Effect of External Calcium Concentration on the Intensity Dependence of Light-Induced Membrane Current and Voltage Signals in Two Defined States of Adaptation in the Photo-Receptor of *Limulus*

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Z. Naturforsch. **41c**, 1092–1110 (1986); received June 27, 1986

Limulus Ventral Nerve Photoreceptor, Light-Induced Receptor Current and Receptor Potential, Light and Dark Adaptation, Stimulus/Response Characteristics, Intensity Dependence

The intensity dependence of the response of the *Limulus* ventral nerve photoreceptor to light flashes was determined in alternating measurements for the membrane current signal (receptor current) under voltage clamp conditions and the membrane voltage signal (receptor potential). Responses were obtained at two reproducible states of adaptation, while the photoreceptor was superfused by physiological saline (10 mmol/l Ca^{2+}), or by salines with either lowered (250 µmol/l) or raised (40 mmol/l) calcium concentration.

For the *dark-adapted* state of the photoreceptor the double logarithmic plot of the response current-time integral F (or the current amplitude) *vs.* flash intensity rises in a steep, supralinear section (slope 2–4) to a curve knee towards a less steep, sublinear section (slope 0.2–0.6), but does not reach saturation in the intensity range tested. *Light adaptation* shifts the response size *vs.* intensity curve towards higher light intensities. This sensitivity shift is enlarged in raised, and almost abolished in low external $[Ca^{2+}]$.

The changes of response latency and time-to-peak with stimulus intensity or adaptation are almost identical for receptor current and receptor potential. The decrease-time of the receptor current response, however, depends much less on the stimulus intensity or the state of adaptation than that of the receptor potential. The relative changes in the time course of the receptor current caused by *light adaptation* are not much influenced by variation of the $[Ca^{2+}]_{ex}$.

Interpretation: The macroscopic receptor current signal consists of a volley of overlapping bumps; the size of these bumps is scaled by a calcium-dependent attenuation function which increases with delay time. This gradual growing attenuation a(t) acts as automatic gain control and may be responsible for the sublinear slope of the intensity dependence of the size of the receptor current. The supralinear slope of this dependence at lower stimulus intensities is probably caused by cooperative effects. Changes in the time course of the macroscopic receptor current due to *light adaptation* or varied calcium concentration are based on changes in the latency distribution of the underlying bump volley, and the size of the attenuation function.

Introduction

The light response of the *Limulus* ventral nerve photoreceptor in the state of *light* and *dark adaptation* has been investigated by many authors [1–5]. One of these investigations indicates that size and duration of the light response and its changes with stimulus intensity and adaptation are primarily determined by the distribution of latencies and sizes of the underlying single photon responses (bumps) evoked by the light stimulus [4]. The number of bumps evoked by a weak light stimulus increases linearly with the light intensity, *i.e.*, with the number of photons absorbed [6, 7]. With stronger light stimuli bumps fuse to the macroscopic receptor current signal [8, 9]. This macroscopic receptor current has a much faster time course than the signal obtained by the linear bump summation [10, 11].

From the proportionality of light-evoked bumps to the number of absorbed photons follows the prediction of a linear rise of the receptor current amplitude with increasing stimulus intensity. According to Brown and Coles [12] (see also [2]) this is the case only for very low stimulus intensities, while in a medium intensity range the increase of the response amplitude becomes supralinear (steepness > 1 in double logarithmic plot) [3, 12–16] until a certain intensity I_{knee}. According to Brown and Coles [12] the stimulus energy I_{knee} corresponds to about 350 (range 91–776) rhodopsin photoisomerisations per cell in the *dark-adapted Limulus* ventral nerve photoreceptor. At still higher light intensities the in-

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0341–0382/86/1100–1092 $ 01.30/0

crease of the response amplitude becomes sublinear (steepness < 1) until final saturation [10, 12, 15, 16].

In previous studies we had measured the characteristics of the membrane voltage response depending on stimulus intensity and state of adaptation [3–5]. The intention of the present investigation was to measure the stimulus/response characteristics upon light flashes applied in two reproducible states of adaptation for the membrane current signal and to compare the results with those obtained for the membrane voltage signal recorded from the same cell. The method applied allowed alternating measurement of the receptor current (ReC, voltage clamp) and the receptor potential (ReP; see [17]). A flash sequence [4] was used to evoke reproducible states of moderate *light* and considerable *dark adaptation*, a procedure made possible by the fast sensitivity recovery of the *Limulus* photoreceptor in the dark.

Abbreviations: PMP [mV], pre-stimulus membrane potential (difference between extracellularly recorded zero line and intracellularly recorded base line; initial and final zero level were averaged; ReC, receptor current (membrane current signal in response to light stimulus); ReP, receptor potential (membrane voltage signal in response to light stimulus); H MAX [mV], peak amplitude of ReP; H SAT [mV], saturated response amplitude; T LAT [ms], latent-period (time from stimulus begin until first measurable increase of response); T 1 [ms], rise-time (from half to response maximum); T MAX [ms], time-to-peak (from stimulus begin); T 2 [ms], decrease-time (from response maximum to half); V_M [mV], membrane voltage; J_M [nA], membrane current; J_D [nA], membrane current in the dark during voltage clamp, 1 s after clamp onset; ΔJ_L [nA], amplitude of light induced current; F [nAs], current-time integral of light-induced membrane current; LA, (moderate) light adaptation; DA, (considerable) dark adaptation; t_α, t_β [ms], delay times between begin of conditioning 2 s illumination and test flashes evoking responses in a state of moderate light adaptation (α) and considerable dark adaptation (β); I_{max}, maximal intensity of conditioning illumination; I_0, maximal energy of test flashes; I_{50}, flash energy evoking a half saturated voltage response; I_{10nA}, flash energy evoking a current response of 10 nA amplitude; I_{1nAs}, flash energy evoking a current-time integral of 1 nAs; I_{knee}, flash energy at curve knee in double log plot of receptor current amplitude or area *vs.* flash energy (the vertex of the extrapolated supra- and sublinear sections); r_{50} [log mV/log I], steepness of log voltage response height *vs.* log flash energy curves at I_{50}; r_1 [log nA/log I], steepness of first curve section of current response *vs.* stimulus energy curve in double log plot (from lowest flash energy to knee); r_2 [log nA/log I], steepness of second curve section from knee to highest flash energy; PS, physiological saline; $[Ca^{2+}]_{ex,i}$, extracellular/intracellular calcium ion concentration; ld, logarithm to the basis 2.

The influence of varied extracellular calcium concentration on the characteristics of the *Limulus* light response has also been the subject of several studies [3, 5, 17–20]. The most prominent effect of the reduction of external calcium concentration consists in a slowing down of the time course of the light response and a diminution of the sensitivity shift of the stimulus/response characteristics (measured by the intensity I_{50} evoking half saturation of the receptor potential) due to *light adaptation*; raise of the $[Ca^{2+}]_{ex}$ has the opposite effect. Here the influence of the $[Ca^{2+}]_{ex}$ on the current response characteristics in dependence of the stimulus intensity was tested in salines with either decreased (250 μmol/l) or increased (40 mmol/l) calcium concentration.

Material and Methods

The experiments were carried out with ventral photoreceptors excised from adult *Limuli* and kept in physiological saline until use. Glass microelectrodes (Ag/AgCl electrodes filled with 0.5 mol/l KCl, resistance between 10 and 15 MΩ) were inserted into the photoreceptor cell for voltage measurements and current injection; an indifferent electrode was placed in the bath. The recording circuit (for a detailed description see [17]) allowed alternating measurement of the unclamped membrane voltage and the membrane current under voltage clamp. The responses were photographed from a dual beam oscilloscope and parallely digitized and recorded on tape. The measuring accuracy was 0.5 nA and 1 mV respectively, the time resolution was 1 ms. A plexiglass test vessel with sylgard bedding was used. The preparation was stimulated from below through the bedding by white light from Xenon lamps (VIX 150); the maximal light energy of the test flashes was ca. 4×10^{14} (540 nm) photons cm^{-2} and the intensity of the conditioning, light-adapting illumination was ca. 1.8×10^{16} photons $cm^{-2}s^{-1}$. The preparation was permanently superfused with a flow rate of ca. 1 ml/min; half-time of saline exchange at the preparation was ca. 1 min. The composition of the salines used is shown in Table I. In the reference saline for the test saline with increased Ca^{2+}-concentration (40 mmol/l), sucrose was added to obtain the same osmotic pressure in both salines. The pH of the salines was ca. 7.5.

All other details of the experimental setup and the processing of the recorded data have been described elsewhere [4, 5].

Table I. Composition of salines [mmol/l].

		Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	Hepes	Sucrose
1	PS	486	10	10	25	561	30	10	–
2	PS (high osm)	486	10	10	25	561	30	10	90
3	high Ca^{2+}	486	10	40	25	621	30	10	–
4	low Ca^{2+}	486	10	0.25	25	541	30	10	–

Procedure

After impalement of the cell with two microelectrodes each experiment started with a pre-period of at least 15 min in physiological saline without background illumination, during which the cell was stimulated at regular intervals until identical stimuli evoked constant responses.

A stimulus cycle consisted of a 2 s conditioning illumination followed by two 10 ms test flashes of identical energy (Fig. 1, first scheme). The test flash energy varied from one cycle to the next, from maximal until the responses became too small to be measured. The first test flash (α) was applied after a delay time t_{DA} varying between 12 and 30 s in different experiments, but constant in one experiment. It evoked responses in a defined state of moderate *light adaptation* due to the preceding conditioning illumination. The second test flash (β), applied after a constant interval t_β 120 s, evoked responses in a state of considerable *dark adaptation*. 60 s after the β-test flash the next cycle began, again consisting of a conditioning illumination and two test flashes. This method has been described in greater detail [4, 5]. The conditions of adaptation were fairly constant throughout the experiments. The states "moderate *light adaptation*" and "considerable *dark adaptation*" will be referred to as "light-adaptated" (LA) and "dark-adaptated" (DA) in the text.

In the experiments described here voltage responses (ReP) were measured alternatingly with current responses (ReC). To this end the cell was voltage-clamped for a period of 2 to 5 s to the value of the pre-stimulus membrane potential (−30 to −60 mV) 1 s after the conditioning 2 s illumination, and the current signals evoked by the test flashes were recorded under otherwise identical conditions as in the preceding voltage measurement. The conditioning, light-adaptating illumination was kept constant throughout the entire experiment and was always applied to the unclamped cell to ensure that the conditions of *light adaptation* were identical for voltage and current measurements.

