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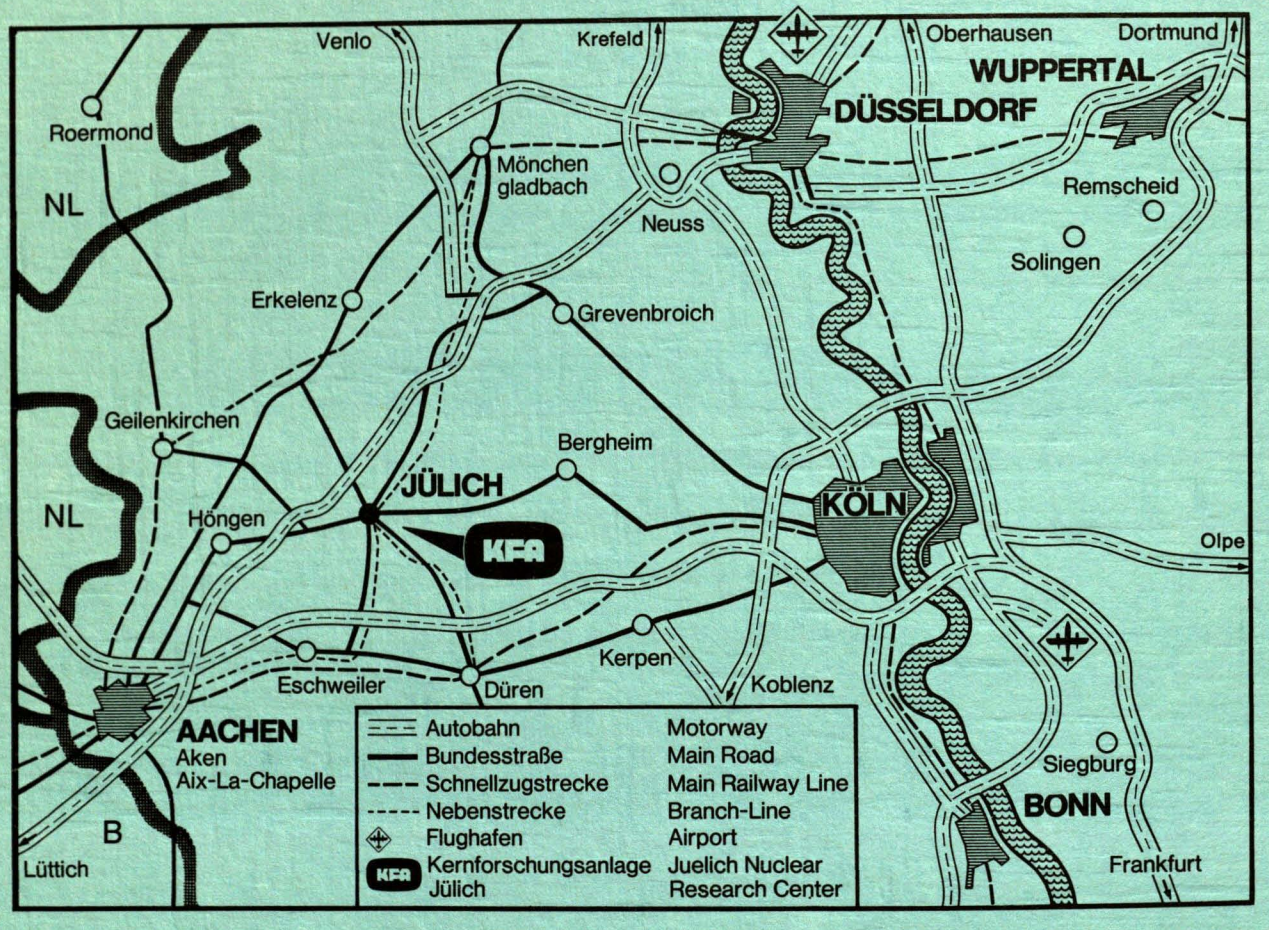

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The action of lanthanum ions in the eyes of Astacus and Limulus: access to the photoreceptor membrane and influence on the electrical response

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C o n t e n t s

1. Abstract

The effect of lanthanum ions on visual cells was studied in the lateral eye of *Limulus* (using intracellular electrodes) and in the retina of *Astacus* (using intracellular and/or extracellular electrodes) after external application of 1 mM/l La^{+++} solution.

1.1 Results

1. In *Limulus* the effective input resistance of the measuring circuit was greatly increased under the action of lanthanum ions, while the membrane potential in the dark and the receptor potential showed only small changes.

2. In *Astacus* lanthanum ions increased the dark resistance and decreased dark potential and receptor potential drastically. The effect was not reversible by following treatment with physiological solution.

3. During superfusion by sodium-free external solution (ca. 1% of normal sodium concentration at the preparation) dark potential and receptor potential were slightly reduced in both preparations. The effective input resistance was increased. The effect of lanthanum ions in sodium-free solution did not differ markedly from the effect in normal sodium solution.

1.2 Interpretation

1. In *Limulus* lanthanum ions do not reach the photoreceptor cell membrane. Only those substances which can traverse the cell-layers surrounding the receptor cells in the *Limulus* ommatidium can reach the photoreceptor cell membrane, at least during the time of application used (ca. 1 hour).

2. In the *Astacus* preparation, lanthanum ions reach the photoreceptor cell membrane including the external surface of the microvillar membrane. Substances from the external solution have direct access to parts of the photoreceptor cell membrane, even though the access to some areas like the

microvillar membrane is restricted by the narrow extracellular cleft.

3. It is assumed that lanthanum ions in *Astacus* are bound strongly to the external surface of the visual cell membrane and block both the dark permeability and the light induced transient increase of the permeability by neutralizing surface charges and changing the potential profile of the visual cell membrane.

2. Introduction

Calcium ions are very important for the function of excitable membranes. Hille (1968) found that calcium binds to external surface charges of the membrane and thus influences the potential profile through the membrane markedly. From a number of experimental results concerning the action of calcium ions on characteristics of arthropod photoreceptor membranes we derived a working hypothesis that the conductance of the membrane is controlled by a calcium/sodium competition for binding sites at the cell membrane (Stieve, 1974).

In the pursuit of this hypothesis we wanted to study the influence of ions known to be able to replace calcium at binding sites. There is a whole series of such ions with increasing affinity to calcium binding sites (see discussion), the most commonly used of these being magnesium, cobalt and lanthanum ions. Lanthanum ions (referred to by Lettvin et al., 1964a, as "super calcium") were chosen for our experiments due to their strong affinity to replace calcium.

The results of other authors (cited in the discussion) suggest the assumption that ionic lanthanum as a rule does not penetrate into intact living cells but diffuses via extracellular pathways. Like in all experiments where extracellular changes are involved it was important to find out whether the lanthanum ions in our experiments actually reached the photoreceptor membrane, or whether they caused secondary effects at the membrane by acting on more distant compartments.

In our experiments slices of lateral eyes of *Limulus* and of retinas of *Astacus* were exposed to superfusion by salines of different ionic composition to measure the effect of lanthanum ions on the electrical properties of the visual cell membrane. Additionally the effect of sodium deficiency in the presence of lanthanum ions was measured for this reason: Lanthanum ions are known to influence the membrane conductance for various ions differently. As an electrical response of arthropod visual cells can still be recorded in sodium-free saline (Stieve et al. 1972) the combined effect of lanthanum ions and sodium deficiency seemed to be interesting. Geduldig and Junge (1968) report that in *Aplysia* neurons only the sodium channels are influenced by lanthanum ions.

3. Material and Methods

3.1 Preparation and measuring technique

Slices of lateral eyes of *Limulus polyphemus* or slices of isolated retinas of the crayfish *Astacus leptodactylus* were kept in streaming saline during the experiments. The method will be described in detail elsewhere (Stieve et al., Jül-Bericht, 1976).
Two different techniques were used:

(a) Measurement by one intracellular electrode of pre-stimulus membrane potential, pMP, (in some figures also referred to as dark potential, DaP), receptor potential, ReP, input impedance of the measuring circuit, R_d, (resistance between intracellular and extracellular electrode in the dark), and the light-induced change of this resistance ΔR_m.

(b) Measurement of the receptor potential of the isolated receptor cell layer of the *Astacus* retina by external electrodes, sometimes simultaneous with intracellular recording from one cell of the same retina.

The salines used to perfuse the test chamber containing the preparation are described in Table 1. Under consideration of the solubility of La^{+++} the pH of the salines was decreased to 6.9 to 7.0, to keep lanthanum ions in solution. The temperature of the preparation during the experiments was ca. $15^{o}C$. The preparation was illuminated by white light at regular intervals (Mercury lamp 8,500 lx $\hat{=}$ $1.38 \cdot 10^{16}$ photons $\cdot$ $cm^{-2} \cdot sec^{-1}$ for JB 79-83, 85-88, 108-113; Xenon lamp 90,000 lx $\hat{=}$ $1.46 \cdot 10^{17}$ photons $\cdot$ $cm^{-2} \cdot sec^{-1}$ for JB 119-123). Every 10 min the preparation was stimulated by a short flash (50 ms in the experiments with *Limulus* and 10 ms for *Astacus*, shorter than the latency period). A longer flash (1000 ms) was applied every 30 min.

3.2 Procedure

The experiments began with a pre-period of 60 min during which the preparation was superfused with normal physiological saline (Table 1, L_1, A_1) or salines of slightly varied ionic composition (L_4, A_4). This pre-period was followed by main periods of varying duration in which the preparation was exposed to different test solutions, and finally by an after-period of at least 60 min in the same saline as in the pre-period.

