The Intensity Dependence of the Receptor Potential of the *Limulus* Ventral Nerve Photoreceptor in Two Defined States of Light- and Dark Adaptation

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Receptor potentials of *Limulus* ventral nerve photoreceptors were evoked in reproducible states of moderate light- and considerable dark adaptation (LA, DA) with light stimulus intensities from threshold to saturation values using a repeated flash sequence.

With increasing light intensities latent period and time-to-peak shortened in the state of both LA and DA. The decrease-time is prolonged by increasing stimulus intensities (LA and DA) and continues to rise even when the amplitude of the receptor potential is already saturated.

The sigmoid response height *vs.* log stimulus intensity curve is shifted upon LA towards higher stimulus intensities, and its steepness is increased. LA prolongs latent period and time-to-peak at low light intensities; at high light intensities, however, the DA-values are longer. The decrease-time of the receptor potential is always shorter in the light-adapted state.

The results are discussed according to the assumption that size and duration of the receptor potential are primarily determined by the distribution of bump latencies. Changes of the receptor potential parameters are explained by changes of bump size and of bump latency distribution due to light adaptation.

Introduction

Receptor cells are able to code different magnitudes of stimuli. As an essential function they can change their sensitivity. This adaptation process is time-dependent. According to Lisman and Brown [1] light adaptation in *Limulus* photoreceptor cells starts about 200 ms after the onset of the light-adapting illumination and is almost completed after 1 s. Dark adaptation of photoreceptors (sensitivity recovery) takes much more time (*e.g.* up to 60 min in *Astacus*), but is relatively fast in *Limulus* (ca. 90% completed in 2 min).

Owing to this fast sensitivity change the *Limulus* photoreceptor is well suited for investigations of the process of adaptation to light and darkness. Adaptation is usually characterized in one of three ways: either by constant stimulus curves (time dependence of response increase and changes of response shape), or by constant response amplitude curves (time dependence of the sensitivity increase, [2], [3]), or by measuring response height versus stimulus intensity curves at different states of adaptation. We adopted this third method in our experiments described here. Using a repeated stimulus sequence consisting of a light-adapting illumination followed by two test flashes of varied intensity at fixed intervals in the dark we recorded two entire response *versus* stimulus intensity curves at two states of adaptation (without background light), the first moderately light-adapted, and the second fairly dark-adapted. We measured the height and the time parameters of the response depending on a) the stimulus intensity and b) the state of adaptation. The experiments should demonstrate adaptation under conditions close to normal and were carried out with physiological saline as bathing solution of the preparation. In a further paper we intend to describe how the intensity *versus* response characteristics of the *Limulus* photoreceptor in the state of light- and dark adaptation is influenced by different calcium- and sodium concentrations of the external saline.

Methods

Limulus ventral photoreceptors were superfused with physiological saline (composition: Na^+ 486, K^+ 10, Ca^{2+} 10, Mg^{2+} 55, Cl^- 561, SO_4^{2-} 30, HEPES

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10 mmol/l). The flow rate of the saline was about 1 ml/min. The membrane potential and its light-evoked change (receptor potential, ReP) were measured intracellularly by a microelectrode filled with 0.5 mol/l KCl solution against an indifferent silver/silver chloride electrode in the bathing saline.

A Varian Xenon lamp, VIX 150 F, white light of maximal intensity, equivalent to ca. 1.5×10^{17} (550 nm) photons $\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$, was used for stimulation; the light intensity was varied by neutral density filters (Schott). The experiments were carried out at 15 °C. The results were parallely recorded by a slow paper writer (Hellige, speed 1 cm/min), photographed, stored on tape, and evaluated by computer. (For details of the methods see [4]; details of the evaluation are described in [5]).

In each measurement the following parameters* of the receptor potential (ReP) were determined by computer: the peak amplitude of the transient (HMAX), the latent-period (TLAT), the time-to-peak (TMAX), and the decrease-time T2. We also determined the decline-quotient QHN (=HN/HMAX; HN measured 500 ms after the maximum of the transient) as a useful measure when the decline of a response is so slow that the decrease-time T2 cannot be recorded within our 5 s recording periods.

Schematic representations of the parameters are shown by the figure insets.

Procedure

After a test period to determine t_α for the respective experiment the preparation was exposed to the stimulus sequence repeated every three minutes. It consisted of a light-adapting stimulus of 2 s duration, with an intensity evoking a response of saturated height, followed by two 10 ms test flashes of identical intensity, varied stepwise from sequence to sequence. The first test flash (α) was applied after a delay time t_α between 10 and 30 s, and the second after $t_\beta = 120$ s after the light-adapting 2 s illumination. A schematic representation of the stimulus sequence is shown in Fig. 5. The time t_α between the light-adapting illumination and the first test flash was adjusted for each preparation to evoke, at a flash intensity of $I_0 \times 2^{-3}$ or $I_0 \times 2^{-4}$ receptor potentials of about half the amplitude of the potential evoked by the second test flash (β) of identical intensity, which was always applied after $t_\beta = 120$ s. The time t_α was kept constant for the respective experiment. The intensity of the light-adapting illumination was constant, while the intensity of the two test flashes was identical in one sequence, but reduced stepwise by half from sequence to sequence starting from maximal intensity, until both test flashes did no longer elicit measurable responses. The intensity of the test flashes was then rised again by the same steps. The values of the parameters of the two corresponding responses measured at each intensity were averaged. There was no background illumination during the experiments which lasted up to 3 or 4 h.

Owing to our stimulus conditions the photoreceptors are only moderately light-adapted, while they are fairly dark-adapted, at least concerning the first phase of dark adaptation. Since this difference in the degree of adaptation remains constant, it will not be mentioned every time in the text, but the terms "light and dark adaptation" used from now on should be understood in this sense.