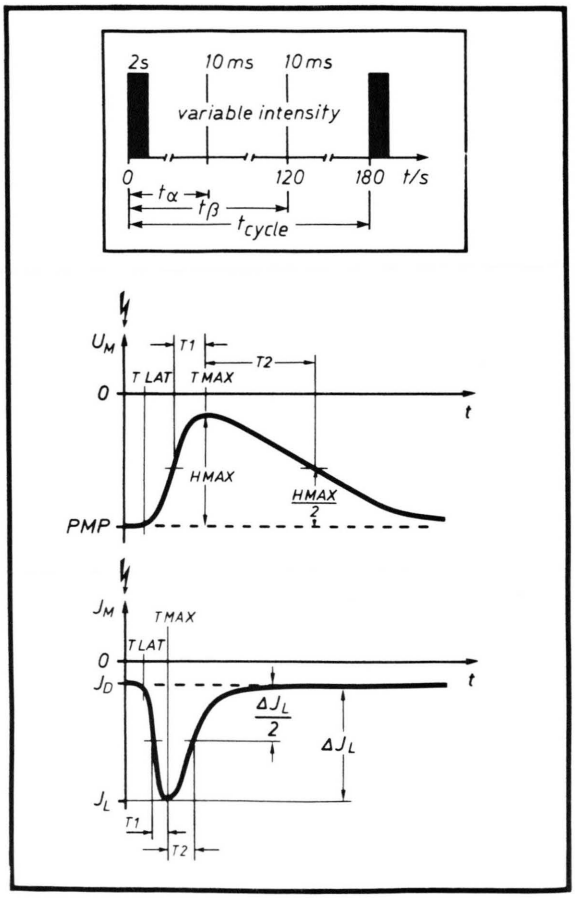


Fig. 1. Stimulus program of one measuring episode (upper scheme) and schemes of membrane voltage (U_M) and membrane current responses (J_M) (lower scheme) including measured parameters T LAT (latent-period), T 1 (increase-time), T MAX (time-to-peak), T 2 (decrease-time) H MAX (maximal amplitude of voltage response) and ΔJ_L (maximal amplitude of current response).

After voltage and current responses had been measured for test stimuli over the entire light energy range in physiological saline (first period), the perfusion of the test chamber was switched to the test saline (second period). When responses to constant stimuli applied to the unclamped cell had again become constant, measurements were continued in the way just described. In the test period the calcium concentration of the saline was either lowered to 250 μmol/l or raised to 40 mmol/l. The experiments lasted 2 to 3 h and were carried out at a temperature of 15 °C. A number of parameters characterizing size and shape of the voltage signal (Fig. 1, second graph) were determined from each recorded response (see also list of abbreviations).

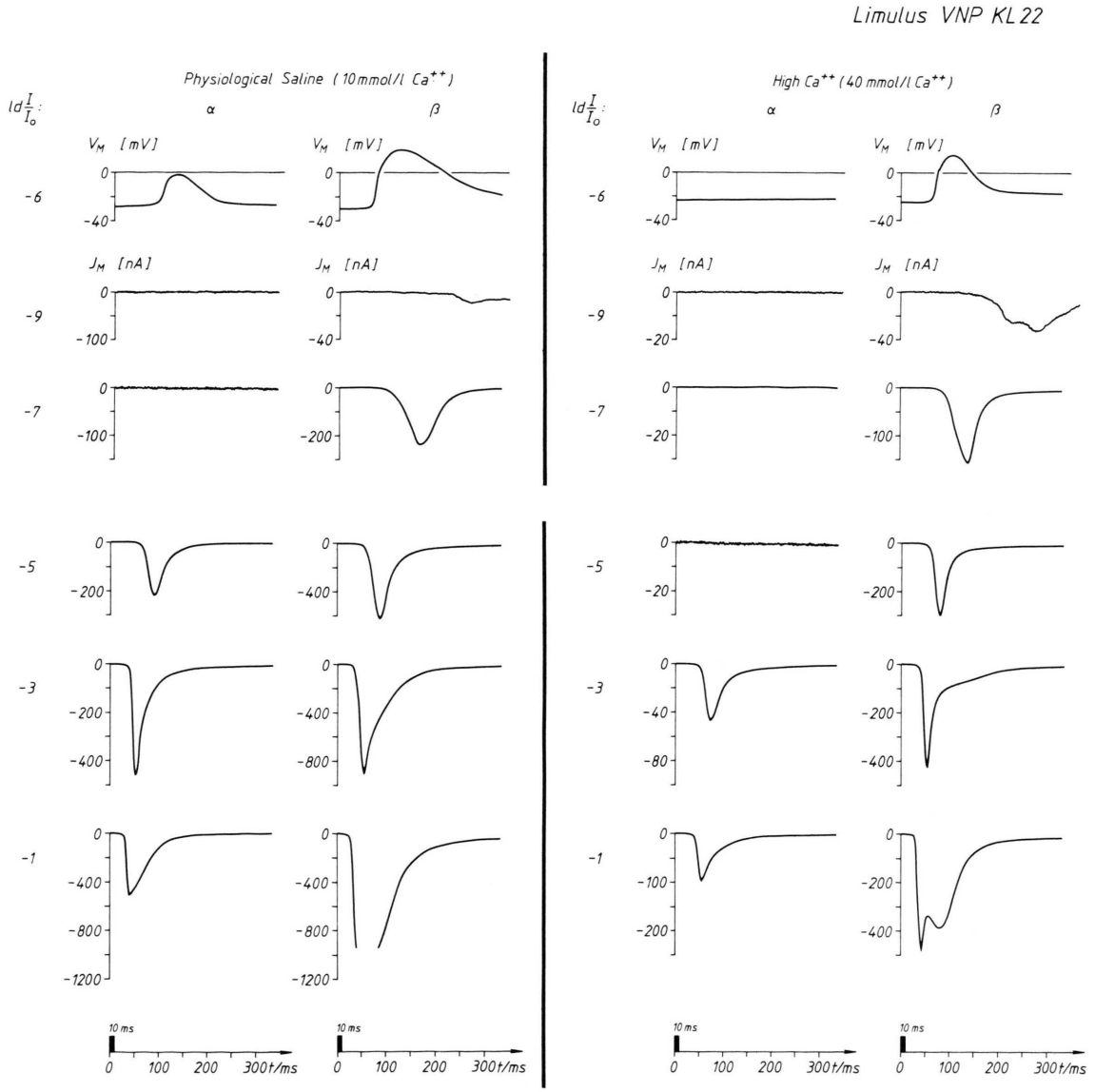


Fig. 2. Receptor potentials (upper row) and receptor currents of *Limulus* ventral nerve photoreceptor recorded in PS (left part) and saline with increased $[Ca^{2+}]_{ex}$ (right part). Responses recorded in a state of moderate light adaptation (α) and considerable dark adaptation (β) at different flash intensities (ld I/I_0). Intensity of 2 s conditioning illumination (white light) equivalent to 1.8×10^{16} (540 nm) photons $cm^{-2}s^{-1}$. Maximal energy of 10 ms α and β flashes (white light) equivalent to 4×10^{14} (540 nm) photons cm^{-2}; t_α 13 s. For composition of salines see Table I. Temperature 15 °C. Exp. KL 22.

Results

Our main intention was to show the characteristics of the receptor current response to light (ReC) of the *Limulus* ventral nerve photoreceptor as compared with those of the voltage response (RePP) under defined conditions. For this reason the data obtained from the *dark-adapted* receptor superfused by physiological saline will be described first, prior to the changes brought about by *light adaptation* and/or changed $[Ca^{2+}]_{ex}$.

The results are demonstrated for two individual experiments in Fig. 2–11, showing the intensity dependence of the response characteristics for increased and lowered $[Ca^{2+}]_{ex}$. The *average values* of evaluations from all experiments (three each with increased and reduced $[Ca^{2+}]_{ex}$) are listed in Tables II and III. Unless otherwise specified the data in the text refer to the average values of Table II and III.

Original registrations of current responses; Fig. 2 and 3

Increasing stimulus intensity causes the receptor *current* (ReC) in the state of *dark adaptation* to become larger and faster (Fig. 2 and 3, β-columns, PS). With the highest stimulus intensities applied the response becomes broader, *i.e.*, the decrease-time is prolonged, while latent-period and time-to-peak are further shortened. *Light adaptation* reduces the size of the receptor current (Fig. 2 and 3, α-columns, PS). The time parameters are shortened. The changes caused by raised and reduced $[Ca^{2+}]_{ex}$ of the test saline will be described later.

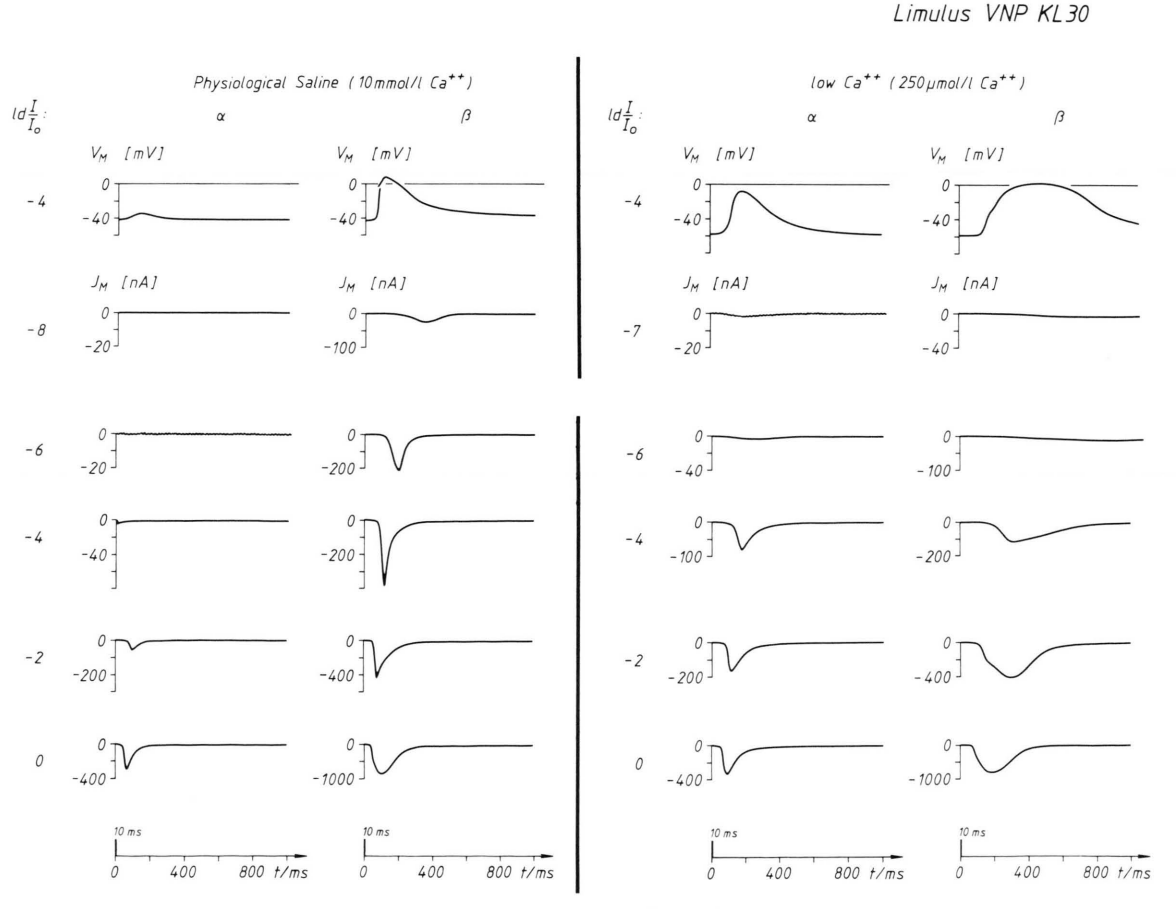


Fig. 3. Receptor potentials (upper row) and receptor currents of *Limulus* ventral nerve photoreceptor recorded in PS (left part) and saline with decreased $[Ca^{2+}]_{ex}$ (right part). Responses recorded in a state of moderate light adaptation (α) and considerable dark adaptation (β) at different flash energies (ld I/I_0). t_α 18 s. Exp. KL 30. Further details as in Fig. 2.