3.3 Evaluation

The following parameters of the receptor potentials were measured:

(a) for short light stimuli

h_{max} (mV) the peak-amplitude value of the transient of the receptor potential (ReP).

t_{lat} (msec) the latency-period - the time from the beginning of the stimulus until intersection of the tangent line in the steepest point of the rising phase of the ReP with the extrapolated level of the membrane potential before the stimulus.

t_{max} (msec) the time-to-peak - the time from the beginning of the stimulus until the maximum of the transient is reached.

t_2 (msec) the decrease-time - the time in which the ReP decreases from h_{max} to $h_{max/2}$.

(b) for long light stimuli, in addition to h_{max}, t_{lat}, and t_{max}, which were measured in the same way as described for short light stimuli,

h_e (mV) the plateau-value - the amplitude measured at stimulus end.

h_a (mV) the after-potential - the amplitude measured 500 ms after stimulus end.

h_{max}/h_e the shape-quotient.

In intracellular recordings the pre-stimulus membrane potential pMP (mV) was measured as difference to the zero reference level recorded before insertion of the electrode into the visual cell. The pMP (which, in this publication, is identical with the membrane potential in the dark, DaP) was measured and corrected to allow for potential drifts during the experiment. (For details of this correction procedure see Stieve et al., Jül-Bericht, 1976).
In the following the stimulus duration τ will appear, if necessary, as subscript to the respective parameter (e.g. $h_{max_{20}}$ = h_{max} after light flash of 20 ms duration). Similarly the subscript "i" or "e" will indicate whether intracellular or extracellular electrodes were used (e.g. h_{max_i} = h_{max} of receptor potential recorded by intracellular electrode).

For evaluation and compilation of groups of experiments the measured parameters of the receptor potentials were normalized (%) with respect to the reference values measured from a ReP recorded at the end of the pre-period (a-values).

3.4 Impedance measurement

Impedance measurements were carried out using current pulses through the intracellular electrode. Two parameters were measured:

R_d (MΩ) the dark resistance, i.e. the effective input resistance of the measuring circuit (the sum of the resistances of intracellular measuring electrode and extracellular indifferent electrode, and the resistance between these electrodes which includes the resistance of the visual cell membrane).

ΔR_m (MΩ) the light-induced change of the dark resistance at the time of the peak amplitude of the receptor potential. This change can be attributed mainly to a change of the resistance of the visual cell membrane.

The measurements of the membrane resistance were generally less reproducible than the measurements of the membrane potential. Therefore the values of the membrane resistance are not summarized in tables; the relative resistance changes recorded in the course of the experiments will merely be mentioned in the text.

4. Results

4.1 *Limulus*

4.1.1 The influence of 1 mM/l La^{+++} in a saline of normal physiological extracellular sodium concentration on the electrical response of the *Limulus* photoreceptor (retinular cells, intracellular measurement)

The effect of 1 mM/l $LaCl_3$, added to a slightly changed saline (L_5, Table 1) on the measured membrane characteristics was studied in five experiments. In the pre-period (60 min) the test chamber was perfused with SO_4^{--} and HCO_3^--free saline (L_4). In the main-period (120 min) the preparation was exposed to a saline containing La^{+++} (L_5); and in the after-period (60 min) again saline L_4 was used. The results are shown in Figs. 1 and 2 and summarized in Table 2.

Only small changes occur under the influence of 1 mM/l La^{+++}, except for a pronounced increase of the dark resistance. The recorded effects change in a way depending on the duration of exposure to the La^{+++}-containing saline: After 60 min main period the membrane potential (pMP) is decreased on the average to 70-80%, after another hour it is increased to 120-125%.

The maximum of the pMP and the maximal amplitude h_{max} of the receptor potential after 30 min main period in Fig. 2, which is not shown in the averaged results (first b-value in the main period after 55 min) is not characteristic for the average response.

Changes of receptor potential following short stimuli

The peak amplitude h_{max} shows a little decrease to 85% after one hour and an increase to 115% in the second hour under the influence of La^{+++}. The time parameters are increased continuously: the latency-period t_{lat} to 165% and the time-to-peak t_{max} to 145%. While these increases are uniform, the increase of the decrease-time t_2, while reaching greater values of up to 500%, is less regular (great standard deviation). The changes of the amplitudes of the receptor potential are more or less parallel to the changes of the pre-stimulus membrane potential pMP, except for the after-period where the pMP remains increased.

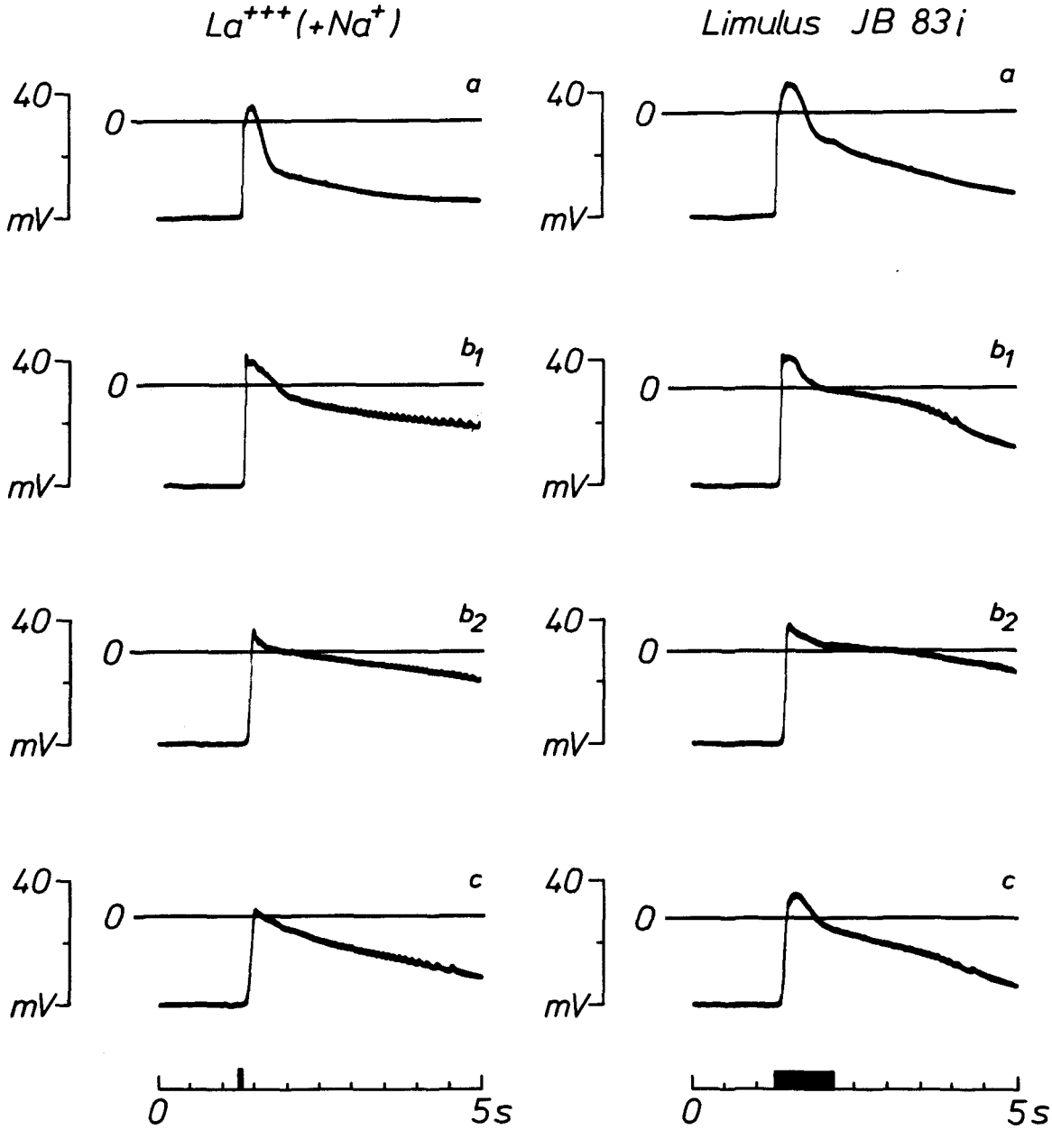

Fig. 1

The influence of external application of 1 mM/l La^{+++} on *Limulus* retinular cell. Receptor potentials intracellularly recorded after 50 msec (left) and 1000 msec (right) constant light stimuli.
a: reference potential after 55 min (left) and 60 min (right) in saline L_4;
b_1: after 55 min and 60 min;
b_2: after 115 min and 120 min in saline L_5 containing 1 mM/l La^{+++};
c: after 55 min and 60 min in saline L_4.
(JB 83i)