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* *List of abbreviations:*

ReP	receptor potential;
PMP [mV]	pre-stimulus membrane potential;
HMAX [mV]	peak amplitude of the transient of the ReP;
HSAT [mV]	saturated response amplitude;
TMP [mV]	transient membrane potential = HMAX-PMP;
TLAT [ms]	latent-period, time from the beginning of the stimulus until the first measurable increase, determined by double noise value;
TMAX [ms]	time-to-peak, from the beginning of the stimulus;
T2 [ms]	decrease-time, time needed from the peak of the response to reach half the maximal response height;
QHN	decline-quotient (= HN/HMAX);
HN [mV]	after-potential 500 ms after response maximum;
LA	light adaptation;
DA	dark adaptation;
t_α, t_β [ms]	delay times between the beginning of the conditioning light-adapting illumination and test flashes evoking responses in a state of moderate light adaptation (α) or considerable dark adaptation (β);
I_0	maximal intensity available of the stimulating light; equivalent to ca. 1.5×10^{17} (550 nm) photons $\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$;
I_{50}	intensity evoking half saturated response height;
r [mV/log I]	steepness of response height versus log stimulus intensity curve at I_{50}.

Results

1. Time course of dark adaptation after a conditioning light-adapting illumination

In these pre-experiments, intended to determine the time course of the dark adaptation, only one constant test flash (α) was applied after a varied delay time. The time course of the increase in response height following the transient decrease caused by the conditioning, light-adapting illumination used in our experiments, is shown in Figs. 1 to 4. We varied t_α, the time between the conditioning, 2 s illumination and the following 10 ms test flash (α), from 2 to 120 s. As shown in Fig. 2 the response is almost saturated 20 s after the end of the light adapting stimulus. The time until saturation varies between 18 and 32 s for different experiments.

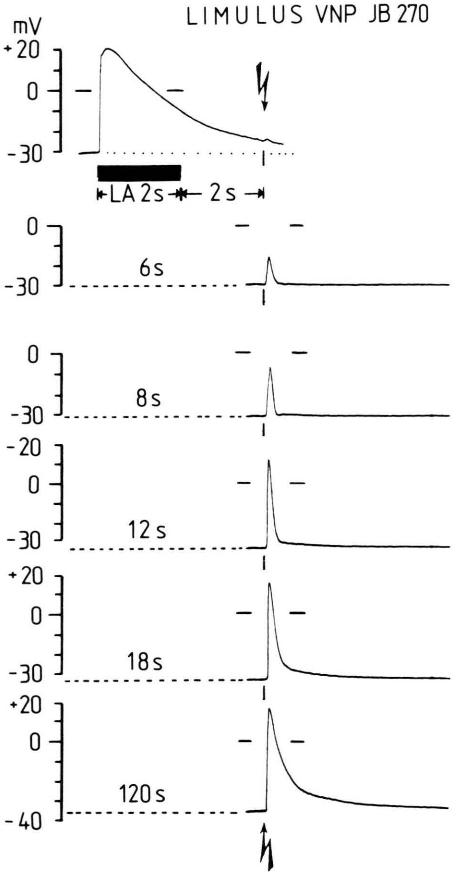

Fig. 1. Dark adaptation in *Limulus* ventral nerve photoreceptor: receptor potentials evoked by a 10 ms flash applied after intervals from 2 s to 120 s following a 2 s light-adapting illumination repeated with increasing intervals from cycle to cycle. Cycle time 3 min. Top record: response to 2 s light-adapting illumination White light, intensity equivalent to 1.5×10^{17} (550 nm) photons $cm^{-2} s^{-1}$.

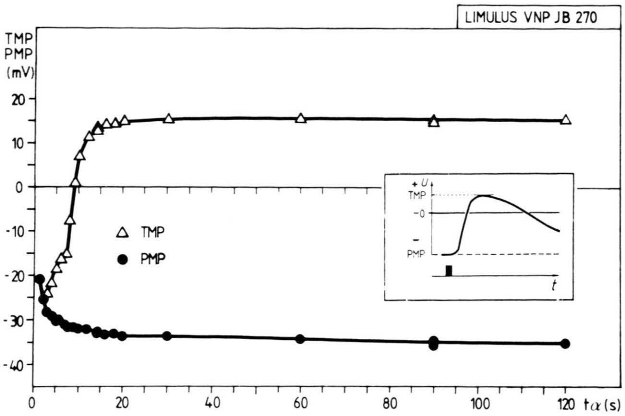

Fig. 2. Time course of dark adaptation: measurement of pre-stimulus membrane potential PMP and transient membrane potential TMP (=HMAX-PMP, see inset) evoked by 10 ms flashes after intervals (t_α) from 2 s to 120 s following a 2 s light-adapting illumination. Same experiment as in Fig. 1, JB 270.

The receptor is not fully dark-adapted 120 s after the light-adapting stimulus, although the major phases of dark adaptation are already completed [6], [7]. The following slow phase of dark adaptation [8], [3] takes additionally about 15 to 20 min and contributes no more than ca. 10% to the height of the fully dark-adapted receptor potential. In our experiments we did not await this slow phase of adaptation in order to apply a convenient 3 min stimulus repetition cycle.

The sensitivity increase following light adaptation shown in Fig. 2 takes place in two phases: a slower increase in the first phase up to dark adaptation times $t_\alpha = 10$ s, followed by a steeper rise toward a first saturation. The two phases of the sensitivity increase, which have been described by Maaz *et al.* [6] and J. Claßen-Linke [3], in *Limulus* ventral nerve photoreceptors, could be demonstrated in all their experiments by selecting the proper experimental conditions, *i.e.*, varying the intensity and duration of the light-adapting stimulus, and the intensity of the test flash. The transition from the first phase of adaptation to the second phase at $t_\alpha = 8$ s which is visible in the plot of the peak amplitude (resp. TMP) of the response in Fig. 2, is observed also in the plots of the other parameters (except for the PMP).

The membrane potential in the dark (PMP, see Fig. 2) changes rapidly by approx. 10 mV towards more negative values in the first phase up to $\alpha = 10$ s. For longer values of t_α it still continues to change into the same direction by some mV, but reaches an almost constant level for values of $t_\alpha > 20$ s. The plot of the latent period TLAT has a minimal value at $t_\alpha = 8$ s and then rises slightly again (Fig. 3). The changes, however, are small. The time-to-peak TMAX changes initially into the reverse direction as compared to TLAT; it passes through a maximum at $t_\alpha = 8$ s. After a transient minimum at $t_\alpha = 18$ to 20 s the course of TMAX is almost parallel to that of TLAT. The decrease-time T2 remains at a low level until $t_\alpha = 8$ s and then increases strongly (approx. 4 fold) from $t_\alpha = 20$ to $t_\alpha = 100$ (Fig. 4), and keeps increasing after HMAX (or the TMP) is already saturated.