Intensity/response characteristics in the dark-adapted state (physiological saline); Table II, Fig. 4 and 5

In the *dark-adapted* photoreceptor the *current response* becomes greater and shorter with increasing stimulus intensity. In physiological saline the *amplitude* ΔJ_L of the light-induced receptor current rises, with a steep slope (2.9 ± 0.4), with the light intensity (double log plot) until the curve knee (I_{knee} ld I/I_0 -7.0 ± 0.9; Table II). For further increased flash intensities the slope of the curve becomes less steep (0.2 ± 0.1). The two curve sections are straight in the double log plot, and the curve does not saturate within the intensity range tested. The steepness > 1 of the first curve section expresses a supralinear rise of the response with the light intensity, *i.e.* a steeper rise than proportional. The intensity dependence of the *current-time integral F* is very similar to that of the maximal current amplitude ΔJ_L. The curve knee is almost at the same intensity (ld I/I_0 -7.8 as compared to -7.0 for ΔJ_L). The slope of the first curve section for the current time integral F (3.0 ± 0.3) is close to that of ΔJ_L (2.9). The second curve section is steeper (0.5 ± 0.1; for ΔJ_L 0.2), *i.e.* in this section the area of the current response increases *steeper* with increasing flash energy than its amplitude. When the *amplitude* H MAX of the membrane *voltage response* (ReP) is plotted from alternating measurements under the same conditions (DA, PS), its increase with increasing flash energy is much steeper in the first curve section than that of ΔJ_L (Fig. 4c and 5c). The amplitude of the receptor potential saturates at intensities as low as ld I/I_0 -8 to -9, as opposed to the size of the receptor current, which surprisingly does not saturate in the range investigated.

Changes of the response characteristics due to light adaptation (physiological saline); Table II, Fig. 4 and 5

Light adaptation reduces the amplitude of the receptor *current* and shifts the intensity/response characteristics towards higher stimulus intensities. The knee of the amplitude of the receptor current *vs.* stimulus intensity curve is shifted towards higher light intensities (from ld I/I_0 -7.0 to -4.2). The steepness of the first curve section remains the same (r_1 2.9 ± 0.6); r_2 is slightly steeper (0.3 ± 0.1) than in the *dark-adapted* state (0.2 ± 0.1). The amplitude ΔJ_L at our maximal intensity available is about half

Table II. Average values of measured parameters from membrane current and voltage responses recorded from *Limulus* ventral nerve photoreceptors in PS, $[Ca^{2+}]_{ex}$ 10 mmol/l, saline with decreased $[Ca^{2+}]_{ex}$ (250 µmol/l), and saline with increased $[Ca^{2+}]_{ex}$ (40 mmol/l) in the state of considerable dark adaptation (DA) and moderate light adaptation (LA). For the *current response vs.* stimulus intensity curves the maximal current amplitude ΔJ_L at I_0 the steepness r_1 and r_2 of the curve sections and the flash energies at the curve knee (ld I_{knee}/I_0) and a criterion 10 nA response (ld I_{10nA}/I_0) are listed. For the *current-time integral F vs.* stimulus energy curves current-time integral at I_0, the steepness r_1 and r_2 of the curve sections and the flash energies at the curve knee (ld I_{knee}/I_0) and a criterion (1nAs) current-time integral F (ld I_{1nAs}/I_0) are listed. For the *voltage response* the saturation amplitude H SAT, the half saturation intensity ld I_{50}/I_0 and the steepness r_{50} are listed. The negative sign for the stimulus intensity values (ld I) has been omitted.

	Ca^{2+}	$n = 3$ 250 µmol/l DA		LA		$n = 6$ 10 mmol/l (PS) DA		LA		$n = 3$ 40 mmol/l DA		LA	
current	ΔJ_{max} [nA]	725	± 190	240	± 83	924	± 209	400	± 88	435	± 42	70	± 26
	ld I_{knee}/I_0	4.1	± 0.5	4.7	± 0.3[2]	7.0	± 0.9	4.2	± 1.3[5]	7.1	± 2.5	4.2	± 1.0[2]
	ld I_{10nA}/I_0	5.5	± 0.5	4.8	± 0.6	8.9	± 1.0	5.3	± 1.3	8.6	± 2.5	3.9	± 1.6
	r_1 (log nA/log I)	2.4	± 0.3	1.7	± 0.2	2.9	± 0.4	2.9	± 0.6	3.4	± 0.7	3.1	± 0.9[2]
	r_2 (log nA/log I)	0.5	± 0.2	0.6	± 0.1[2]	0.2	± 0.1	0.3	± 0.1[4]	0.17	± 0.1	0.2	± 0.1[2]
current-time integral	F (nAs)	241	± 109	36	± 12	153	± 51	23	± 7.3	43	± 13	5	± 0.7
	ld I_{knee}/I_0	5.9	± 0.5	5.9	± 1.0	7.8	± 3.2	4.9	± 1.4[5]	8.0	± 2.8	4.6	± 0.9[2]
	ld I_{1nAs}/I_0	6.9	± 0.4	6.3	± 0.4	9.1	± 1.0	5.2	± 2.1	8.5	± 2.9	3.7	± 1.6
	r_1 (log nAs/log I)	4.0	± 0.8	2.4	± 0.2	3.0	± 0.3	2.1	± 0.6	3.7	± 1.1	2.6	± 0.7[2]
	r_2 (log nAs/log I)	0.9	± 0.2	0.7	± 0.1	0.5	± 0.1[4]	0.5	± 0.1	0.6	± 0.3	0.3	± 0.2[2]
voltage	HSAT [mV]	63	± 4.4	60	± 1.9	65	± 3.4	62	± 2.5	56	± 12.3	43	± 6.5
	ld I_{50}/I_0	5.3	± 0.3	3.8	± 0.8	8.5	± 1.0	5.1	± 1.2	7.8	± 2.5	3.7	± 1.0
	r_{50} (log mV/log I)	85	± 10	55	± 17	63	± 11	84	± 20	38	± 12	32	± 7

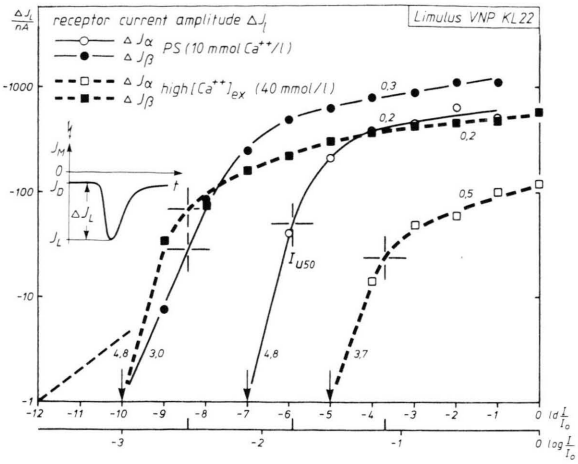


Fig. 4a. Current response height *vs.* stimulus intensity curves of *Limulus* ventral nerve photoreceptor in the state of moderate light adaptation (α) and considerable dark adaptation (β) in PS and saline with *increased* $[Ca^{2+}]_{ex}$. Steepness of curves indicated. The vertical bars of the crosses indicate the stimulus intensity (I_{U50}) evoking half-saturated *voltage* responses (4c) of the same experiment. The darts indicate values of ΔJ_L below 1 nA. For experimental detail see Fig. 2. Exp. KL 22.

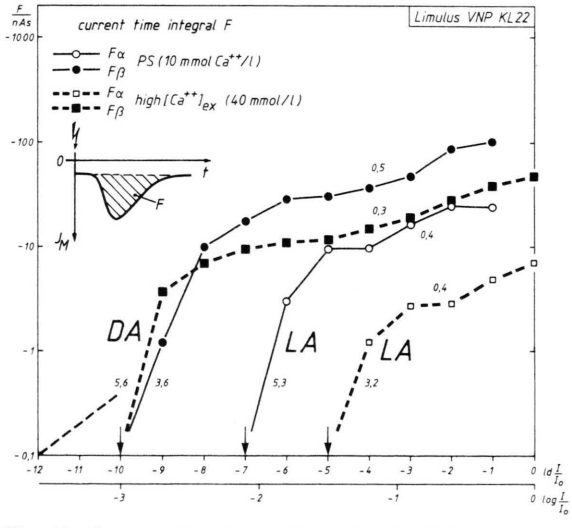


Fig. 4b. Current-time integral *vs.* stimulus intensity curves of the same experiment as shown in Fig. 4a. The darts indicate values of F below 0.1 nAs.

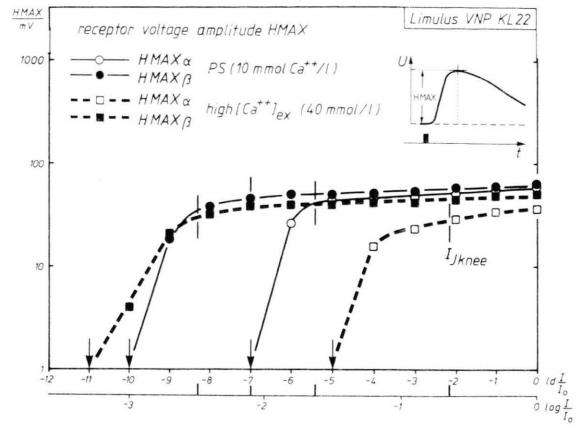


Fig. 4c. Voltage response height *vs.* stimulus intensity curves of the same experiment as shown in Fig. 4a and 4b. The vertical bars indicate the intensity $I_{J\,knee}$ of the curve knee of the *current* response characteristics (4a) of the same experiment. The darts indicate values of H MAX below 1 mV.