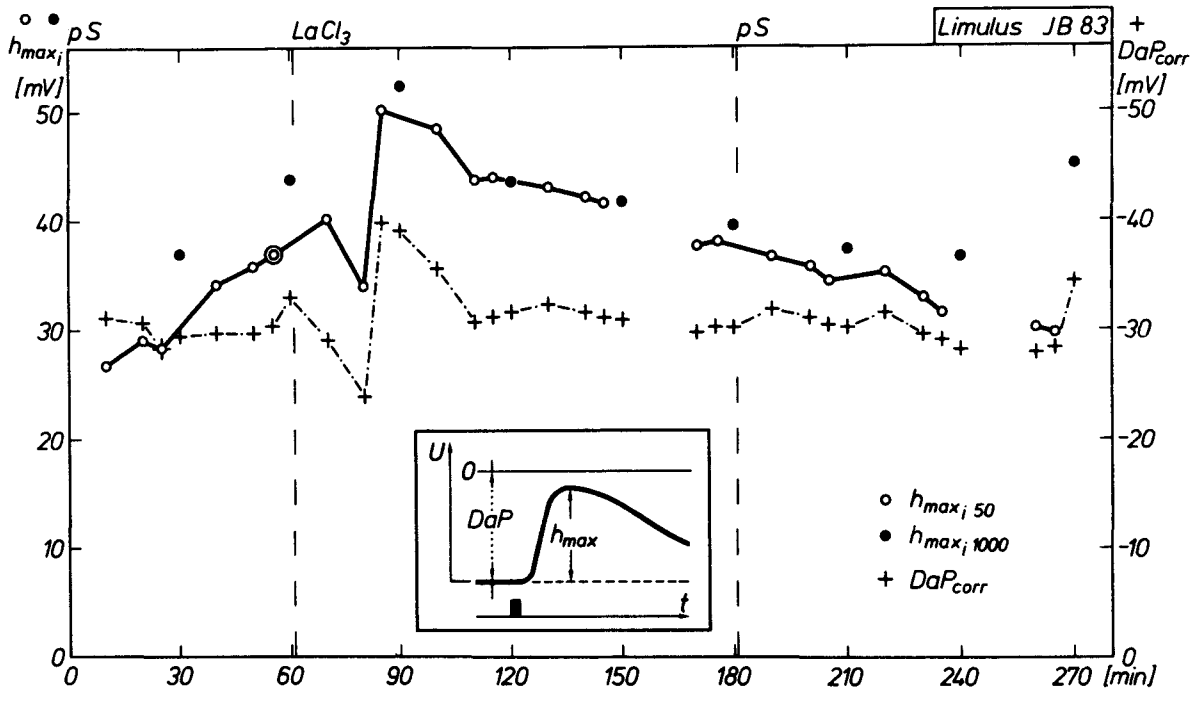

Fig. 2

The influence of external application of 1 mM/l La^{+++} on *Limulus* retinular cell. Plot of peak amplitude (h_{max}) of receptor potentials and pre-stimulus membrane potential (pMP = DaP). Intracellular recording after 50 msec and 1000 msec constant light stimuli.
pS: perfusion with saline L_4;
$LaCl_3$: perfusion with saline L_5 containing 1 mM/l La^{+++}.
(JB 83i)

Changes of receptor potential following long stimuli

All measured amplitudes h_{max}, h_e and h_a show parallel changes. Therefore the shape-quotient h_{max}/h_e does not change significantly. The changes of the time-to-peak t_{max} are parallel to those recorded for short stimuli, but less regular.

The changes of the amplitude parameters h_{max}, h_e, and h_a are partially reversible after washing out the La^{+++}-containing saline in the after-period. The changes of the pre-stimulus membrane potential, the dark resistance, and the time parameters are not reversible.

4.1.2 The influence of 1 mM La^{+++} in a sodium-free saline on the electrical response of the *Limulus* photoreceptor (retinular cells, intracellular measurement)

In four experiments we tested whether the unexpectedly weak effect of lanthanum on membrane characteristics of the Limulus photoreceptor could be due to a special property of the visual cell membrane, in analogy to the conditions described by Geduldig and Junge (1968), who found that in the neuron of Aplysia only one type of membrane potential dependent channels, a calcium channel is blocked by cobalt, while the other type, a sodium channel, is unaffected. If similar channels exist in the visual cell membrane, but quantitatively so many more sodium channels than calcium channels that the blocking of the latter by lanthanum would have only little effect, this effect should become decisive in the absence of extracellular sodium.

The experiments were done in the following way:

After a pre-period of 60 min in normal saline (L_1) the test chamber was perfused for 60 min (b-period) with a sodium-free saline (L_2) and thereupon for 90 min (c-period) with a sodium -free saline containing 1 mM/l La^{+++} (L_3). Due to the perfusion of the test chamber with sodium-free streaming saline in the b-period the sodium concentration at the preparation is less than 5 mM/l (1% of the normal concentration) 30 min after the switch from saline L_1 to L_2. In two successive after-periods

the chamber was first perfused with sodium-free saline (L_2) for 30 min (d-period, not shown in the table) and finally with normal saline (L_1) for 60 min (e-period).
The results of these experiments are described in Figs. 3 and 4 and summarized in Table 3.

The effect of sodium deficiency

In the sodium-free saline (b-period) the pre-stimulus membrane potential pMP decreases to ca. 70% and the peak amplitude h_{max} of the receptor potential to ca. 60% of the reference values. The changes of the time parameters are not significant; the latency period t_{lat} increases slightly, while the time-to-peak t_{max} and the decrease-time t_2 are shortened. All amplitude values of the receptor potential (long stimuli) decrease by about the same degree. The dark resistance increases to about 150%. These results agree with those described by Wulff (1973), and Wulff et al. (1975).

The effect of lanthanum together with sodium deficiency

The results correspond entirely with those obtained in normal sodium concentration. Due to the influence of La^{+++} (c-period) the depolarization of the pre-stimulus membrane potential pMP is not further increased, but there is some additional increase of the dark resistance (to 150-200 MΩ). There is a slight further decrease of the peak amplitude h_{max} for short stimuli. For long stimuli h_{max} does not change, but the plateau-value h_e and the after-potential h_a are further decreased. Consequently the shape-quotient h_{max}/h_e increases (to 160%). The latency-period t_{lat} is markedly prolonged (from 111 to 188%), the time-to-peak t_{max} remains practically unchanged, and the decrease-time t_2 is strongly shortened (from 71 to 27%).

While the dark resistance R_d was irregularly increased in sodium-free saline, it increases still more strongly under the influence of lanthanum. The membrane resistance change ΔR_m was difficult to measure exactly, but could still be recorded.

The amplitude of the receptor potentials recorded in the saline containing La^{+++} (L3) fluctuates considerably in the experiment shown in Fig. 4, much more than in the La^{+++}-free salines. This fluctuation is not typical, i.e. it does not occur in all experiments; generally the value of the S.E. of the mean is low for h_{max}.

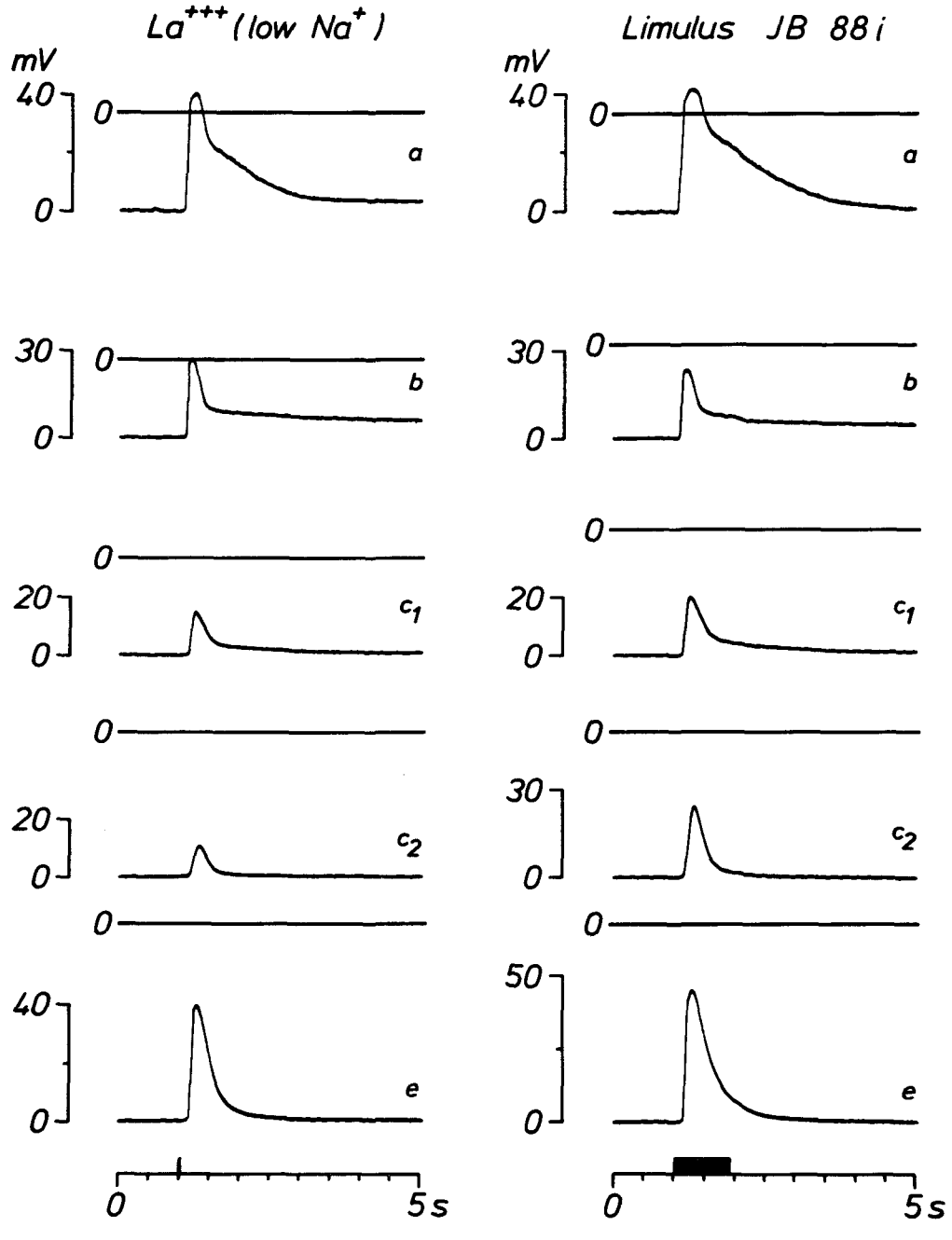

Fig. 3

The influence of external application of 1 mM/l La^{+++} together with extracellular Na^{+}-deficiency on *Limulus* retinular cell. Receptor potentials intracellularly recorded after 50 msec (left) and 1000 msec (right) constant light stimuli.
a: reference potential after 55 min (left) and 60 min (right) in saline L_1;
b: after 55 min and 60 min in saline L_2 (Na^{+}-free);
c_1: after 55 min and 60 min;
c_2: after 85 min and 90 min in saline L_3 (Na^{+}-free, containing 1 mM/l La^{+++});
e: after 55 min and 60 min in saline L_1.
(JB 88i)