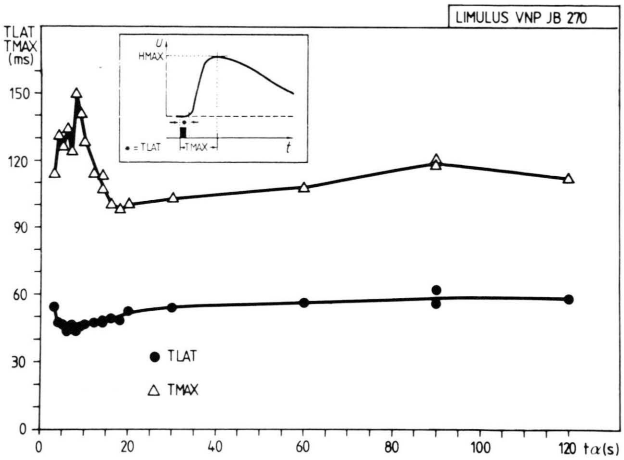

Fig. 3. Time course of dark adaptation: measurement of latent period TLAT and time-to-peak TMAX (see inset) of receptor potentials evoked by 10 ms flashes after $t_\alpha = 2$ s to 120 s following a 2 s light-adapting illumination. Same experiment as in Fig. 1, JB 270.

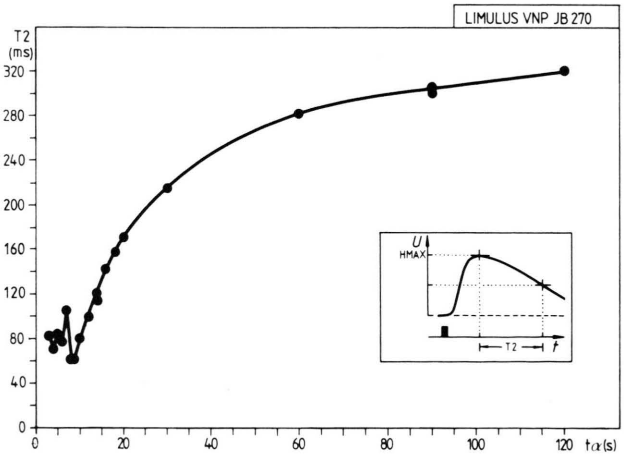

Fig. 4. Time course of dark adaptation: measurement of the decrease-time T2 (see inset) of receptor potentials evoked by 10 ms flashes after $t_\alpha = 2$ s to 120 s following as 2 s light-adapting illumination. Same experiment as in Fig. 1, JB 270.

2. *States of moderate light and considerable dark adaptation after a conditioning light adapting illumination; two response versus stimulus intensity curves*

2.1. Response amplitude

The course of part of an experiment to determine response versus stimulus intensity curves for two reproducible states of adaptation following a constant conditioning, light-adapting illumination is shown in the paper recording (Fig. 5). The α-peaks represent responses in the state of moderate light adaptation and the β-peaks those in the state of dark adaptation.

With decreasing intensity of the test flashes, first the α- and then the β-responses become smaller and finally disappear. Examples are shown in Fig. 5 (and Fig. 6 with greater time resolution). The response of the fairly dark-adapted receptor (β) at the lowest light intensity demonstrates in Fig. 6 the composition of the ReP of individual bumps typical for low light stimulus intensities. At the time t_α when the α-test stimulus is applied, the prestimulus membrane potential PMP is still slightly (maximally 5 mV) depolarized in some experiments with very short t_α. On the average the difference in PMP at t_α and t_β was negligible (legend of Table I).

The response height (HMAX) *versus* log stimulus intensity curves recorded in the state of light and dark adaptation show that the light adaptation caused by the 2 s conditioning illumination primarily causes a transient sensitivity shift (Fig. 7 and Table 1 A). The half saturation stimulus intensity (I_{50}) of the curve recorded in the state of moderate light adaptation (α) is shifted by 6 ld units toward higher intensities as compared to the curve obtained in the state of dark adaptation (curve β).

The extent of the shift depends upon the intensity and duration of the conditioning light-adapting