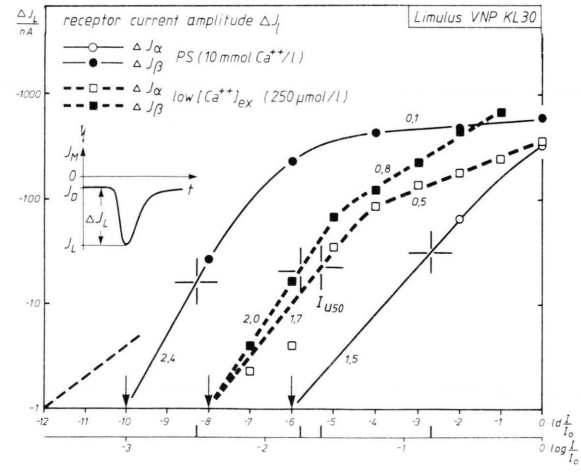


Fig. 5a. Current response height *vs.* stimulus intensity curves of *Limulus* ventral nerve photoreceptor in the state of moderate light adaptation (α) and considerable dark adaptation (β) in PS and saline with *decreased* $[Ca^{2+}]_{ex}$. Steepness of curves indicated. The vertical bars of the crosses indicate the half saturation intensity I_{U50} of the *voltage* response characteristics (5c) of the same experiment. The darts indicate values of ΔJ_L below 1 nA. For experimental details see Fig. 3. Exp. KL 30.

the value of that in the state of *dark adaptation* (400 ± 88 nA compared to 924 ± 209 nA). The curve of the receptor *current-time integral F vs.* the stimulus intensity is shifted due to *light adaptation* towards higher flash energies by about the same amount as the current amplitude plot (I_{knee} shifted from ld I/I_0 −7.8 to −4.9). The steepness r_1 of the first curve section is reduced by *light adaptation* (2.1 ± 0.6 as

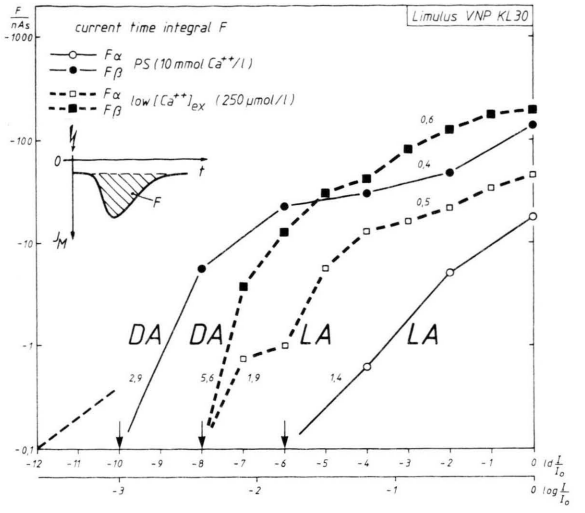


Fig. 5b. Current time integral *vs.* stimulus intensity curves of the same experiment as shown in Fig. 5a. The darts indicate values of *F* below 0.1 nAs.

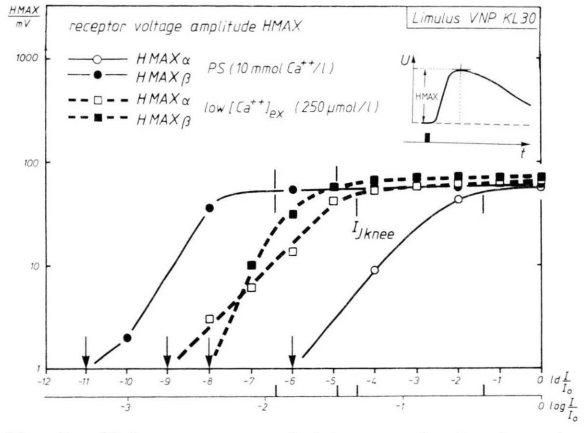


Fig. 5c. Voltage response height *vs.* stimulus intensity curves of the same experiment as shown in Fig. 5a, b. The vertical bars indicate the intensity $I_{\text{J knee}}$ of the curve bend of the *current* response characteristics (5a) of the same experiment. The darts indicate values of H MAX below 1 mV.

compared to 3.0 ± 0.3 for DA). The steepness of the second curve branch is the same for LA and DA (r_2 in both cases 0.5 ± 0.1). This value of r_2 is again steeper than the respective value of the current amplitude curve, which means that also in the state of *light adaptation* the area of the current response increases more steeply with the flash energy than the current amplitude. Under the same conditions as for the current measurements the *receptor potential* height *vs.* stimulus intensity curve is shifted by about the same amount (I_{50} from ld I/I_0 -8.5 to -5.1) as the receptor current curve. The saturation amplitude (H SAT) of the receptor potential is not significantly reduced by *light adaptation* (LA-value 62 mV, DA-value 65 mV), in contrast to the membrane current amplitude, which is reduced by half as described before. The voltage response height *vs.* stimulus intensity curve saturates as clearly in the state of *light adaptation* as in the state of *dark adaptation.*

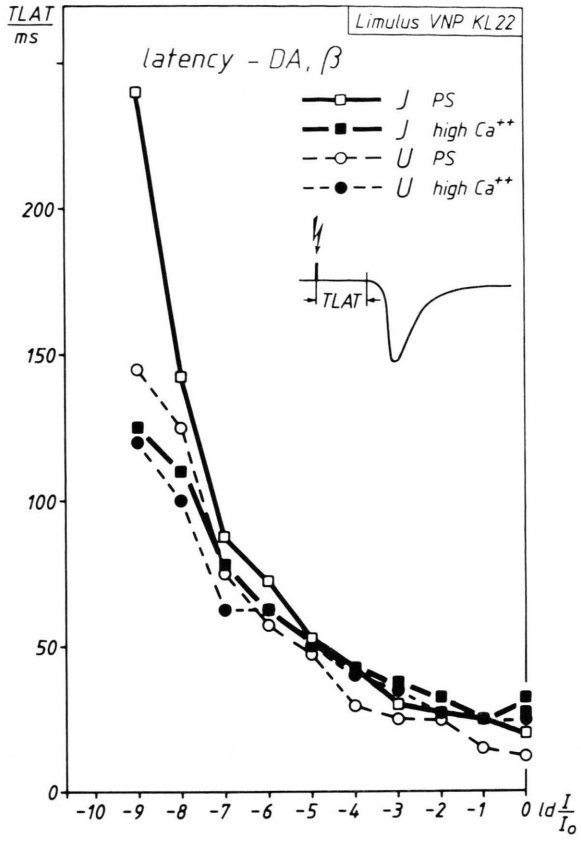
A most striking difference between the plots of current and voltage response height *vs.* stimulus intensity is that the current response apparently must saturate at considerably higher light intensities (not reached in most of our experiments) than the voltage response.

Changes of the time parameters of the receptor current of the dark- and light-adapted photoreceptor (physiological saline); Table IIIa, b; Fig. 6 to 11

The time parameters were chosen to characterize the shape of the light response. Changes of the time parameters of the *receptor potential* with increasing stimulus intensity and with *light adaptation* have been described in a previous publication [4]. Generally the time parameters of the *receptor current* signal, except for the decrease-time T 2, are shortened with increasing stimulus intensity, *i.e.* the response appears faster and sharper. However, the changes of the decline of the receptor current (characterized by T 2), are much less pronounced than those of the receptor potential. *Light adaptation* in most cases shortens all the time parameters of the receptor current.

Latent-period (Table IIIa, b; Fig. 6 and 7)

The latent-period of the receptor current of the *dark-adapted* photoreceptor (Table IIIa) decreases with increasing light intensities. At the highest intensities applied a saturated, shortest value of ca. 30 ms is reached. *Light adaptation* causes a slight reduction of T LAT (to 23 ms at I_0). In our measurements the average latent-period of the voltage response (Table III) is always ca. 10 ms shorter than that of the current response, for both the *light-* and the *dark-adapted* state. (This was discussed in detail by Nagy *et al.*, [21].) The dependence of the increase time T1



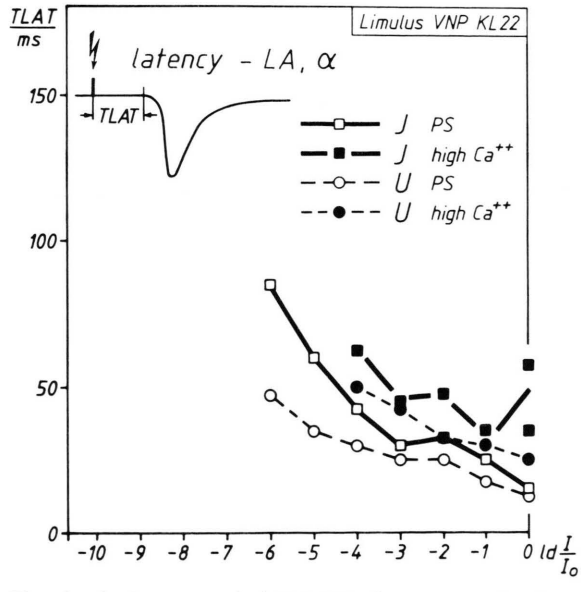
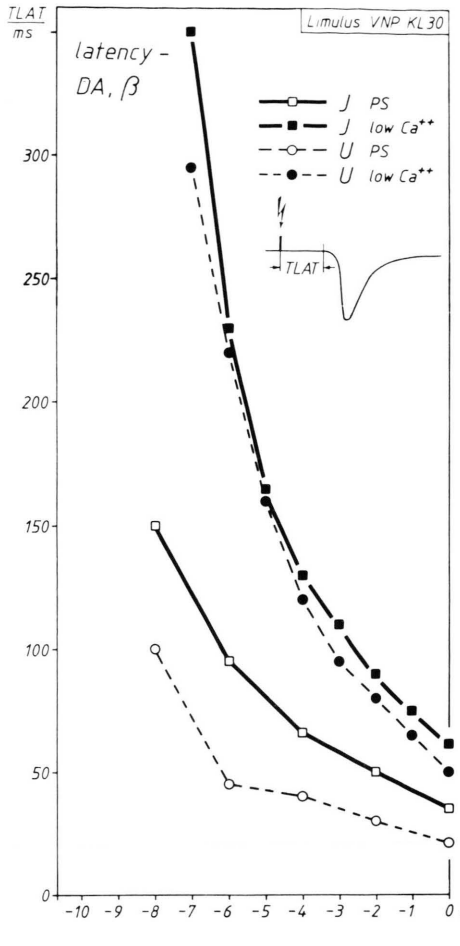


Fig. 6a, b. Latent-period T LAT of current and voltage responses evoked by test flashes of different light intensities in PS and saline with *increased* $[Ca^{2+}]_{ex}$ in the state of considerable dark adaptation (Fig. 6a, DA) and moderate light adaptation (Fig. 6b, LA). Same experiment as in Fig. 2 and 4, see there for details. Exp. KL 22.