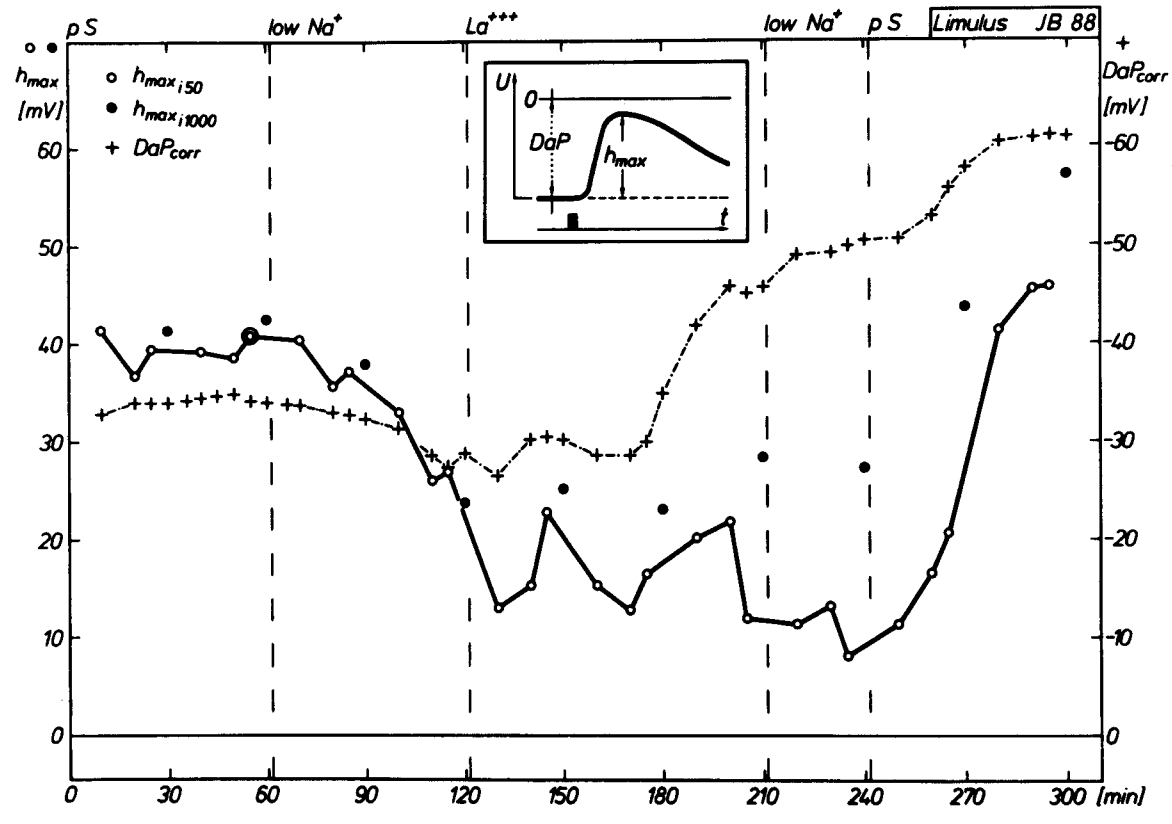

Fig. 4

The influence of external application of 1 mM/l La^{+++} together with extracellular Na^{+}-deficiency on *Limulus* retinular cell. Plot of peak amplitude (h_{max}) of receptor potentials and pre-stimulus membrane potential (pMP = DaP). Intracellular recording after 50 msec and 1000 msec constant light stimuli.
pS: perfusion with saline L_1;
low Na^{+}: perfusion with saline L_2;
La^{+++}: perfusion with saline L_3 containing 1 mM/l La^{+++}.
(JB 88i)

The effects due to lanthanum are only partially reversible. The dark resistance decreases slightly from 176% in the sodium-free saline containing La^{+++} (L3) to finally 141% in normal saline (L1). The pre-stimulus membrane potential pMP recovers well (95-100% in the e-period). The amplitude values of the receptor potential recover relatively well in some, but not in all, experiments, i.e. the S.E. of the mean is great for the pMP as well as for the amplitude values in the e-period. The latency -period remains irreversibly prolonged, while the shortening of the decrease-time t_2 is relatively well reversible (c-period 27%, e-period 78%). The time-to-peak t_{max}, not very much affected by La^{+++} (80-90%) is somewhat prolonged in the after -period (120-125%).

In summary the changes due to the application of lanthanum are almost similar in sodium-free as in normal sodium solution. The changes develop slowly in both cases. The effects of lanthanum together with sodium deficiency (difference between b and c values) are not very strong, and there is no marked parallelity between the changes of the pre-stimulus membrane potential and of the amplitude of the receptor potential (Fig. 4).

4.2 *Astacus*

4.2.1 The influence of 1 mM/l La^{+++} in a saline of normal extracellular sodium concentration of the electrical response of the *Astacus* retina

In five experiments the influence of 1 mM/l La^{+++} (saline A_5) on the receptor potential of the crayfish retina was measured by extracellular electrodes (Figs. 5 and 6, summarized in Table 4), and in one experiment simultaneously by intra- and extracellular electrodes (Fig. 7, Table 5).

In the pre-period (60 min) the test chamber was perfused with HCO_3-free saline (A_4). In the main period (90 min) the preparation was exposed to a La^{+++}-containing saline with normal sodium concentration (A_5), and in the after-period again normal saline (A_4) was used.

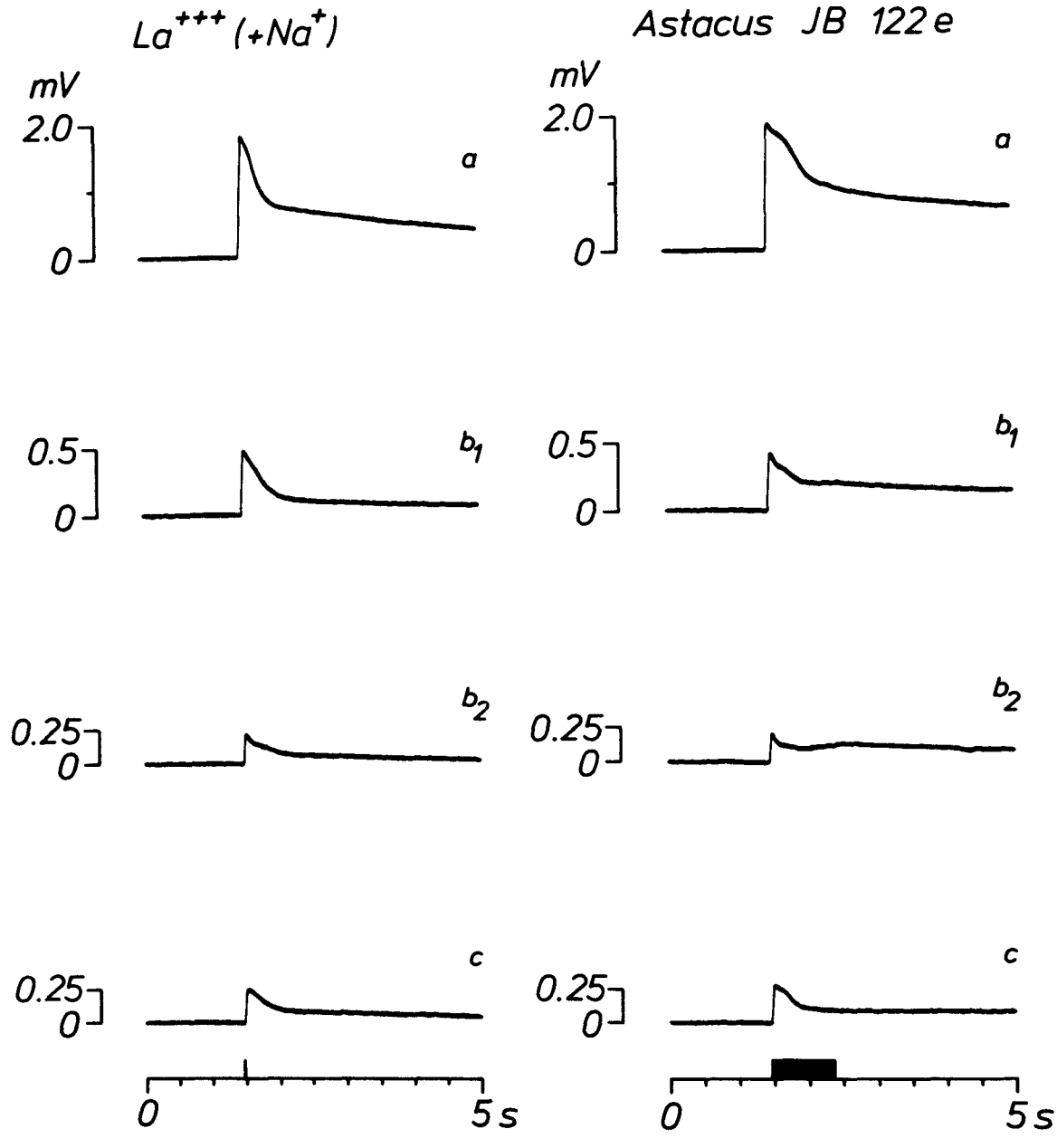

Fig. 5

The influence of external application of 1 mM/l La^{+++} on an isolated crayfish retina. Receptor potentials extracellularly recorded after 10 msec (left) and 1000 msec (right) constant light stimuli.
a: reference potential after 55 min (left) and 60 min (right) in saline A_4;
b_1: after 55 min and 60 min;
b_2: after 85 min and 90 min in saline A_5 (containing 1 mM/l La^{+++});
c: after 55 and 60 min in saline A_4.
(JB 122e)