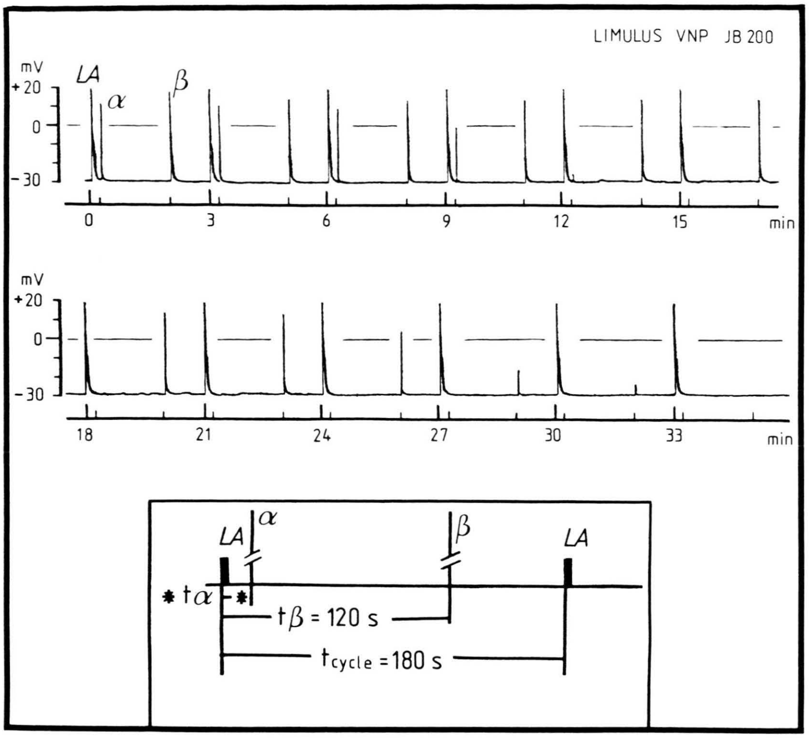

Fig. 5. Light intensity dependence of receptor potentials of the Limulus ventral nerve photoreceptor in two defined states of light- and dark-adaptation; paper writer recording of part of the experiment. Stimulus sequence used (inset): Light-adapting illumination of 2 s duration, white light, intensity equivalent to 1.8×10^{16} (550 nm) photons $cm^{-2} s^{-1}$. Test flashes (10 ms) applied after $t_\alpha = 14$ s (evoking responses in the state of moderate light adaptation, LA) and $t_\beta = 120$ s (evoking responses in state of considerable dark adaptation, DA). Maximal intensity I_0 of test flashes (white light) equivalent to 9×10^{16} (550 nm) photons $cm^{-2} s^{-1}$, starting with α and β at I_0 and reduced by half from cycle to cycle ($t_{cycle} = 3$ min). 15 °C, JB 200.

illumination and the duration of t_α (for the criterion used to choose t_α, see Procedure). The average value of t_α in the 16 experiments described here was 26.7 ± 2.8 s.

The light-adapted α-curve is somewhat steeper and saturates at a slightly lower HMAX level than the β-curve; both curves are sigmoid. Also the averaged steepness of the α-curve of 16 experiments (see Table IA) is somewhat steeper than that of the β-curve.

The curves drawn were calculated using the equation

$$\text{HMAX} = \frac{\text{HSAT} \cdot I^n}{I_{50}^n + I^n}. \qquad (1)$$

This equation which was used by Pak *et al.* [9] is an arbitrary modification of the Naka-Rushton relation [10] and allows adjustment of the slope.

The saturated response height HSAT was chosen by eye; the half saturation intensity I_{50} and the exponent n were obtained by a computer program which selects the values for these parameters by least square deviation fit of relation (1) to the measured data. The steepness r (mV/log I) at I_{50} is given by

$$r = \text{HSAT} \frac{n \cdot \ln 10}{4}. \qquad (2)$$

The symmetric form of this relation in the semilogarithmic plot does not quite fit the data in the range of low and high flash intensities, but it gives reliably reproducible values for the half saturation intensity I_{50} and the curve steepness r. The actually measured curves are asymmetric and rise more steeply from the zero level. The dark-adapted β-curve reaches a plateau in the range of high flash intensities, but tends to rise again at the highest flash intensities used. The light-adapted α-curve seems to approach saturation at the highest flash intensity available.

2.2. Time course of the response

The changes of the time parameters depending on the light intensity of the test flash in the state of light and dark adaptation are illustrated in Figs. 8 and 9 and Table I B. The stimulus intensities which evoke half saturated response amplitudes (I_{50}) are

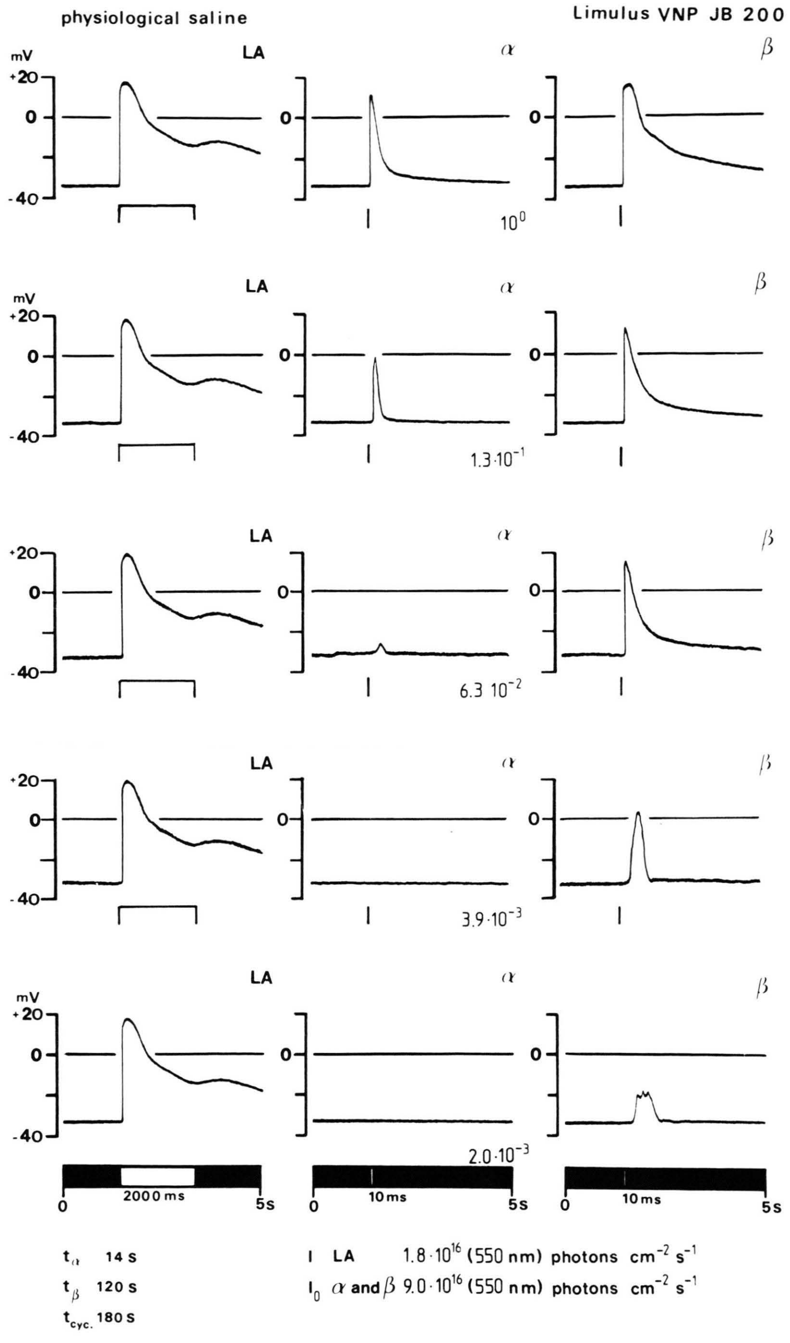

Fig. 6. Receptor potentials at different light intensities and different states of adaptation. First plots in each lines: responses to conditioning light-adapting illumination. Second plots (α): responses to α test flashes; state of moderate light adaptation. Third plots (β): responses to β test flashes; state of considerable dark adaptation. Intensity I/I_0 of test flashes identical in one line, reduced from maximal (first line), as indicated below α-traces. Details as in Fig. 5, JB 200.