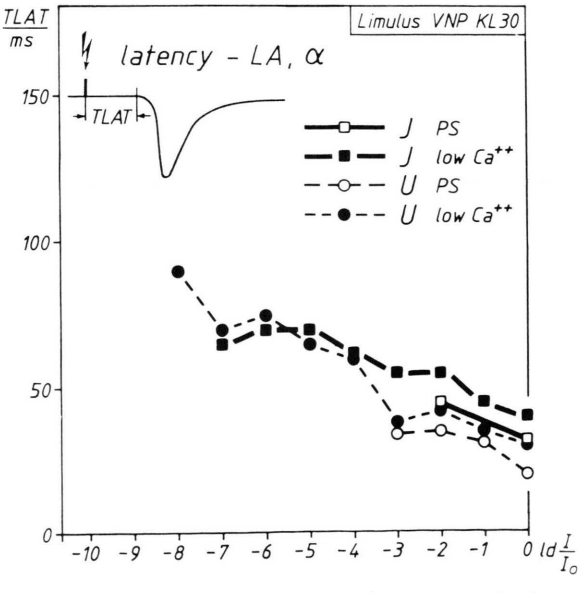


Fig. 7a, b. Latent-period T LAT of current and voltage responses evoked by test flashes of different light intensities in PS and saline with *decreased* $[Ca^{2+}]_{ex}$ in the state of considerable dark adaptation (Fig. 7a, DA) and moderate light adaptation (Fig. 7b, LA). Same experiment as in Fig. 3 and 5, see there for details. Exp. KL 30.

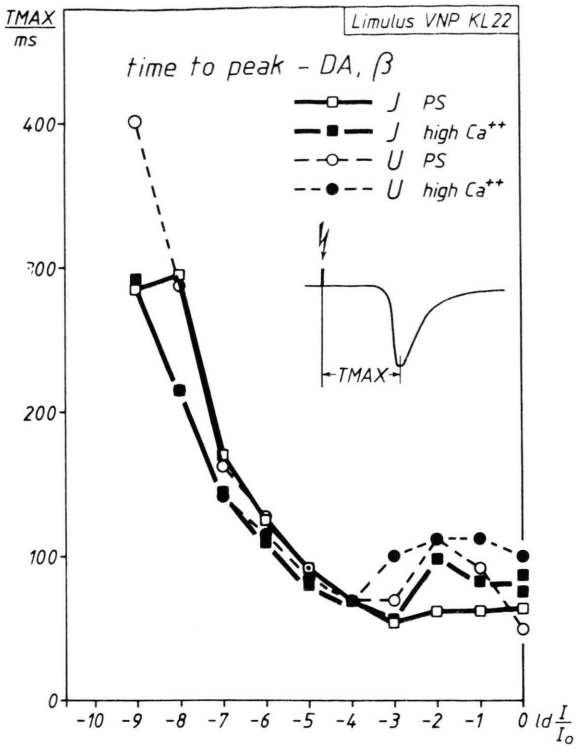
Table III. Average values of time parameters from membrane current and voltage responses of Table II. The time parameters T LAT (latent-period), T 1 (rise time), T MAX (time-to-peak), and T 2 (decrease-time) are listed for the maximal flash energy (I_0) and at specific flash energy values originating from the response characteristics. For the *current response* (Table III a) these are the values of the curve knee (I_{knee}) and 10 nA resp. 1 nAs criterion values, both of DA and LA curves. For the *voltage response* (Table III b) the time parameters and the response amplitude are listed at I_0 and at the half saturation intensity I_{50} for DA and LA curves.

	Ca^{2+}	$n = 3$ 250 mmol/l		$n = 6$ 10 mmol/l (PS)		$n = 3$ 40 mmol/l	
a		DA	LA	DA	LA	DA	LA
at I_0	T LAT [ms]	75 ± 14	51 ± 1	30 ± 7.7	23 ± 7.0	18 ± 6.5	25 ± 11
	T 1 [ms]	101 ± 11	38 ± 5.4	37 ± 12[5]	15 ± 2.9	9 ± 3.1	11 ± 0.7
	T MAX [ms]	233 ± 46	112 ± 6.9	82 ± 20	51 ± 8.2	69 ± 16	46 ± 5.5
	T 2 [ms]	150 ± 13	65 ± 1.6	93 ± 15	43 ± 6.3	41 ± 9.1	31 ± 8.3
at I_{knee} LA curve	T LAT [ms]	223 ± 89[2]	77 ± 10[2]	70 ± 8.8[5]	70 ± 9.1[5]	35 ± 4.0[2]	47 ± 1.5[2]
	T 1 [ms]	223 ± 127[2]	19 ± 18[2]	18 ± 3.2[3]	17 ± 1.6[5]	8 ± 0.3[2]	12 ± 1.0[2]
	T MAX [ms]	565 ± 185[2]	215 ± 10[2]	111 ± 15[5]	111 ± 9.4[5]	55 ± 3.0[2]	80 ± 0.0[2]
	T 2 [ms]	323 ± 53[2]	126 ± 36[2]	49 ± 11[5]	39 ± 5.4[5]	12 ± 0.5[2]	21 ± 5.0[2]
at I_{knee} DA curve	T LAT [ms]	191 ± 31	86 ± 5.5	166 ± 54	120[1]	77 ± 35	–
	T 1 [ms]	173 ± 25	42 ± 2.5	49 ± 21	43[1]	21 ± 6.7	20[1]
	T MAX [ms]	446 ± 64	205 ± 23	310 ± 96	220[1]	159 ± 60	43[1]
	T 2 [ms]	258 ± 4	124 ± 38	67 ± 18	63[1]	38 ± 9.9	26[1]
at I_{10nA} LA curve	T LAT [ms]	265 ± 23	89 ± 8.7	97 ± 26	102 ± 32[5]	30 ± 13	48 ± 19
	T 1 [ms]	289 ± 52	74 ± 25	29 ± 7.1	27 ± 5.8	10 ± 1.2	16 ± 2.2
	T MAX [ms]	695 ± 55	227 ± 15	172 ± 44	166 ± 37[5]	56 ± 12	83 ± 22
	T 2 [ms]	401 ± 75	141 ± 22	45 ± 22	52 ± 4.2[5]	15 ± 2.7	32 ± 6.0
at I_{10nA} DA curve	T LAT [ms]	356 ± 57	98 ± 12	247 ± 66	–	68 ± 60[2]	–
	T 1 [ms]	326 ± 31	109 ± 9	68 ± 32	–	22 ± 6.2[2]	–
	T MAX [ms]	831 ± 44	294 ± 36	470 ± 124	–	182 ± 136[2]	–
	T 2 [ms]	504 ± 48	179 ± 28	88 ± 29	–	62 ± 38[2]	–
b							
at I_0	H MAX I_0 [mV]	59 ± 6.8	58 ± 2.6	62 ± 2.8	58 ± 2.1	53 ± 10	39 ± 5.4
	T LAT [ms]	58 ± 7.6	43 ± 3.7	20 ± 6.8	16 ± 5.4	15 ± 5.8	15 ± 5.2
	T 1 [ms]	8 ± 1.7	31 ± 28	4 ± 0.6	4 ± 0.8	3 ± 0.8	7 ± 0.8
	T MAX [ms]	252 ± 52	137 ± 7	90 ± 32	54 ± 9.7	72 ± 18	48 ± 8.8
	T 2 [ms]	1383 ± 514	600 ± 332	940 ± 194	202 ± 74	407 ± 186	57 ± 10
at I_{50} LA curve	H MAX I_0 [mV]	53 ± 2.8	30 ± 0.9	55 ± 4.7	31 ± 1.3	41 ± 7.2	22 ± 3.3
	T LAT [ms]	153 ± 21	63 ± 7.7	79 ± 32	65 ± 30	26 ± 12	31 ± 11
	T 1 [ms]	35 ± 13	49 ± 8.6	8 ± 2.1	20 ± 2.1	5 ± 0.5	15 ± 1.4
	T MAX [ms]	495 ± 13	213 ± 23	175 ± 51	155 ± 42	54 ± 14	78 ± 16
	T 2 [ms]	587 ± 47	180 ± 25	238 ± 37	70 ± 10.3	113 ± 39	48 ± 7.1
at I_{50} DA curve	H MAX I_0 [mV]	32 ± 2.3	13 ± 3.3	32 ± 1.7	1.2 ± 0.3[4]	28 ± 6.0	9[1]
	T LAT [ms]	285 ± 82	91 ± 21	171 ± 54[5]	183 ± 60[3]	70 ± 33	13[1]
	T 1 [ms]	99 ± 7.8	111 ± 13	29 ± 9.5[5]	80[1]	12 ± 3.7	30[1]
	T MAX [ms]	877 ± 99	310 ± 27	433 ± 129[5]	431 ± 142[2]	164 ± 66	61[1]
	T 2 [ms]	490 ± 38	208 ± 38	222 ± 108[5]		69 ± 17	58[1]

upon the stimulus intensity and adaptation (Table III a, b, not shown in a figure) is rather similar to those of the latent-period and the time-to-peak.

Time-to-peak (Table III a, b; Fig. 8 and 9)

The time-to-peak of the current response of the *dark-adapted* photoreceptor decreases with increasing flash intensity in much the same way as the latent-period. At high light intensities T MAX shows a tendency to saturation. In some cases T MAX becomes longer again at very high stimulus intensities, so that the curve of T MAX *vs.* the stimulus intensity passes through a minimum (Fig. 8a). In the state of *light adaptation* T MAX of the current response is slightly shorter than in the state of *dark adaptation.*



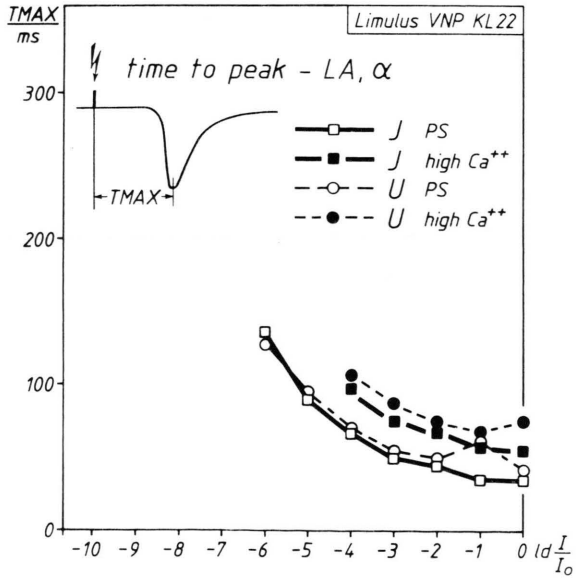
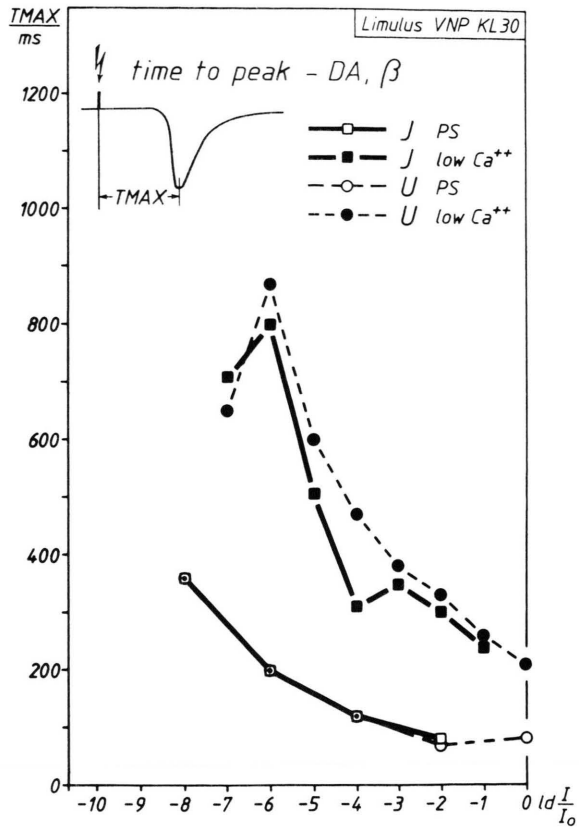


Fig. 8a, b. Time-to-peak T MAX of current and voltage responses evoked by test flashes of different light intensities in PS and saline with *increased* $[Ca^{2+}]_{ex}$ in the state of considerable dark adaptation (Fig. 8a, DA) and moderate light adaptation (Fig. 8b, LA). Same experiment as in Fig. 2 and 4. Exp. KL 22.