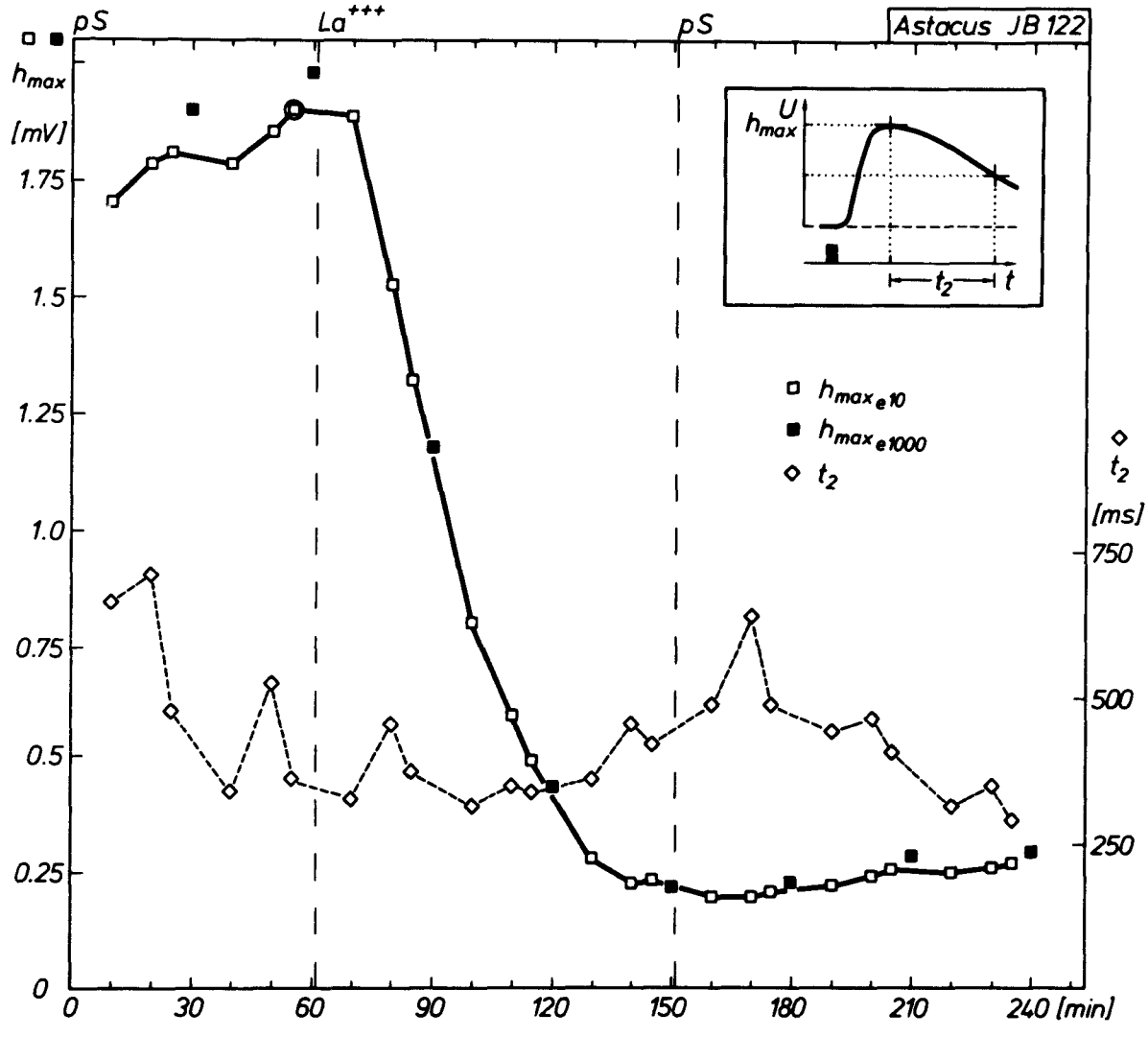

Fig. 6

The influence of external application of 1 mM/l La^{+++} on an isolated crayfish retina. Plot of peak amplitude h_{max} and decrease-time t_2 of receptor potentials. Extracellular recording after 10 msec and 1000 msec constant light stimuli.
pS: perfusion with saline A_4;
La^{+++}: perfusion with saline A_5 containing 1 mM/l La^{+++}.
(JB 122e)

Extracellular measurement

Under the influence of La^{+++} the receptor potential is slowly but markedly reduced in size and, after approximately 90 min, reaches a level of ca. 20% of the maximal amplitude h_{max}. The other amplitude parameters behave similarly. The time parameters show only minor changes, except for the decrease-time t_2 which reaches about 140% of its reference value.

After washing out the lanthanum solution the effect on the height of the receptor potential shows on the average no recovery; in some cases the amplitudes were even further decreased, while in others a slight recovery of the maximal amplitude (Fig. 6) was observed. The average value of the shape-quotient h_{max}/h_e is slightly increased in the after-period (117%). The latency-period t_{lat} is not markedly changed throughout the experiment. The time-to-peak t_{max} is increased to 110 to 120% of the reference value in the after-period (for short and long stimuli), while the decrease-time t_2 recovers beyond the reference value (to 77%).

The long time which is needed until the action of lanthanum develops is remarkable. The time until the maximal amplitude $h_{max_{10}}$ is reduced to 50% ($t_{1/2}$) is 29 $\pm$ 4.1 min. This value can be compared with values from former experiments (Stieve and Wirth, 1971), where the influence of increased external potassium concentration (217 mM/l) on the receptor potential of the crayfish retina was measured under similar conditions (Fig. 8). This K^+-increase also causes a reduction of the receptor potential amplitude. The time needed for a 50% decrease ($t_{1/2}$) was 3.5 $\pm$ 0.17 min (n = 15), i.e. the action of potassium on the receptor potential develops almost 10 times as fast as that of lanthanum in the crayfish retina.

Simultaneous intra- and extracellular measurement

The extracellularly measured parameters of the RePs in the one experiment (Table 5, Fig. 7) with simultaneous intra- and extracellular recording are on the whole similar to those just described (Table 4). (The reversibility of the effects cannot

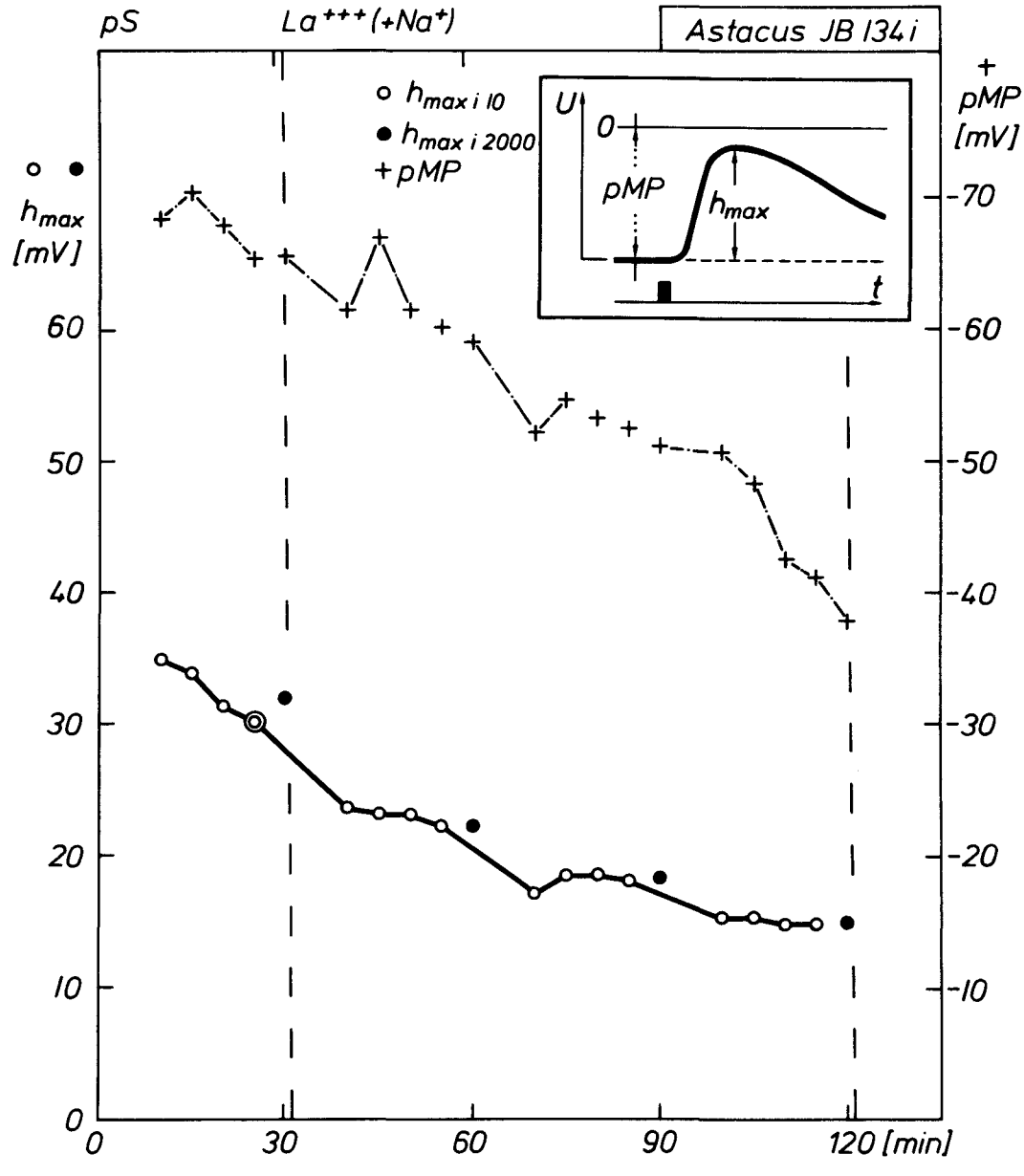

Fig. 7

The influence of external application of 1 mM/l La^{+++} on an isolated crayfish retina. Plot of pre-stimulus membrane potential (pMP) and peak amplitude (h_{max}) of receptor potentials. Intracellular recording after 10 msec and 1000 msec constant light stimuli.
pS: perfusion with saline A_4;
La^{+++} (+ Na^{+}): perfusion with saline A_5 containing 1 mM/l La^{+++}.
(JB 134i)