marked on the abscissa of the plots for comparison. In Table IB the average values of the time parameters measured in 16 experiments of the kind just described are listed. The dependence of the latent period TLAT from the intensity of the test flash in a single experiment is shown in Fig. 8a.

In the experiment shown the latent period is longer in the state of light adaptation (α) than in the state of dark adaptation (β), at least for light intensities smaller than the half saturation intensity I_{50} for the amplitude of the light-adapted receptor (α). With increasing light intensity TLAT is

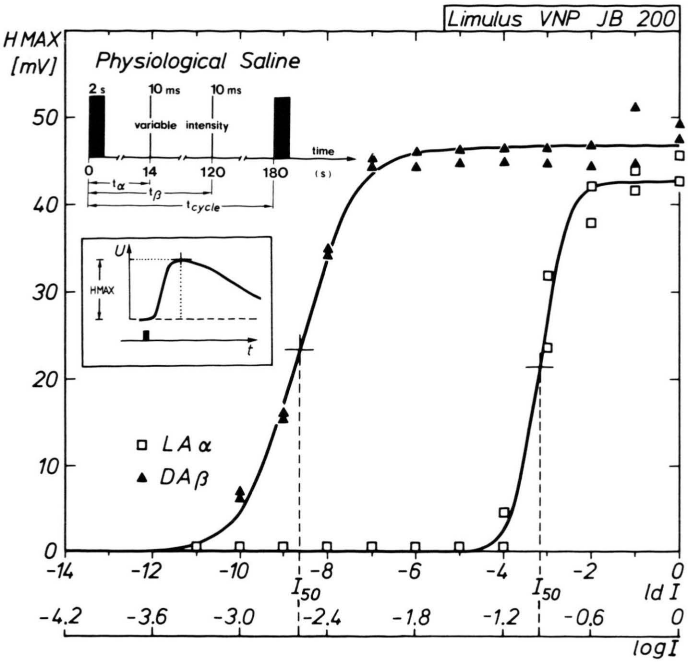

Fig. 7. Response height HMAX versus stimulus intensity curves in the state of moderate light (LA, α) and considerable dark-adaptation (DA, β). Saturated response amplitude HSAT 42.8 mV (LA) and 46.7 mV (DA). Half saturation intensity $\log I_{50} = -0.96$ (LA) and -2.6 (DA). Steepness $r = 34.3$ mV/log I (LA) and 18.7 mV/log I (DA). Exponent n (see equation (1)) 1,39 (LA) and 0.69 (DA). Curves drawn are calculated (see text). For details see legend of Fig. 5. Same experiments as in Fig. 5, JB 200.

Table I a and b. Average values (± S. E. of the mean) of parameters characterizing photoreceptor sensitivity and shape of receptor potentials in the state of moderate light- (α) and considerable dark-adaptation (β). Experimental conditions as described in legend of Fig. 5; 16 experiments, number of averaged values in brackets. – The prestimulus membrane potential PMP at the times of the testflashes was 41 ± 0.3 mV at t_α and 41 ± 2.8 mV at t_β.

Table I a. Amplitude of receptor potential. I_{50}: stimulus intensity evoking half saturated response height; HSAT: saturated response amplitude; r: steepness of response amplitude *vs.* log stimulus intensity curve at I_{50}; n: exponent from equation (1).

	$\log(I_{50}/I_0)$	HSAT [mV]	r [mV/log I]	n
α	-1.44 ± 0.09	50.5 ± 0.8	44 ± 6.4	1.6 ± 0.27
β	-2.42 ± 0.11	53.2 ± 2.1	32 ± 4.2	1.1 ± 0.90

Table I b. Time parameters of receptor potential TLAT: latent period; TMAX: time-to-peak; T2: decreased time; QHN: decline quotient. The values are obtained by interpolation from the parameter *vs.* log stimulus plots at 3 points: 1) At the maximal stimulus intensity I_0 (first group); 2) at $I_{50\alpha}$, the stimulus intensity evoking half saturation of the response amplitude of the light-adapted (α) photoreceptor (second group); 3) at $I_{50\beta}$, the stimulus intensity evoking half saturation of the amplitude of the dark-adapted (β) photoreceptor (third group).

I_0	TLAT [ms]	TMAX [ms]	T2 [ms]	QHN
α	44 ± 6.4 (16)	95 ± 4.6 (16)	462 ± 116 (16)	0.37 ± 0.05 (16)
β	52 ± 6.3 (16)	208 ± 26 (16)	1217 ± 166 (16)	0.71 ± 0.04 (16)

$I_{50\alpha}$	TLAT [ms]	TMAX [ms]	T2 [ms]	QHN
α	106 ± 14 (15)	257 ± 26 (15)	139 ± 20 (15)	0.13 ± 0.03 (12)
β	111 ± 19 (16)	258 ± 35 (16)	358 ± 63 (16)	0.33 ± 0.05 (16)

$I_{50\beta}$	TLAT [ms]	TMAX [ms]	T2 [ms]	QHN
α	No α-Response			
β	235 ± 36 (16)	588 ± 76 (16)	237 ± 36 (16)	0.27 ± 0.06 (13)

