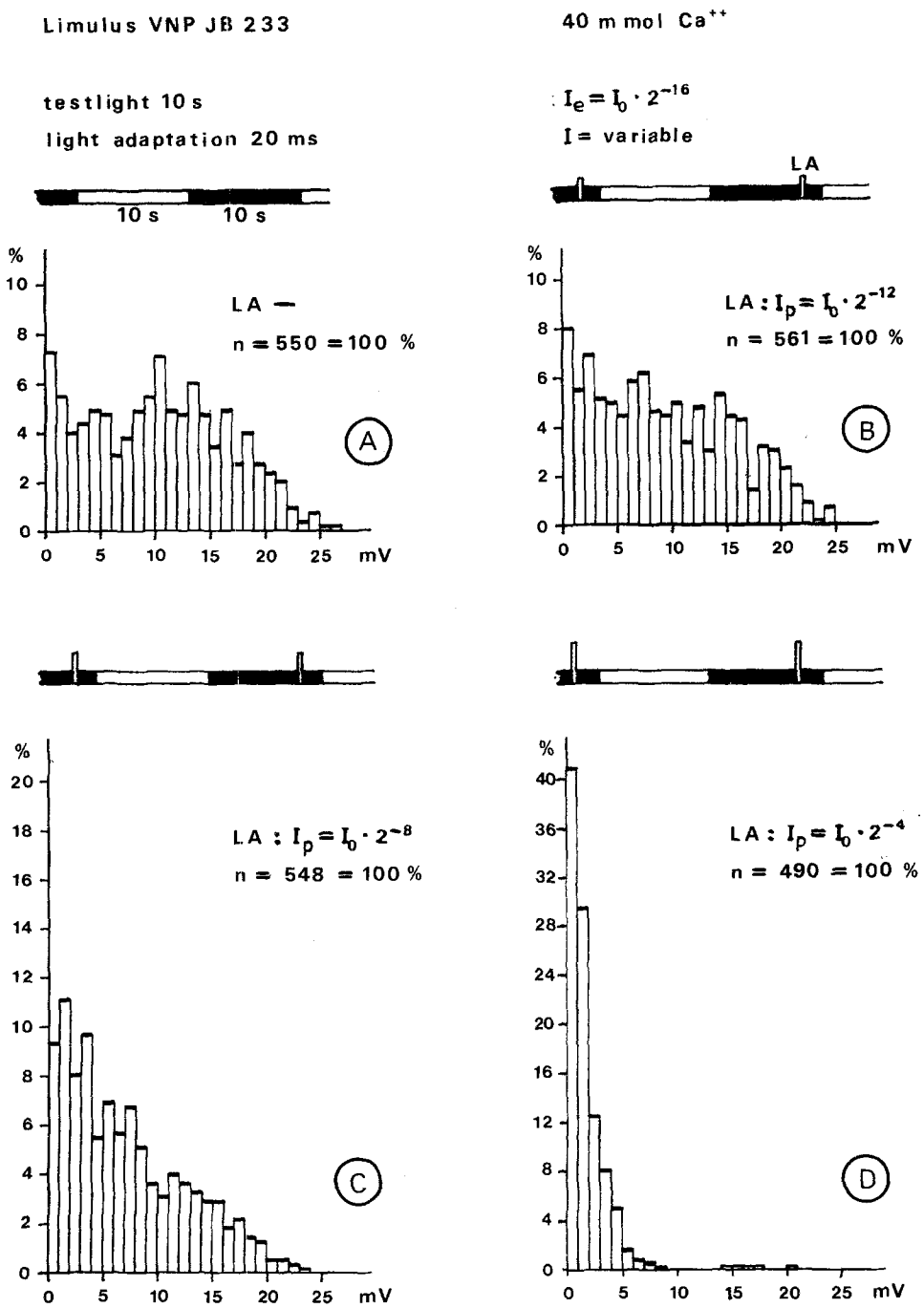


Fig. 4 A–D. Frequency distribution of "light bump" amplitudes at different levels of adaptation of the same photoreceptor as Figs. 2 and 3 superfused by raised (40 mmol/l) Ca^{2+}-concentration. Multiple bumps 15–20% (Table 1). Rest as in Fig. 3