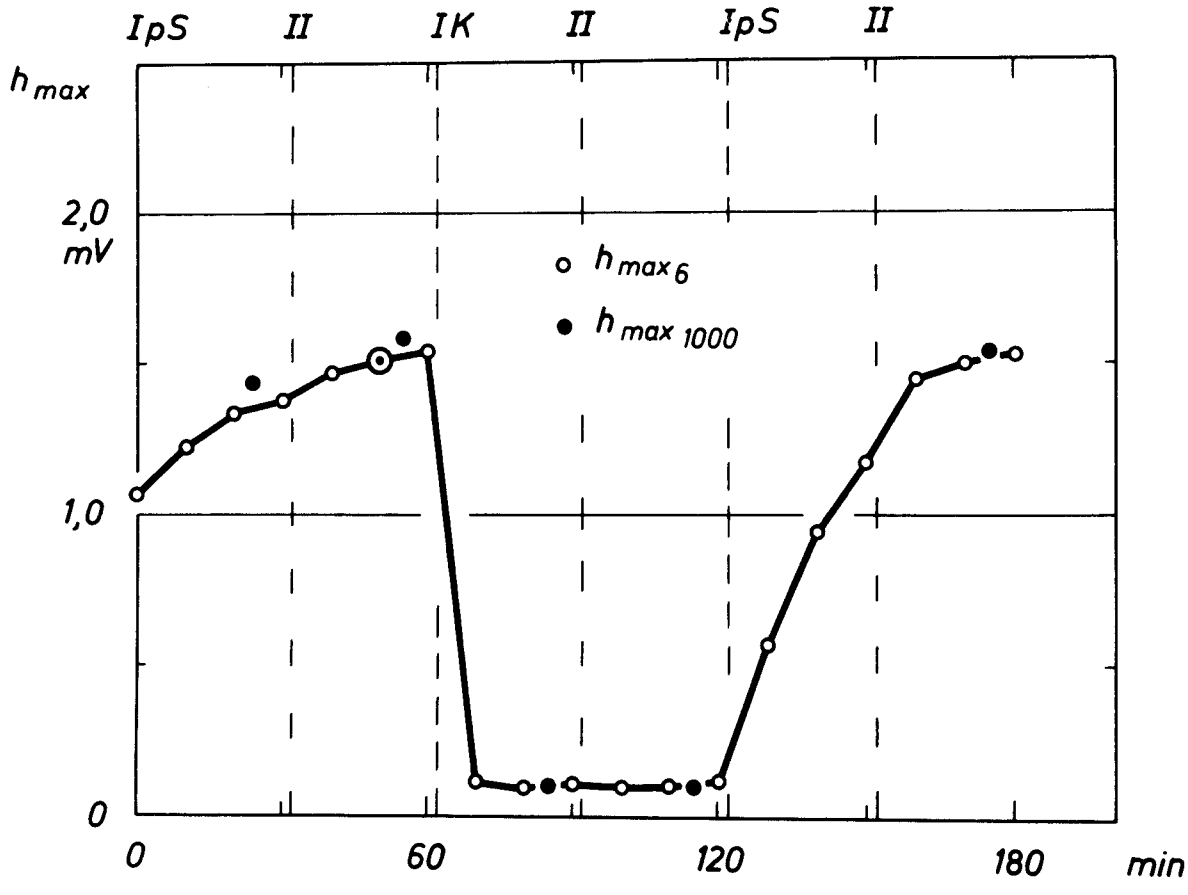

Fig. 8

The influence of external application of high external K^+ concentration on an isolated crayfish retina. Plot of peak amplitude h_{max} of extracellularly recorded receptor potentials after 6 msec and 1000 msec constant light stimuli.
pS: perfusion with normal physiological saline;
K: perfusion with 217 mM/l K^+ containing saline;
I and II: different modes of perfusion.
(A_{57})
(from Stieve and Wirth, 1971)

be compared because there is no after-period in the experiment shown in Fig. 7, due to failure of the intracellular electrode.) The main difference between intra- and extracellular recording in Table 5 is that the effect of lanthanum is more drastically shown by the extracellular measurement (decrease of peak amplitude h_{max} to 15-20% after 90 min) than by the intracellular measurement (decrease of h_{max} to 45-50% after 90 min). The difference is in the same direction, but less marked, for the other amplitude parameters. The time-to-peak t_{max} is increased under the influence of lanthanum in the intracellular measurement (ca. 140% after 90 min) and decreased in the extracellular measurement (50-80% after 90 min).
As can be seen in Fig. 7 the decrease of the peak-amplitude h_{max} (intracellular recording) is roughly speaking parallel to the decrease of the pre-stimulus membrane potential pMP, although h_{max} seems to have reached a level value toward the end of the experiment, whereas the pMP shows a tendency to decrease still more.

4.2.2 The influence of 1 mM/l La^{+++} in a sodium-free saline on the electrical response of the *Astacus* retina

In six experiments the influence of lanthanum ions on the receptor potential of the *Astacus* retina in sodium-free saline was tested. The receptor potential was measured simultaneously by intra- and extracellular electrodes in one experiment (intracellular recording shown by Figs. 9 and 10 and Table 6) and extracellularly in six experiments (Figs. 11 a, b and 12 a, b; summarized in Table 7).

Following the pre-period (60 min in normal saline (A_1) the test chamber was perfused in the main-periods first with a sodium -free saline (A_2) for 60 min (b-values) and then with a sodium -free saline containing 1 mM/l La^{+++} (A_3) for 60 min (c-values). In two successive after-periods the preparation was exposed again to the sodium-free saline (A_2) for 30 min (d-values) and finally to the normal saline (A_1) for 60 min (e-values).