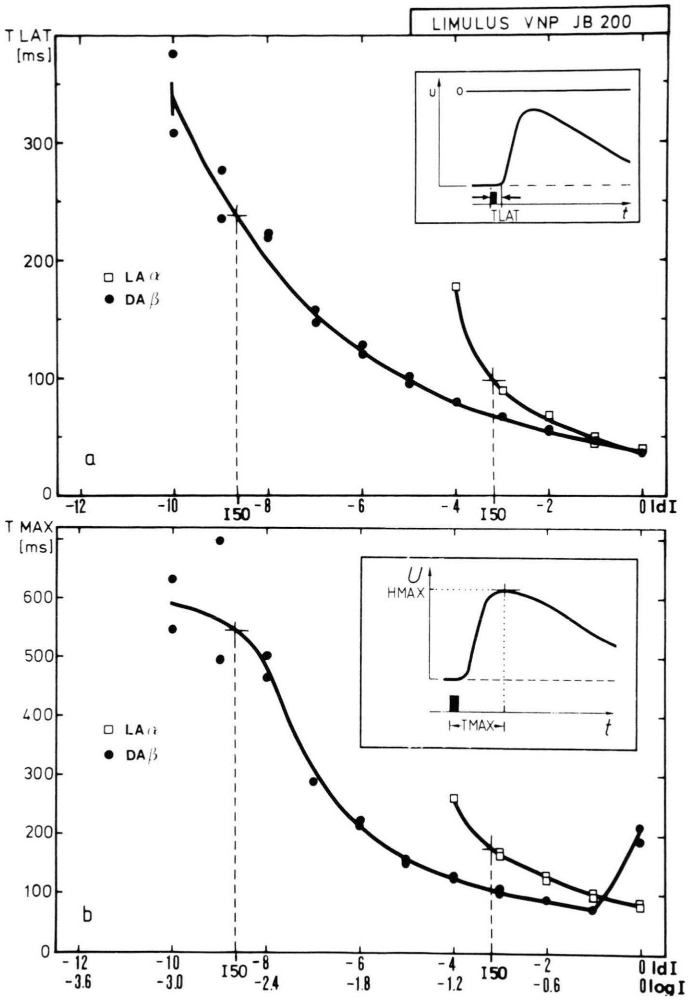

Fig. 8a, b. Latent-period TLAT (a) and time-to-peak TMAX (b) of receptor potentials recorded at different light intensities in the state of moderate light (LA, α) and considerable dark (DA, b) adaptation. The values of the half saturation intensity I_{50} of the response amplitude (Fig. 7) are marked on the abscissa. Curves are drawn by hand. For details see legend of Fig. 5. Same experiment as in Fig. 5, JB 200.

shortened. In some experiments a crossing over the curves recorded in the state of light and dark adaptation occurs at the highest light intensities. At the highest flash intensity available some curves saturate, others do not yet reach saturation. At light intensities still lower than those applied here (Fig. 8a) the values of the latent period of the dark-adapted photoreceptor can be expected not to rise further but to level off in the range of bump latencies, *i.e.* about 200 to 300 ms. The TLAT curves recorded in the state of light and dark adaptation are not shifted along the intensity axis with respect to each other. As compared to the single experiment shown in Figs. 6–9 the tendency of the changes of the average values of TLAT (Table I B) is basically the same; however, on the average TLAT of the light-adapted responses is shorter than that of the dark-adapted ones at the highest light intensity used in our experiments. At the half saturation intensity for the α-response amplitude the light- and dark-adapted TLAT values are almost equal.

Our findings in the range of low and high light intensities are similar to those of Clark and Duncan [11] in the cephalopode *Sepiola* but only for high stimulus intensities they are similar to those of Fuortes and Hodgkin [12] who report the latent period of the *Limulus* reticular cell to be shorter in the state of light adaptation.

The dependence of the time-to-peak, TMAX, on the stimulus intensity is roughly similar to that of

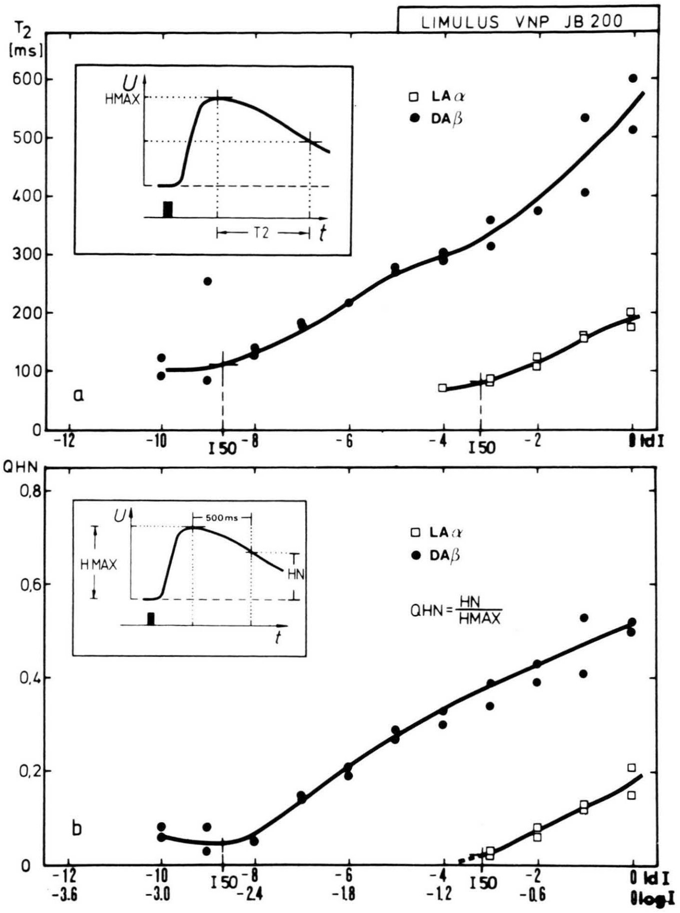

Fig. 9a, b. Decrease-time T2 (a) and decline quotient QHN (b) of receptor potentials recorded at different light intensities in the state of moderate light (LA, α) and considerable dark (DA, β) adaptation. The values of the half saturation intensity I_{50} of the response amplitude (Fig. 7) are marked on the abscissa. Curves are drawn by hand. For details see legend of Fig. 5. Same experiments as in Fig. 5, JB 200.