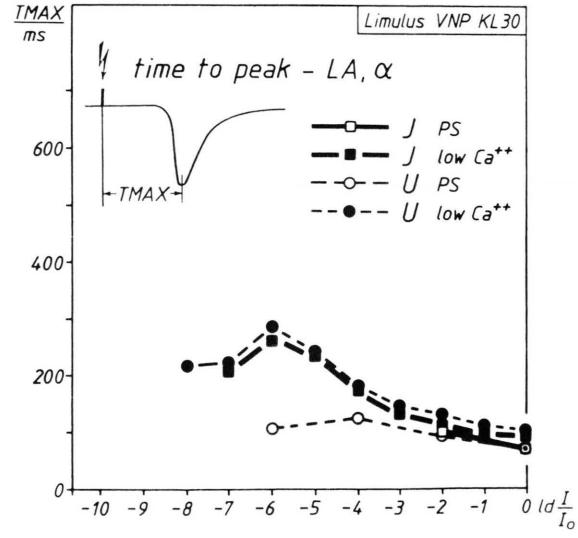


Fig. 9a, b. Time-to-peak T MAX of current and voltage responses evoked by test flashes of different light intensities in PS and saline with *increased* $[Ca^{2+}]_{ex}$ in the state of considerable dark adaptation (Fig. 9a, DA) and moderate light adaptation (Fig. 9b, LA). Same experiment as in Fig. 3 and 5. Exp. KL 30.

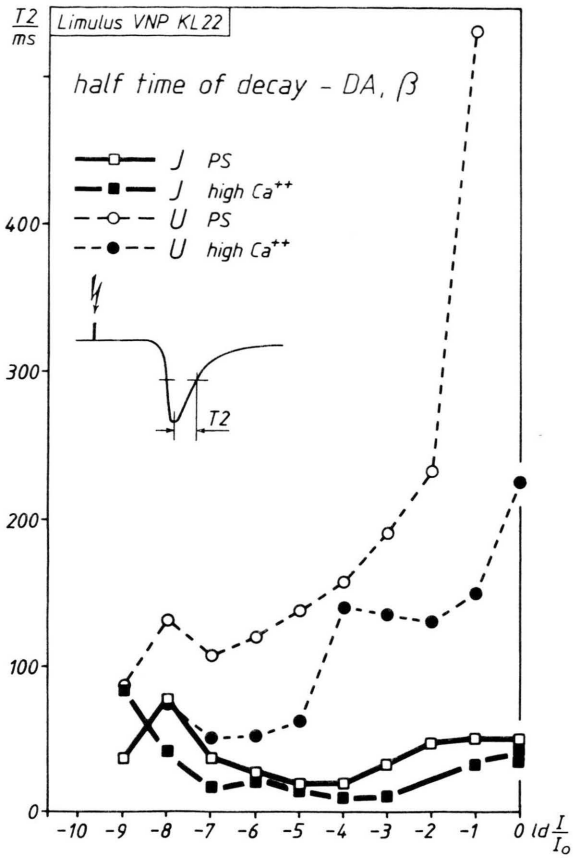
While the values of the latent-period of the voltage response had been generally ca. 10 ms shorter than those of the current response, this does not apply to the time-to-peak. In both states of adaptation the values of T MAX of the current response differ not significantly from those of the voltage response.

Decrease-time (Table IIIa, b; Fig. 10 and 11)

The decrease-time T 2 of the receptor current of the *dark-adapted* photoreceptor does not change much with the flash intensity, as opposed to latent-period and time-to-peak (Table IIIa). The flash intensity dependence of the decrease-time often tends to be U-shaped (Fig. 10a), in Table IIIa T 2 is ca. 90 ms for both the highest and a low flash intensity (I_{10nA}, DA-curve) and has a minimum of about half this value in the range of medium light intensities (I_{knee}, LA-curve). In the state of *light-adaptation* the value of the decrease-time is shorter (ca. 40 ms) and changes only slightly over the range of stimulus intensities measured. The decrease-time of the voltage response of the *dark-adapted* photoreceptor is much longer and increases strongly with increasing flash intensity (opposite intensity dependence as compared to T LAT and T MAX) in both states of adaptation. *Light adaptation* shortens the decrease-time T 2 of the voltage response much more than that of the current response.

Influence of increased (40 mmol/l) and decreased (250 µmol/l) concentration of the external calcium on the receptor current

Increased $[Ca^{2+}]_{ex}$, as compared to physiological saline (Fig. 2) causes the receptor current signal of the *dark-adapted* photoreceptor to become faster and sharper. In some cases the transient of the current response showed two components, as described in detail by Maaz *et al.* [22]. Calcium reduction (Fig. 3) results in a considerable slowing down of the current response. The desensitizing effect of *light adaptation* is greater when the $[Ca^{2+}]_{ex}$ is increased and smaller when it is decreased (Fig. 4 and 5). Although lowering of the $[Ca^{2+}]_{ex}$ almost abolishes the



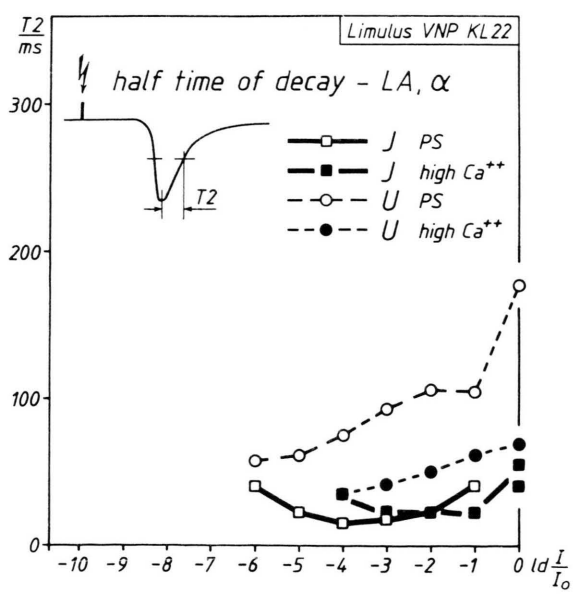
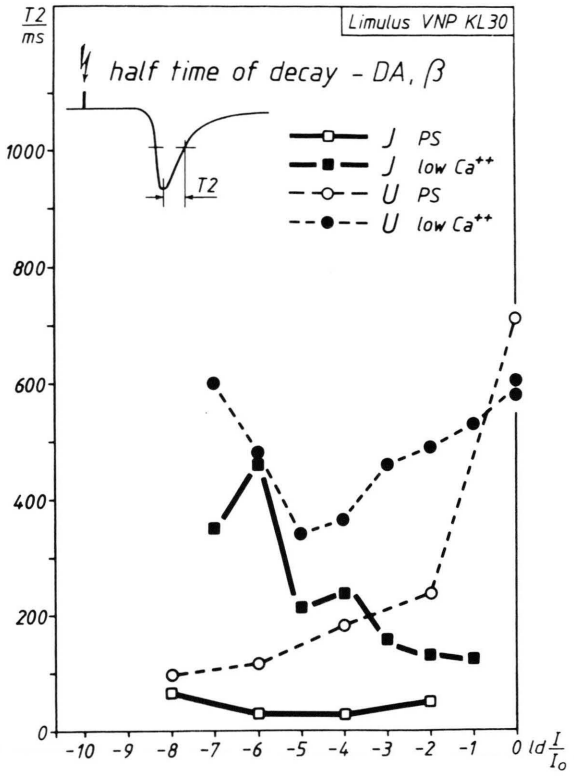


Fig. 10a, b. Decrease-time T 2 of current and voltage responses evoked by test flashes of different light intensities in PS and saline with *increased* $[Ca^{2+}]_{ex}$ in the state of considerable dark adaptation (Fig. 10a, DA) and moderate light adaptation (Fig. 10b, LA). Same experiment as in Fig. 2 and 4. Exp. KL 22.



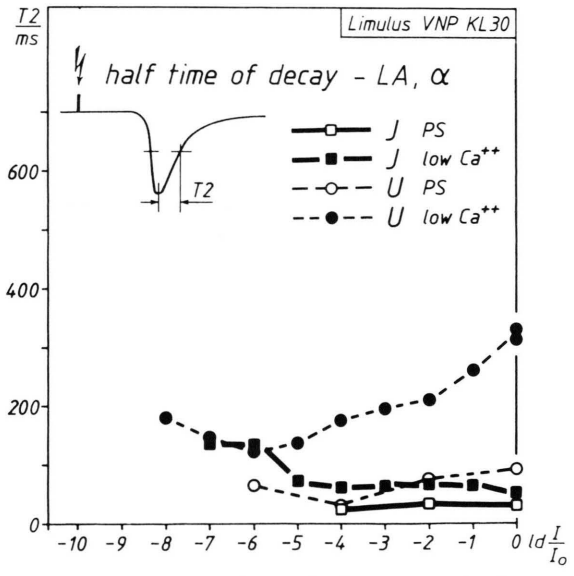


Fig. 11a, b. Decrease-time T 2 of current and voltage responses evoked by test flashes of different light intensities in PS and saline with *increased* $[Ca^{2+}]_{ex}$ in the state of considerable dark adaptation (Fig. 11a, DA) and moderate light adaptation (Fig. 11b, LA). Same experiment as in Fig. 3 and 5. Exp. KL 30.

sensitivity shift of the current response/intensity characteristics (Fig. 5) it does not substantially influence the relative changes of the shape (time course) of the receptor current due to *light adaptation*. The acceleration and sharpening of the response brought about by *light adaptation* seem not much to depend on the $[Ca^{2+}]_{ex}$.