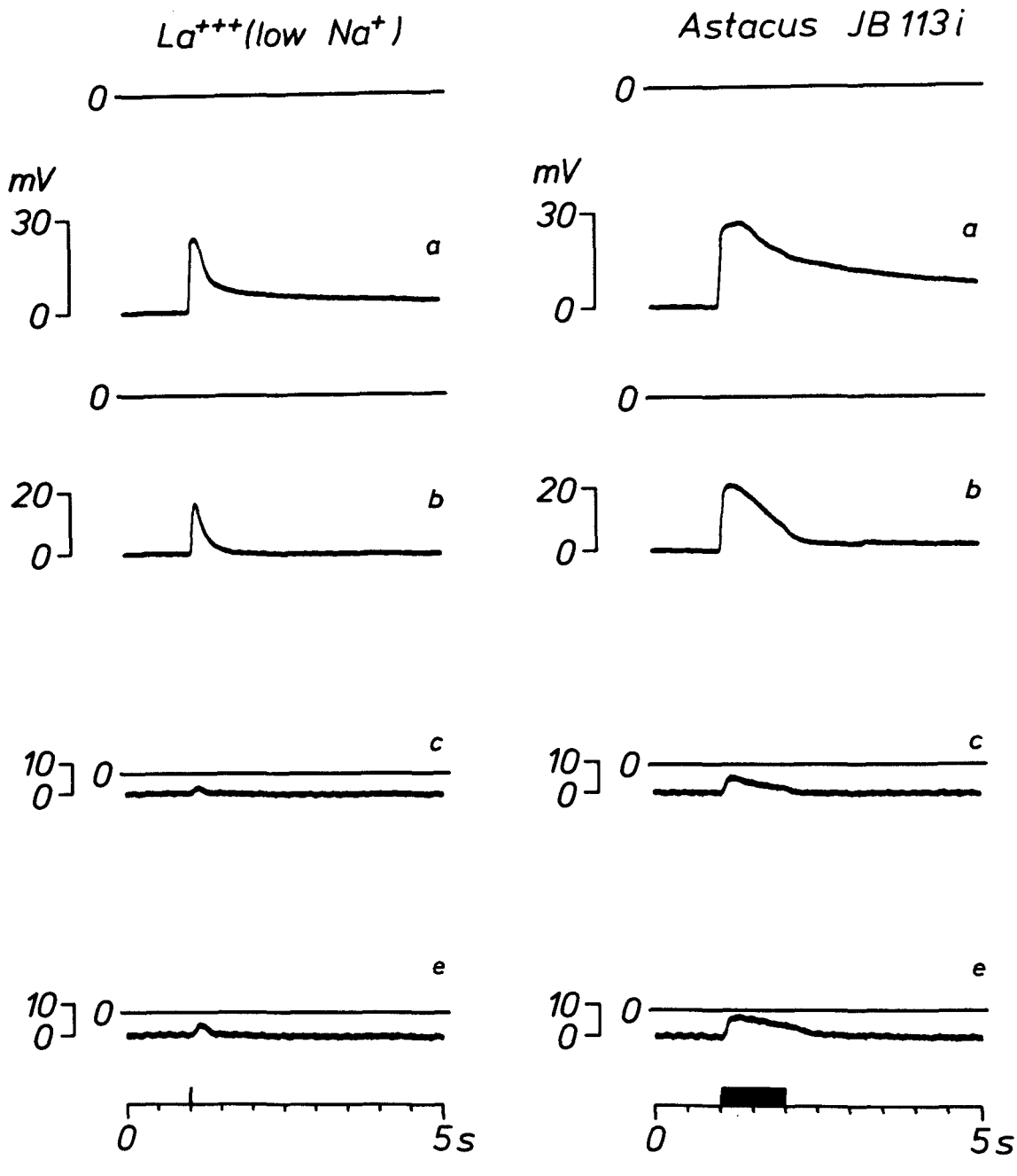

Fig. 9

The influence of external application of 1 mM/l La^{+++} together with extracellular Na^{+}-deficiency on an isolated crayfish retina. Receptor potentials intracellularly recorded after 10 msec (left) and 1000 msec (right) constant light stimuli.
a: reference potential after 55 min (left) and 60 min (right) in saline A_1;
b: after 55 min and 60 min in saline A_2 (Na^{+}-free);
c: after 55 min and 60 min in saline A_3 (containing 1 mM/l La^{+++});
e: after 55 min and 60 min in saline A_1.
(JB 113i)

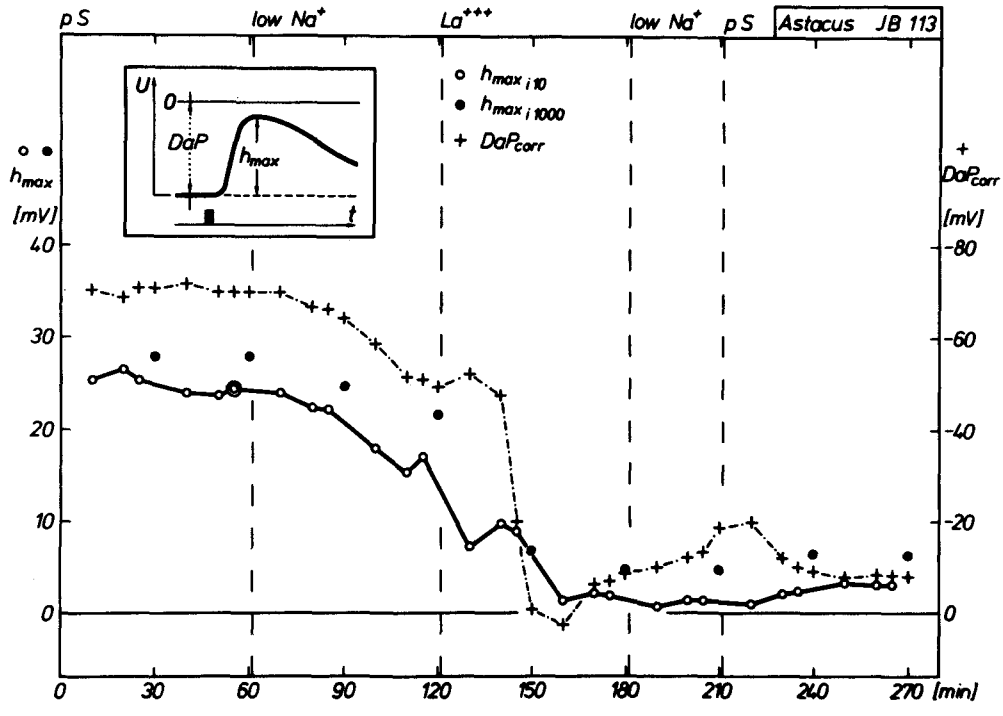

Fig. 10

The influence of external application of 1 mM/l La^{+++} together with extracellular Na^{+}-deficiency on an isolated crayfish retina. Plot of pre-stimulus membrane potential (pMP = DaP) and peak amplitude (h_{max}) of intracellularly recorded receptor potentials.
pS: perfusion with saline A_1;
low Na^{+}: perfusion with Na^{+}-free saline A_2;
La^{+++}: perfusion with saline A_3 containing 1 mM/l La^{+++}.
(JB 113i)

The effect of sodium deficiency, intracellular recording (Table 6)

External sodium deficiency leads to a decrease of the pre-stimulus membrane potential pMP to ca. 70%, and to a slight increase of the dark resistance to ca. 115%. The amplitudes of the receptor potential decrease, the plateau-value h_e and the after-potential h_a relatively more than the maximal amplitude h_{max}, which is reduced only to ca. 70-80%. The shape-quotient h_{max}/h_e increases to ca. 150%. The pre-stimulus membrane potential pMP is decreased to the same extent as the maximal amplitude h_{max} of the ReP (by ca. 20 to 30%). The latency-period t_{lat} is increased to 150%, while the time-to-peak t_{max} and the decrease-time t_2 become shorter. The decrease of t_{max} is relatively greater for long stimuli.

The effect of sodium deficiency, extracellular recording (Table 7)

The changes of the extracellularly measured receptor potentials differ somewhat from those of the intracellular recordings: Under the influence of sodium deficiency all amplitudes of the extracellularly recorded receptor potentials decrease (h_{max} for short stimuli to 35%, for long stimuli to 54%, h_e and h_a are still more reduced to less than 30%; shape-quotient h_{max}/h_e therefore increased to 225%). As in the intracellular recordings the latency-period t_{lat} is increased under the influence of sodium deficiency, while the time-to-peak t_{max} and the decrease-time t_2 are shortened.

Following the last long stimulus after 60 min perfusion with sodium-free saline the response of the preparation is very much decreased (Fig. 9). This demonstrates the increased adaptation sensitivity in sodium-free saline concentration which has already been described earlier (Stieve and Wirth, 1971; Wulff et al., 1975). The effect of sodium deficiency on the response of the *Astacus* retina (intracellular measurement) is similar to the effect on the *Limulus* photoreceptor (see 4.1.2), except that (1) the latency period is prolonged in *Astacus*, and (2) the reduction of $t_{max_{1000}}$ is more pronounced than that of $t_{max_{10}}$ in

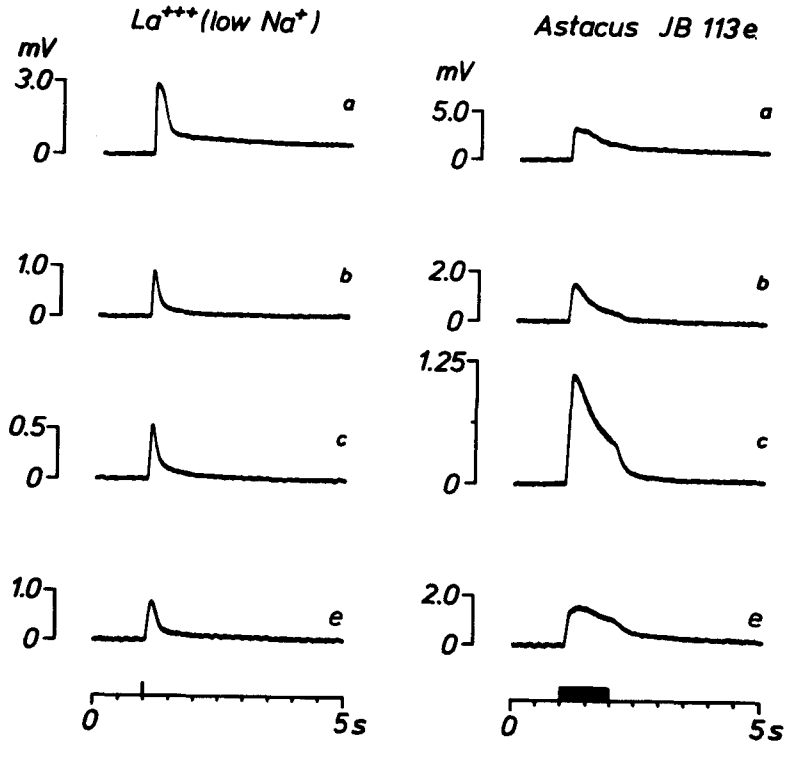

Fig. 11a

The influence of external application of 1 mM/l La^{+++} together with extracellular Na^{+}-deficiency on an isolated crayfish retina. Receptor potentials extracellularly recorded after 10 msec (left) and 1000 msec (right) constant light stimuli.
a: reference potential after 55 min (left) and 60 min (right) in saline A_1;
b: after 55 min and 60 min in Na^{+}-free saline A_2;
c: after 55 min and 60 min in saline A_3 containing 1 mM/l La^{+++};
e: after 55 min and 60 min in saline A_1.
(JB 113e)