TLAT with a greater scaling (Fig. 8b and Table IB). At very low intensities the values of the dark-adapted response scatter. With increasing light intensity the curve of TMAX decreases in a sigmoid course. In the experiment shown the values of the α curve (light-adapted) are longer than those of the β curve (dark-adapted), except for the highest light intensities where the curves cross each other.

In Table IB the averaged values of TMAX are almost equal for α and β at the stimulus intensity $I_{50\alpha}$, evoking half saturated response amplitudes in the light-adapted receptor ($I_{50\alpha}$). At the intensity I_0 the β-values of TMAX of the dark-adapted receptor are about 2-fold longer than the α-values of the light-adapted receptor. At lower light intensities the opposite is true. Also these findings are similar to those of Clark and Duncan, cited before.

While the values of TLAT and TMAX are shortened with increasing intensity of the light stimulus both in the state of light- and dark adaptation, the opposite applies to the decrease-time T2 or the decline quotient QHN (Fig. 9, a, b; Table IB). The decrease-time of the photoreceptor becomes continuously longer with increasing light intensity both in the light- and the dark-adapted state. There is no sign of saturation at the highest stimulus intensity available. The α-curve of the light-adapted receptor appears to be shifted towards higher light intensities as compared to the β-curve recorded in the state of dark adaptation, but the slope of the α and β curve is not identical. The average values of the decrease-time (Table I B) correspond with those of the single experiment. The changes of the decline quotient QHN with the intensity are in the same direction as those of the decrease-time, T 2, both in the single experiment (Fig. 9 b) and in Table I B for the state of light- and dark adaptation.

Discussion

We measured the effect of adaptation under largely constant conditions. Due to the flash sequence the dark-adapted (β) responses are slightly influenced by the preceding α-flash at high intensities of the test flashes. By measuring the light-adapted responses without background illumination we achieved comparable conditions for responses in the two states of adaptation which had the additional advantage that the responses of the photoreceptor to the test-flashes at very low light intensities were not disturbed by superimposition of bumps in response to background light.

We discuss our results under the assumption that the receptor potential is primarily composed by superimposing bumps [13] and only secondarily modified by voltage-sensitive membrane conductances.

Intensity dependence of the response height in the state of light- and dark adaptation

The response height versus stimulus intensities curves of the light-adapted receptor are shifted, by an extent depending on the extent of light adaptation, towards higher flash intensities as compared to the dark-adapted (β) state. The curves are not symmetrical: The increase at low intensities is steeper than that near saturation. In a previous publication [14] we have shown, that the light-adapted response-height versus stimulus intensity curve rises steeper than linear at low light intensities, which is seen in a plot with linear scale for both voltage and light intensity. This holds also for the curves shown here. This "supralinearity" was also reported by Hanani and Hillmann [15] and under voltage clamp conditions, by Fein and Charlton [16].

The "supralinearity" of the curve section may be explained by assuming that in this low light range the number of bumps rises more than proportionally with the light intensity, which means an increased quantum efficiency. According to Stieve and Bruns [18], [13] moderate light adaptation increases he bump evoking quantum efficiency and also the bump size.

Intensity dependence of the time parameters of the response in the state of light and dark adaptation

Our measurements of the time parameters of the receptor potential can be correlated with the bump latency distribution shown in Fig. 10. The latent period of a receptor potential (composed of many bumps) is determined by the latent period of the earliest bumps; for stimulus intensities below response saturation the most frequently observed bump latency should determine the time-to-peak, and the width of the bump latency distribution after the maximum should correspond, in first approximation, to the decrease-time of the receptor potential (not regarding the voltage sensitive conductances).

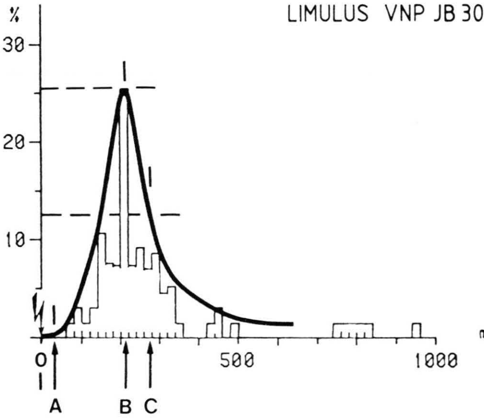

Fig. 10. Frequency distribution of time-to-peak of bumps in *Limulus* ventral nerve photoreceptors following very weak, repeated light flashes. 50 µs 550 nm light stimuli; energy ca. 3×10^7 photons cm^{-2}; physiological saline; 15 °C; Experiment JB 307. TA: time-to-peak of bumps from stimulus onset (average 274 ± 19 ms); $N + N_r = 81$ (total number of bumps including riding bumps). The frequency distribution of TA was chosen here because TA is the only parameter which can be reliably measured both for first and riding bumps. With the time scale used here, the distribution is almost identical with that of the bump latencies; the time-to-peak of bumps is 20 to 40 ms longer than the bump latency. The time parameters of the ReP are determined by characteristics of the frequency distribution of bump latencies or times-to-peak: The latency of the ReP by the first bump occurring (A); the time-to-peak of the ReP by the most frequently observed bump TA (B); the half-time T2 of the ReP decline by the width of the frequency distribution after B which may be characterized by BC.

The first bump after a flash determines the latent period of the ReP. For higher stimulus intensities the latent period becomes shorter. The shorter bump latencies (of the first bumps) are observed because increasing bump numbers make also the more improbable (very early) bumps regularly observable. According to Hamdorf and Kirschfeld [18] and Payne (personal communication) the latent period of the ReP becomes still much shorter with still higher stimulus intensities due to the effect of photon density: Absorption of photons by rhodopsin molecules close to each others (located in the same microvillus or in neighbouring microvilli) causes shorter bump latencies than absorption by the same number of photons by rhodopsin molecules separated by longer distances.