Raised calcium concentration (Table II, Fig. 2 and 4)

The supralinear section of the current response amplitude characteristics of the *dark-adapted* photoreceptor is steeper in the saline with increased $[Ca^{2+}]_{ex}$ (r_1 3.4 ± 0.7) than in PS (2.9 ± 0.4). The position of the curve knee is not substantially changed and the steepness r_2 is decreased (0.17 as compared to 0.2). The average amplitude of receptor currents to flashes of maximal light energy (I_0) is reduced by half (435 nA as compared to 924 nA in PS, Table II). Increased $[Ca^{2+}]_{ex}$ causes the supralinear increase r_1 of the current-time integral F with the flash intensity to become steeper (3.7 ± 1.1) than in PS (3.0 ± 0.3) but the curve knee and the sublinear slope r_2 are not substantially changed. The current-time integral F at the maximal flash energy in the high calcium saline is ca. 1/3 of that obtained in PS. Raised calcium causes a reduction of the size (amplitude or area) of the current responses recorded at flash energies above I_{knee}. The voltage response amplitude *vs.* stimulus intensity plot (Fig. 4c and Table II) shows smaller changes upon raised $[Ca^{2+}]_{ex}$. The steepness r_{50} is significantly diminished (38 ± 12 compared to 63 ± 11 in PS) and the saturation amplitude is slightly smaller for the *dark adapted* photoreceptor.

While the effect of increased $[Ca^{2+}]_{ex}$ on the *dark-adapted* photoreceptor is relatively small, there is a marked effect on the changes due to *light adaptation* of the receptor. The enlargement of the shift of the current response amplitude *vs.* stimulus intensity curve due to the *light adaptation* shown in Fig. 4a, however, is not confirmed by the average values of I_{knee} (Table II), where the same average value (ld I_{knee}/I_0 −4.2) is obtained for the *light-adapted* photoreceptor both in PS and saline with increased $[Ca^{2+}]_{ex}$. But for I_{10nA} a corresponding, markedly enlarged shift due to *light adaptation* is seen for high calcium saline (Table II). The same is true for the current-time integral: the shift caused by *light adaptation* is greater for I_{1nAs} than for I_{knee} in the high calcium saline. The reduction of the size of the cur-

rent response due to *light adaptation* is stronger in the high calcium saline (the amplitude ΔJ_L is reduced to 16% as compared to 43% in PS; the area F is reduced to 11% as compared to 15% in PS). The sensitivity shift due to *light adaptation* measured as I_{50} is enlarged by 0.7 ld units for the receptor *voltage response* characteristics in high calcium saline.

Decreased calcium concentration
(Table II; Fig. 3 and 5)

On the *dark-adapted* photoreceptor calcium decrease apparently has effects which seem to be contrary to each other: Some effects are similar to those of *light adaptation*; others are similar to *dark adaptation*. The former consist in a sensitivity decrease, *i.e.* a shift to higher stimulus intensities (from ld I/I_0 −7.0 to −4.1 for I_{knee} of the receptor current amplitude *vs.* stimulus intensity curve, Table II) and a reduction of the receptor current amplitude by a factor of about 0.8 at the highest light intensity used (from 924 nA in PS to 725 nA). The effects which appear to be similar to DA cause a slowing down of the time course of the response. The slope r_1 is not significantly changed, r_2 is increased. The *current-time integral vs.* the stimulus intensity curve shows a somewhat smaller sensitivity shift of the *dark-adapted* photoreceptor in low calcium saline as compared to the current amplitude curve for I_{knee} from ld I/I_0 −7.8 (PS) to −5.9. The current-time integral F of the *dark-adapted* photoreceptor at I_0, however, is greater by a factor of about 1.6 in low calcium saline (241 nAs) than in PS (152 nAs). This is due to the considerable prolongation of the current response, which has a reduced maximal amplitude in the low calcium saline. Here the slopes r_1 and r_2 are both enlarged due to the lowering of the $[Ca^{2+}]_{ex}$. The half saturation intensity ld I_{50}/I_0 of the *voltage response* of the *dark-adapted* receptor is shifted to higher intensities from -8.5 ± 1.0 in PS to -5.3 ± 0.3 and the slope r_{50} is steeper in low calcium saline. The saturated response amplitude H SAT is not significantly changed in low calcium saline both for the *dark-adapted* and the *light-adapted* photoreceptor, as opposed to the amplitude of the current response.

In addition to having an effect similar to *light adaptation*, and some effects similar to an even further *dark adaptation*, calcium lowering reduces the effects of *light adaptation*, *i.e.* the sensitivity shift due to *light adaptation* is almost abolished. This is observed for the sensitivity measured by I_{knee} in the current amplitude curve or that of the current time integral, or by I_{50} for the amplitude of the receptor potential. The average value of I_{knee} of the current-time integral *vs.* stimulus intensity curves is ld I/I_0 −5.9 for both the *dark-* and the *light-adapted* photoreceptor. A slight shift of I_{1nAs} (DA 6.9 ± 0.4, LA 6.3 ± 0.4) is observed in the same curves. The *current-time integral* F of the receptor current at our maximal flash intensity in the state of *light adaptation* is again greater in the low calcium saline (36 ± 12 nAs) than in PS (23 ± 7 nAs), while the opposite applies to the *current amplitude* (240 ± 83 nA in low calcium saline and 400 ± 88 nA in PS). This shows that also in the state of *light adaptation* low calcium leads to a reduced, but much longer receptor current. The slope r_1 of both receptor current characteristics is somewhat more diminished by *light adaptation* in lowered $[Ca^{2+}]_{ex}$ than in PS, whereas r_2 is not significantly altered. The sensitivity shift measured by I_{50} of the intensity characteristics of the *receptor potential* due to *light adaptation* is reduced (from −3.4 in PS to −1.5 ld units) in lowered calcium saline. The slope r_{50} is reduced by *light adaptation* in lowered calcium saline as opposed to PS.

The sensitivity diminution of the *dark-adapted* photoreceptor due to lowering the external calcium observed here for current and voltage responses is in contrast to our previous results [5], obtained for the receptor potential of the same type of photoreceptor. They showed the same calcium effects on the sensitivity shift due to *light adaptation* as observed here, but practically no calcium effect on the sensitivity of the *dark-adapted* photoreceptor. (The stimulus sequence and the time of *dark adaptation* were equal in both sets of experiments.)

Changes of the time parameters of the receptor current; effect of increased (40 mmol/l) and decreased (250 μmol/l) external calcium concentration; Table IIIa, b; Fig. 6 to 11

Raised calcium concentration

Calcium increase acts similar on the *latent-period* of the current response − and also of the voltage response − as on the *time-to-peak*. The course of T LAT and T MAX *vs.* the flash intensity in the test saline with increased $[Ca^{2+}]_{ex}$ is parallel to that described for physiological saline, but the values are

shorter. The shortening is more marked in the *dark-adapted* state than in the *light-adapted* state (Table III a, contrary to the single experiment shown in Fig. 6b and 8b). In addition the shortening of T LAT and T MAX of the receptor current is stronger at low stimulus intensities than at high intensities. Also the *decrease-time* T 2 of the receptor current (and of the receptor potential, Table III b) becomes shorter in the saline with increased $[Ca^{2+}]_{ex}$, which corresponds to the reduction of the current-time integral under the same conditions (see above). The values of T 2 in the saline with increased $[Ca^{2+}]_{ex}$ are shorter than in PS in both states of adaptation (Table III a), again somewhat more in the state of dark adaptation. In contrast to T LAT and T MAX the influence of raised calcium on T 2 is stronger at high stimulus intensities than at low intensities.

Decreased calcium concentration

The effect of calcium reduction in the external saline on the time parameters of the response is much greater than that of calcium increase. It consists in a pronounced prolongation of all time parameters (apart from few exceptions) and is again more marked in the state of dark adaptation. Both *latent-period* and *time-to-peak* of the current (and voltage) response of the *dark-adapted* photoreceptor are prolonged in the low calcium saline, on the average to ca. twice their value in physiological saline. For the *light-adapted* photoreceptor T LAT and T MAX of receptor current (and voltage) response are slightly prolonged for low flash intensities, but close to the values in PS in the range of high light intensities. The relative changes of latent-period and time-to-peak with the flash intensity in the low calcium saline are similar to those observed in physiological saline. The *decrease-time* T 2 of the receptor current does not depend markedly on the flash intensity in PS in both states of adaptation. However, in reduced calcium saline the prolonged T 2 is progressively shortened with increasing stimulus intensity, especially in the *dark-adapted* state. The prolongation due to reduced calcium is more marked for low light intensities than for high intensities. The decrease-time of the *voltage response,* as opposed to that of the current response, increases with increasing flash intensities in the state of *dark adaptation* in PS. In calcium-deficient saline the intensity dependence of the prolonged T 2 may become U-shaped (Fig. 11a).

Discussion

The stimulus/response characteristics of the *Limulus* ventral nerve photoreceptor measured in our experiments show a supralinear increase with the test flash intensity, in agreement with other publications quoted in the introduction, followed by a sublinear curve section. Saturation, as described for the membrane voltage amplitude [3–5] was reached in most cases neither for the current-time integral nor for the receptor current amplitude with the highest flash energy available in our experiments (equivalent to ca. 4×10^{14} (540 nm) photons cm^{-2}). The current-time integral is a measure of the actual charge flow and represents, for this reason, a more direct measure of the response size than the maximal amplitude, the measurement of which does not register changes of the shape of the response. The supralinearity of the first branch of the intensity dependence of the amplitude of the receptor current is not due to increasing bump synchronization with rising flash intensity since it is also seen when the current-time integral is plotted; see also [16]. The degree of the observed supralinearity differs largely in the reports of various authors and perhaps depends on the conditions of the experiment and the state of the preparation. While in our experiments and those of Gryzwacs [16] the slope was definitely above 2, values between 1 and 2 appear in the results of other authors [12]. A linear rise has been observed in the low intensity range by Lisman and Brown [2]. Our own data in this low light intensity range are too wide apart from each other to show such an effect.

The *gating mechanism* of the light-activated ion channels is not yet comprehensively understood. A calcium/sodium antagonism and perhaps a light-induced affinity change of a channel gating structure seem to play an important part in the gating process [17, 18]. It seems reasonable to assume that the ion channels are opened following binding of an internal transmitter T as messenger. The number of T molecules which have to be bound in order to cause the opening of an ion channel is not known. (Yau *et al.* [23] report a Hill coefficient of 2.2 for the light-controlled ion channels of the toad rod cell which suggests that the binding of more than 2 cyclic guanosin monophosphate (cGMP) molecules is necessary for the opening of a light-activated channel in photoreceptors of vertebrates.) The assumption that the binding of more than one T molecule is needed to

open a channel provides a possible *explanation* for the observed *supralinearity* of the response characteristics [10, 11] see also [24, 25]. In the case of two transmitter molecules necessary for the opening of a light activated channel the photosensory membrane would contain three classes of channels after weak illumination:

1. many closed channels (Ch);
2. some channels which have bound one T-molecule and are still closed, but "unlocked" (Ch−T1);
3. a small number of open channels which have bound two T-molecules (Ch−T2).

Under these conditions the number of open channels (Ch−T2) would rise supralinearly with increasing stimulus energy within a certain stimulus energy range.