This increase in sensitivity E caused by moderate light adaptation may be identical with a phenomenon which was called facilitation by Hanani and Hillman (1976) who first described it for the barnacle photoreceptor. A similar feature in *Limulus* ventral nerve photoreceptor was reported as enhancement by Fein and Charlton (1977) and as causing the supralinearity of the response height vs stimulus intensity curve by Stieve and Pflaum (1978).

Illumination by preadaption light flashes thus has two major effects:
– Diminution of the bump amplitudes to a degree which depends on the intensity of the preadapting light flash (weak pradapting light flashes can cause an increase in average bump amplitude).
– Increase in number of bumps elicited by identical bump evoking stimuli.

The extent of light adaptation is influenced by the extracellular Ca^{2+}-concentration. In experiments not described here we have measured the sensitivity shift caused by light adaptation in *Limulus* ventral nerve photoreceptors bathed in different extracellular Ca^{2+}-concentrations. The sensitivity was measured as the stimulus intensity I_{50}, necessary to evoke half saturation of the amplitude of the receptor potential. Lowering the external Ca^{2+}-concentration to ca. 40 µmol/l reduces (Stieve and Pflaum 1978) whereas raising external Ca^{2+}-concentration to 40 or 100 mmol/l increases (Stieve and Bruns 1980) the sensitivity shift due to the same light adapting illumination without much affecting the sensitivity of the dark adapted photoreceptor.

Here we studied the influence of raised external Ca^{2+}-concentration on the changes in the bump amplitude vs frequency distribution caused by light adaptation. Figure 4 shows the amplitude vs frequency distribution of the same experiment as Figs. 2 and 3, after the Ca^{2+}-concentration was raised to 40 mmol/l. The histogram of the light bumps recorded without a preadapting light flash (Fig. 4A) differs from the corresponding in 10 mmol/l Ca^{2+}-concentration (Fig. 3A) insofar as greater bumps, up to 25 mV, are recorded in 40 mmol/l Ca^{2+}-concentration (see also Table 2). This is also seen in the other experiments (Table 3).

Light adaptation in raised external Ca^{2+}-concentration causes a stronger diminution of average bump amplitudes $\bar{A}$ and of sensitivity E by the same preadapting light intensity (Tables 2 und 3). The changes in shape of the bump amplitude vs frequency distribution caused by light adaptation are generally similar to those in normal Ca^{2+}-concentration but occur at weaker intensities of the preadapting flash (Figs. 3 and 4). Raising external Ca^{2+}-concentration can also cause an increase in the number of bumps occuring during the bump evoking 10 s stimulus without a preadapting light flash (in our example by ca. 30%, Fig. 2, Tables 1 and 2, and Fig. 3a). Under these conditions no significant increase in bump frequency and no raise in sensitivity E is seen in moderate light adaptation (Fig. 2, Tables 2 and 3) in contrast to measurements in physiological Ca^{2+}-concentration.

Raising external Ca^{2+}-concentration to 40 mmol/l doubles the dark bump frequency (Fig. 2, Table 1). However the increase in light bump frequency in raised external Ca^{2+}-concentration is somewhat greater than can be accounted for by an increase in spontaneous bump rate alone. The prestimulus membrane potential is not significantly changed by raising the Ca^{2+}-concentration to 40 mmol/l or by the applied light adaptations.

Increase of external Ca^{2+}-concentration thus has the following effects:
– Augmentation of the light adapting action of the preadapting flash in desensitizing the photoreceptor.
– Increase in the frequency of the light bumps elicited by the same bump evoking stimulus.