The time-to-peak will be shortened with stimulus intensities increasing above ReP-amplitude-saturation because earlier portions of the rising phase of the bump latency distribution will suffice to saturate the Rep amplitude. Opposite to the latent-period and time-to-peak the decrease-time becomes longer with increasing light stimulus intensity. The decrease-time T2 of the ReP still increases when the ReP amplitude (HMAX) is already saturated (HSAT). The voltage response (ReP) saturates when the reversal potential is reached. The number of the most frequently observed bumps, however, is not yet saturated under these conditions, *i.e.*, while the form of the bump latency distribution may be still the same (and differs only by frequency scaling from the distribution for low flash intensities), there are absolutely more bumps of longer latencies which account for the prolongation of the decrease-time for stimulus intensities at which the peak amplitude is already saturated.

In the state of light adaptation the decrease-time T2 of the ReP is shorter than in the dark-adapted state. This corresponds to the fact that light adaptation sharpens the bumps latency distribution [13]: The most frequently observed bump latency becomes shorter and the bump latency distribution becomes narrower. Additionally the decrease of a light response evoked by a bright flash is steeper (*i.e.*, the decrease-time shorter) than expected from the sum of overlapping bumps for two reasons:

1. The light-adapting effect of the flash itself reduces the bump size for bumps following a stronger flash with latencies which are longer than ca. 200 ms [19].
2. The voltage-sensitive conductance increase accelerates the decrease phase of the ReP and thus also shortens T2 [20].

Phases of dark adaptation

As pointed out by Maaz *et al.* [6] and Claßen-Linke [3] the two phases of dark adaptation which we observed in the pre-experiment (Fig. 2) are due to the two components of the *Limulus* photoreceptor potential. The first phase of the dark adaptation is determined mainly by the recovery of the first component, and the second phase by that of the second component. According to Maaz *et al.* [6] light adaptation reduces the size of the second component much more than that of the first.

As shown in Fig. 4 the decrease-time T2 does not change much in the first phase of dark adaptation and increases rapidly and continuously in the second phase. This means that with increasing dark adaptation relatively more and perhaps larger bumps occur at a later time, *i.e.*, the bump latency distribution must be considerably broadened especially for latencies which are longer than the most frequently observed latency.

Conclusions

Shape and size of the receptor potential are based on the time distribution of bumps evoked by the stimulus. The measurement of the latency distribution of bumps evoked by very low light stimulus intensities (Fig. 10) can be compared with responses to higher light intensities (where bumps are no longer directly discernible). The decrease-time T2 of the receptor potential in response to high light stimulus intensities is shorter than might be expected from the bump latency distribution measured after very dim flashes for two reasons:

(1) light adaptation reduces the size of bumps occurring in the decline of the response and (2) the receptor potential (as opposed to the receptor current) is accelerated in its decline phase by voltage-sensitive conductance.

The size reduction of the individual bumps is primarily responsible for the change in the response size and the sensitivity shift caused by light adaptation. The increased steepness of the response height versus stimulus intensity curves in the state of light adaptation may be explained by assuming the generation of bumps becomes more probable when several photons are absorbed in the same area of the photosensory membrane (raised quantum efficiency). The supralinearity of the stimulus *vs.* response characteristics has its correspondence in the facilitation, the increase in bump size and bump probability after very weak flashes due to light adaptation [13]. We do not yet know the mechanism responsible for this facilitation. By further studying the bump statistics we want to check whether the interpretation of the correlation between bump distribution and shape of the receptor potential outlined in this paper is correct.

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[1] J. E. Lisman and J. E. Brown, J. Gen. Physiol. **66,** 473–488 (1975).

[2] R. M. Benolken, Vision Research **2,** 103–124.

[3] I. Claßen-Linke, Messung der Hell-Dunkel-Adaption und ihre Beeinflussung durch die extrazelluläre Calciumkonzentration. Elektrophysiologische Messungen am Ventralnerv Photorezeptor von Limulus polyphemus. Thesis, RWTH-Aachen-Jülich 1982.

[4] H. Stieve and M. Bruns, Z. Naturforsch. **33c,** 574–597 (1978).

[5] H. Stieve, M. Bruns, and H. Gaube, Z. Naturforsch. **38c,** 471–483 (1983).

[6] G. Maaz, K. Nagy, H. Stieve, and J. Klomfaß, J. Comp. Physiol. **141,** 303–310 (1981).

[7] K. Nagy and H. Stieve, Biophys. Struct. Mech. **9,** 207–223 (1983).

[8] I. Claßen-Linke and H. Stieve, Biophys. Struct. Mech. **7,** 336–337 (1981).

[9] W. L. Pak, S. R. Grabowski, and L. H. Pinto, Adaptation and receptor interactions: some results of intracellular recordings (preliminary note). In: Biochemistry and Physiology of Visual Pigments (H. Langer, ed.), pp. 225–228, Springer-Verlag, Berlin-Heidelberg-New York 1973.

[10] K. J. Naka and W. A. H. Rushton, J. Physiol. **185,** 536–555 (1966).

[11] R. B. Clark and G. Duncan, Biophys. Struct. Mech. **4,** 263–300 (1978).

[12] M. G. F. Fuortes and A. F. Hodgkin, J. Physiol. **172,** 239–263 (1964).

[13] H. Stieve and M. Bruns, Biophys. Struct. Mech. **9,** 329–339 (1983).

[14] H. Stieve and M. Pflaum, Vision Res. **18,** 747–749 (1978).

[15] M. Hanani and P. Hillman, J. Gen. Physiol. **69,** 553–569 (1977).

[16] A. Fein and J. S. Charlton, Increased intracellular sodium mimics some but not all aspects of photoreceptor adaptation in the ventral eye of Limulus (1977).

[17] H. Stieve and M. Bruns, Biophys. Struct. Mech. **6,** 271–295 (1980).

[18] K. Hamdorf and K. Kirschfeld, Z. Naturforsch. **35c,** 173–174 (1980).

[19] H. Stieve and M. Bruns, Biophys. Struct. Mech. **7,** 344 (1981).

[20] P. M. O'Day, J. E. Lisman, and M. Goldring, J. Gen. Physiol. **79,** 211–232 (1982).