This concept could explain, besides the supralinearity, also the observed *facilitation,* if the Ch−T1 state has a life-time in the order of seconds. As facilitation we describe the fact that after conditioning stimuli of low intensities the light responses are larger than without a preceding conditioning illumination [10]. A weak conditioning flash will generate a number of channels in the "unlocked" Ch−T1 state. A following flash will evoke an enlarged response if applied during the life-time of the Ch−T1 state, causing a supralinear increase (slope ≤ 2) in the number of open Ch−T2 channels with rising stimulus intensity. The cooperativity, which is responsible for the supralinearity and perhaps also for facilitation need not occur at the channel gating; it could occur additionally or alternatively in earlier stages of the transduction process. Since the slope in our experiments is often found to be definitely steeper than 2 (see above) a cooperativity of more than two transmitter molecules would be required. This cooperative effect could be located at different stages of the transduction chain.

The curve knee and the following sublinear curve section of the receptor current size *vs.* stimulus intensity curves at higher intensities were observed in all experiments reported here (in agreement with the results of other authors [12, 16]), irrespective of the degree of supralinearity of the preceding curve section.

This *sublinear* slope suggests several possible explanations. One of them could be that the effect of the absorption of a photon is reduced at high light intensities, since the single photon-evoked events (bumps) may increasingly interact with increasing stimulus intensity in the following way: According to Brown and Coles [12], a light-activated rhodopsin molecule in the *dark-adapted Limulus* ventral nerve photoreceptor causes the opening of sodium channels in regions of the cell membrane "beyond the microvillus in which the photon was absorbed, but not necessarily more than 2 μm away". Rhodopsin photoisomerization causes opening of ion channels in a surface area, the "bump-speck", which may include about (estimated) 1000−2000 light-activated ion channels corresponding to about the same number of microvilli. For the spreading of information over the bump speck the hypothesis of diffusing internal transmitter molecules, first proposed by Cone [26] seems to be plausible. An excitatory internal transmitter, however, has not yet been identified, although cGMP may be a suspect. The knee in the intensity response characteristics of the receptor current amplitude is found for the *dark-adapted* photoreceptor at a stimulus intensity evoking a volley in the order of 100−800 overlapping bumps [12]. This intensity may correspond to a density of absorbed photons in the photosensory membrane where the bump specks start to touch and overlap. The sublinear slope of the curve then would be due to the *overlapping of the bump specks* resulting in a reduced increase in the number of ion channels activated per absorbed photon. In *light adaptation* the bumps become smaller, because the amplification is lowered, *i.e.* the number of channels involved in a bump becomes smaller. This might be accompanied or caused by a corresponding decrease in size of the bump specks as suggested by the shift of the knee between the two slopes in the intensity dependence of the receptor current towards higher stimulus intensities. Since higher photon density is required for the touching of the smaller bump specks the curve knee should be consequently shifted.

A different explanation for the sublinearly rising region of the stimulus/response characteristics might be that at higher light intensities an *automatic gain control* mechanism causes a reduction of the current response in a way described in the following paragraph, see [11]. This assumption can also explain why saturation of the size of the receptor current is reached only at very high stimulus intensities.

The macroscopic receptor current evoked by a flash of light is the result of a volley of many bumps. However, it is not simply caused by linear bump

summation. The time course of the bump sum is much slower and longer than that of the macroscopic receptor current [10]. One can simulate the shape of the macroscopic response by convoluting the bump sum with an "attenuation function" a(t), the value of which increases with time. The very early bumps following a light flash in a *dark-adapted* photoreceptor are not, or only very slightly, attenuated, whereas consecutive bumps are the more attenuated the later they occur. The attenuation function a(t) which is used by the cell to scale the size of the bumps constituting the macroscopic receptor current could be the current-time integral of the receptor current, or the intracellular calcium concentration signal, as measured as Arsenazo response [27–31] which roughly resembles the receptor current-time integral.

By such convolutions we obtained naturalistic shapes of simulated macroscopic receptor currents [11], which have many characteristic features in common with experimentally recorded receptor currents, when changes of stimulus intensity and *light adaptation* are simulated. The attenuation function a(t) is a negative feedback signal which may cause an automatic gain control. It reduces the size of the response, shifts saturation to higher light intensities and results even in a shortening of the time-to-peak (by increasing attenuation of later occurring bumps, see [11]). The effect of the attenuation function a(t) causes the response to "swallow its own tail", *i.e.* to shorten the decrease-time T 2. The influence of this attenuation function a(t) grows gradually with increasing stimulus intensity. Convolution of the bump sum with the attenuation function simulates the measured intensity dependence of the receptor current-time integral, in so far as to show a slope knee and a sublinear section for higher light intensities. Moreover this convolution unexpectedly produces the two components C1 and C2 of the current response, similar to the experimentally observable components described by Maaz *et al.* [22] and also observed in the present experiments under the influence of increased $[Ca^{2+}]_{ex}$ (Fig. 2).

The voltage response *vs.* stimulus intensity curves saturate, as opposed to the current response curves, well within the flash intensity range applied in our experiments. The voltage amplitude saturates when the light-induced conductance becomes large compared with the membrane dark conductance, so that the membrane potential in the peak of the receptor potential is mainly determined by the ion specifity of the light-modulated ion channels. Saturation of current responses is not measured in most cases, but should be expected for very bright stimuli. The half saturation intensity of the voltage responses (I_{50} marked in Fig. 4a and 5a) is markedly below the intensity I_{knee} of the curve knee of the current/response characteristics.

Effects of changed extracellular calcium concentration

The changes of the receptor current response observed under decreased as well as increased $[Ca^{2+}]_{ex}$ are similar to those observed for the voltage response (except for T 2) [5]. They may be caused primarily by two effects:

(a) The external calcium concentration influences the bump latency distribution, probably via the intracellular calcium concentration. Raised $[Ca^{2+}]_{ex}$ shortens and narrows, lowered $[Ca^{2+}]_{ex}$ prolongs and broadens the bump latency distribution [10].

(b) If, as proposed by us [11], the light-induced increase of the intracellular calcium concentration represents the attenuation function a(t), its size would depend markedly upon the external calcium concentration. This would explain two observations:

1. The extent of the feedback control which results in the shortening of the decrease-time T 2, increases with the $[Ca^{2+}]_{ex}$.

2. The slope r_2 of the sublinear section of the receptor current characteristics depends on the $[Ca^{2+}]_{ex}$; it is steeper in calcium-deficient saline.

The sensitivity of the *dark-adapted* photoreceptor is not much changed when the $[Ca^{2+}]_{ex}$ is raised; it is, however, diminished when the $[Ca^{2+}]_{ex}$ is lowered in the present experiments. In earlier experiments of us [5] the sensitivity of the *dark-adapted* photoreceptor was not much calcium-dependent both for raised and for lowered $[Ca^{2+}]_{ex}$. The calcium independence of the sensitivity of the *dark-adapted* photoreceptor may be explained by assuming that the *dark-adapted* photoreceptor cell can largely adapt to changes of the $[Ca^{2+}]_{ex}$, keeping the $[Ca^{2+}]_i$ constant (see [31]), except for very low $[Ca^{2+}]_{ex}$. On the other hand prolonged stay in salines of very low calcium concentration in combination with strong light stimulation reduces the responsiveness of the *Limulus* photoreceptor [31]. We have, however, no conclusive explanation for the discrepancy of the two sets of experiments concerning the effect of lowered $[Ca^{2+}]_{ex}$ on the sensitivity of the *dark-adapted* photoreceptor.

The sensitivity shift due to *light adaptation*, however, in agreement with the earlier experiments mentioned before [5], depends strongly on the $[Ca^{2+}]_{ex}$, *i.e.* it is decreased by calcium deficiency of the external saline. According to Levy and Fein [32] the desensitation of the cell is greater when the $[Ca^{2+}]_i$ becomes higher. This is consistent with our assumption that, upon illumination, calcium flows into the cell through light-activated ion channels driven by its electrochemical gradient [30]. We propose that this intracellular calcium increase constitutes the attenuation function a(t) described above.

Lowering the external calcium concentration reduces the sensitivity shift but not the relative changes in the shape of the receptor current signal caused by *light adaptation*. These are probably due to an additional desensitizing mechanism of *light adaptation* described elsewhere [29, 33, 34], which is not calcium-controlled.

The quantitative comparison of the time parameters of current and voltage response in dependence of light stimulus intensity and adaptation in our experiments shows that the shortening of the latent-period with rising stimulus intensity and increased $[Ca^{2+}]_{ex}$ is almost parallel with the shortening of the time-to-peak both for receptor current and voltage responses. The decline (T 2) of the current response is much faster (by a factor 2 to 10) than that of the voltage response. As opposed to the decrease-time of the voltage response, which is prolonged with rising stimulus intensity, the decay of the current response remains fast, depending only very weakly on the stimulus intensity. This indicates that the decay of the voltage response is not mainly determined by the bump distribution but is rather dependent on, and greatly prolonged by, the action of voltage-sensitive ion conductance of the cell membrane of the *Limulus* photoreceptor [35, 36]. Calcium deficiency leads to a prolongation of the decrease-time of both current and voltage response (by a factor 2−10). The prolongation of the decrease-time of the current response in low calcium saline (and conversely its shortening in saline with increased $[Ca^{2+}]_{ex}$) is most probably due to the calcium influence on the bump latency distribution (broadening in low $[Ca^{2+}]_{ex}$ and narrowing in high $[Ca^{2+}]_{ex}$) as observed by Stieve and Bruns [37]; see also [5, 10]. Additionally the action of the attenuation function a(t) is probably very much calcium-dependent since it consists presumably in a rise of the $[Ca^{2+}]_i$. It therefore causes a shortening of the decrease-time T 2, which depends strongly on the $[Ca^{2+}]_{ex}$.

The discussion of the results can be summarized under the following aspects: We have tried to explain the stimulus/intensity dependence of the receptor current response on the basis of a bump generation model. The supralinear section of the current/response characteristics is explained by cooperative effects *e.g.* at the channel gating. In our experiments the supralinearity is marked (r_1 as high as 4). The curve knee and the sublinear curve section for higher stimulus intensities may be explained by bump speck overlapping and/or (to us more likely), by the effect of an automatic gain control of the light response. Probably for the same reason (automatic gain control) saturation of the current response, as opposed to the voltage signal, was not reached.

Both for the receptor current and the receptor potential the extent of the sensitivity shift obtained by *light adaptation* depends on the external calcium concentration, since the calcium influx into the photoreceptor cell depends on the electrochemical gradient. The change in the time course of the macroscopic receptor current with the stimulus intensity, with the state of adaptation, and with variation of the $[Ca^{2+}]_{ex}$ can be explained plausibly by underlying changes in the bump latency distribution and by changes in the size of the attenuation function a(t) which probably acts via the intracellular calcium concentration.

Acknowledgements

We thank R. Schanzer for technical help, A. Eckert and B. R. Stieve for considerable help with the manuscript. H. Reuss, B. Schlösser, J. Schnakenberg we thank for critical reading. This work was supported by the Deutsche Forschungsgemeinschaft, SFB 160.

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