– Increase in the frequency of the dark bumps.
– Increase in average bump size.

The first effect probably occurs via an intracellular action of Ca-ions in accordance with the hypothesis of Lisman and Brown (1972, 1975). It seems most likely that intracellular Ca^{2+} regulates the bump size by controlling the amplification factor which determines the extent of the conductance increase (corresponding to the number of transiently opened light channels) following the photo-stereoisomerisation of a single rhodopsin molecule. The higher the intracellular Ca^{2+}-concentration, the smaller the amplification factor, i.e., the smaller the light induced conductance increase or the number of light activated channels per bump.

The second effect may be explained by assuming that calcium ions (probably also intracellularly) are necessary for bump generation. I.e., the probability that a photo-stereoisomerisation leads to the generation of a bump depends on the concentration of intracellular Ca^{2+}. If the Ca^{2}-concentration is very low, the bump probability is relatively low. Illumination causes an increase in intracellular Ca^{2+}-concentration when the photoreceptor is bathed in physiological saline (Brown and Blinks 1974; Brown et al. 1977; Maaz and Stieve 1980); so, under these conditions, light adaptation can cause an increase in bump probability and thereby cause facilitation. We therefore assume that calcium acts among others on the coupling mechanism which couples rhodopsin reactions with the opening of the light activated ion channels (as was suggested by Kramer and Eckert 1979; Eckert 1979). Our data also indicate that the coupling factor connecting photo-stereoisomerisation with bump generation may vary under physiological conditions. Hanani and Hillman (1976) suggested that a decrease in the intracellular Ca^{2+}-concentration might be responsible for facilitation. The raise in sensitivity based on increase in bump frequency observed in our experiments however may be caused by an increase in intracellular Ca^{2+}-concentration. We do not have a simple plausible explanation for the observed enlargegement in bump size due to facilitation.

In Table 1 can be seen that light adaptation or raising extracellular Ca^{2+}-concentration causes a greater increase in multiple bumps than in single bumps. This observed increase in multiple bumps caused by light adaptation is somewhat greater (0.19 : 0.16), the observed increase caused by raising external calcium remarkably greater (0.23 : 0.14), than expected for a Poisson process (uncorrelated events). This difference could be an indication that bump occurance is not strictly independent but rather that the bump probability is slightly raised in the time span following a bump. The calcium dependence of the coupling factor together with a bump accompanying increase in intracellular calcium could provide an explanation for such a phenomenon.

The observed increase in dark bump frequency in raised external Ca^{2+}-concentration as well as our observation that the dark bump frequency is diminished to about half its value when the external Ca^{2+}-concentration is lowered to 1 nmol/l (Stieve et al. 1978) indicate the need for calcium in bump generation. By exerting its effect on the dark bump frequency calcium may perhaps act on the same coupling process which is responsible for the described facilitation.

The observed increase in bump size in raised external Ca^{2+}-concentration may be due to a calcium influx through voltage dependent calcium channels as observed by Fain and Lisman (personal communication).

Concluding Remarks

Our results are in general agreement with the adapting bump model. Two results of our experiments observing individual bumps were unexpected.

1) Weak light adaptation causes an increase in quantum efficiency for light induced bump generation.

2) The voltage bump – amplitude distribution changes its shape due to light adaptation (within the small range tested) in a characteristic, non trivial manner. Similar bump amplitude distributions with similar changes due to light adaptation as observed in our experiments, have been predicted by a model of Eckert and Kramer (1978). For the calculation of bump amplitude, – duration and – rate during steady illumination, using noise analysis, one usually assumes that the shape of the bump amplitude vs frequency distribution is changed in light adaptation only by a diminution of the scaling factor of the amplitude axis. The adaptational range used in our experiments is relatively small. We cannot state whether our results can be extrapolated to higher light adaptation nor how different results will be obtained by similar future measurements performed under voltage clamp conditions.

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