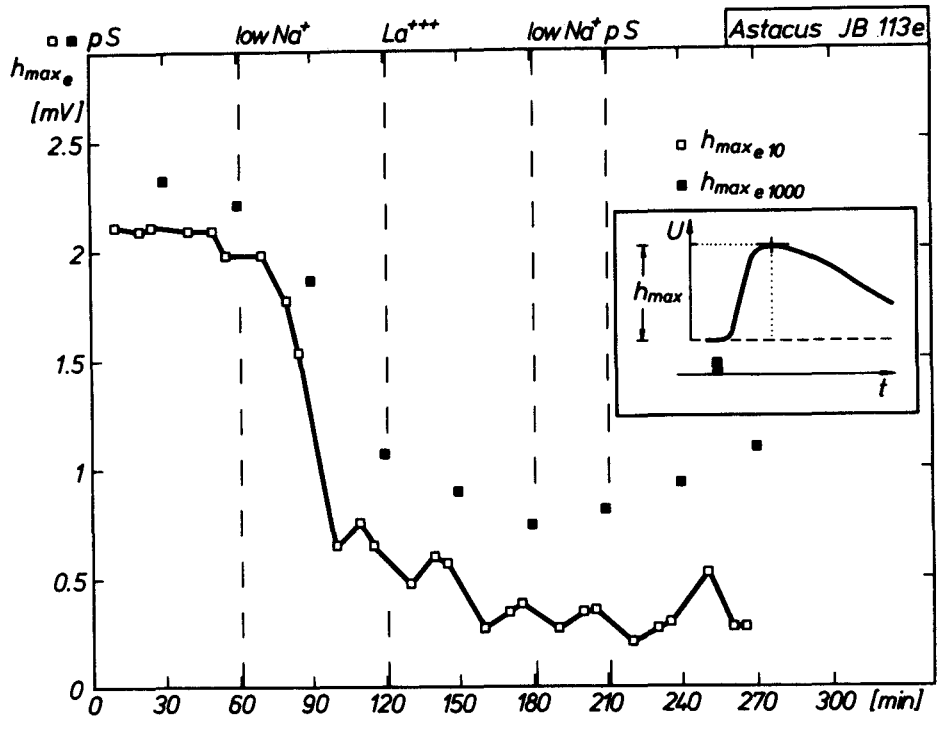

Fig. 11b

The influence of external application of 1 mM/l La^{+++} together with extracellular Na^{+}-deficiency on an isolated crayfish retina. Plot of peak-amplitude h_{max} of extracellularly recorded receptor potentials after 10 msec and 1000 msec constant light stimuli.
pS: perfusion with saline A_1;
low Na^{+}: perfusion with Na^{+}-free saline A_2;
La^{+++}: perfusion with saline A_3 containing 1 mM/l La^{+++}.
(JB 113e)

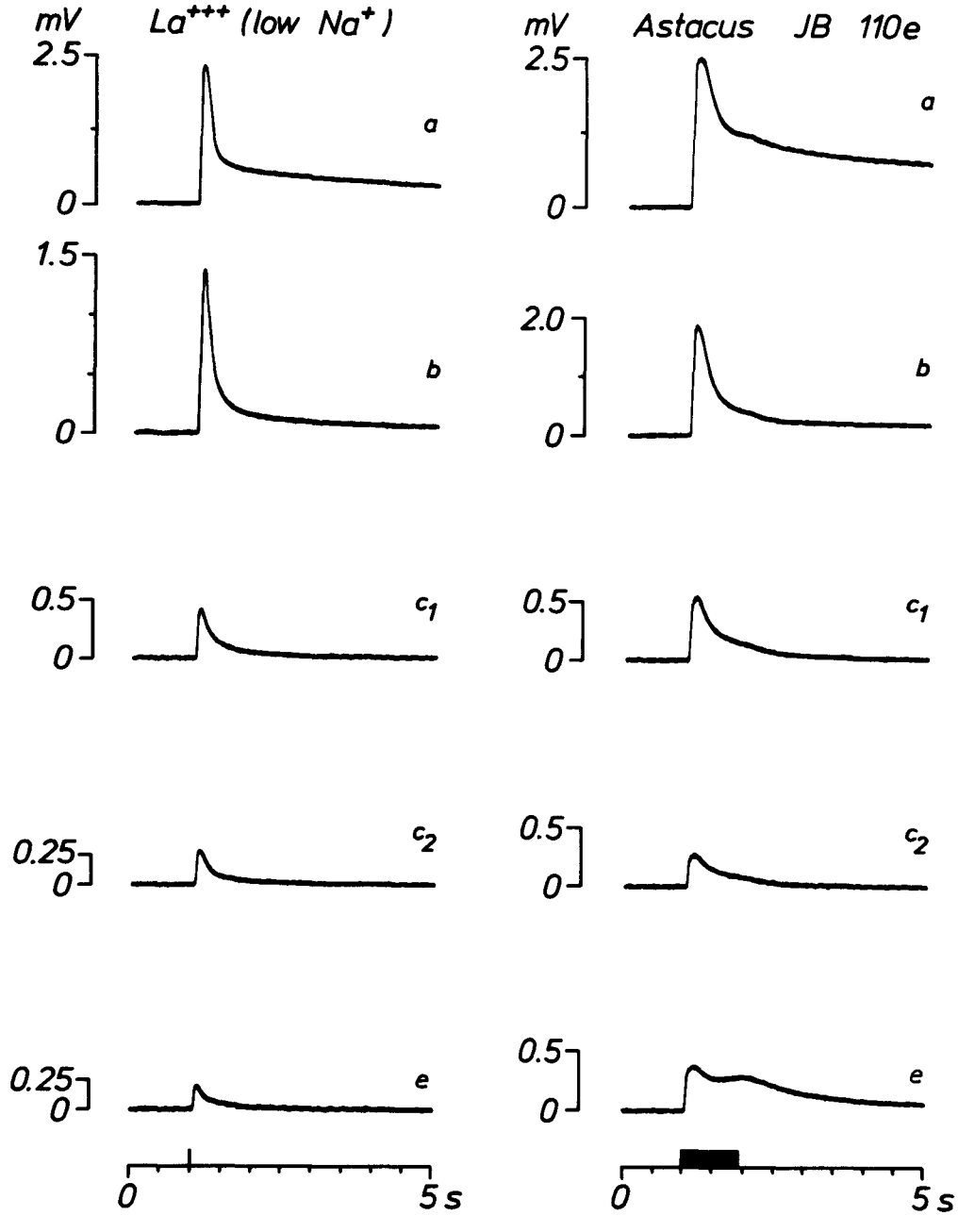

Fig. 12a

The influence of external application of 1 mM/l La^{+++} together with extracellular Na^{+}-deficiency on an isolated crayfish retina. Receptor potentials extracellularly recorded after 10 msec (left) and 1000 msec (right) constant light stimuli.
a: reference potential after 55 min (left) and 60 min (right) in saline A_1;
b: after 55 min and 60 min in Na^{+}-free saline A_2;
c_1: after 55 min and 60 min;
c_2: after 85 min and 90 min in saline A_3 containing 1 mM/l La^{+++};
e: after 55 min and 60 min in saline A_1.
(JB 110e)

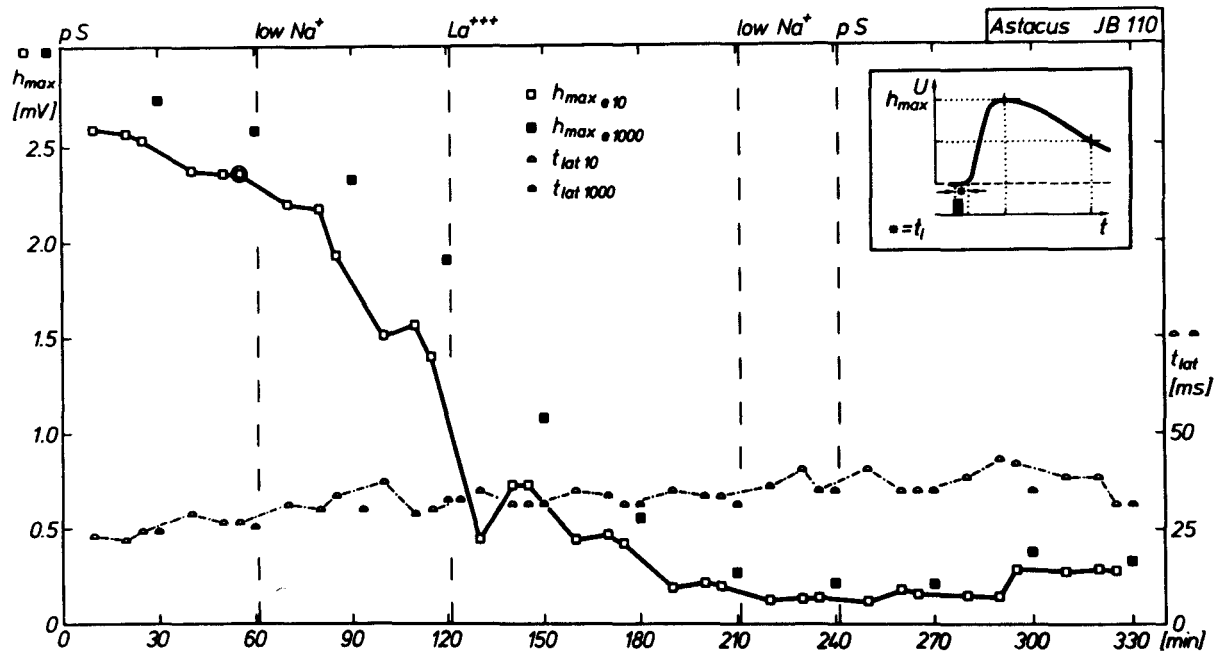

Fig. 12b

The influence of external application of 1 mM/l La^{+++} together with extracellular Na^{+}-deficiency on an isolated crayfish retina.
Plot of peak-amplitude h_{max} and latency-period t_{lat} of extracellularly recorded receptor potentials after 10 msec and 1000 msec constant light stimuli.
pS: perfusion with saline A_1;
low Na^{+}: perfusion with Na^{+}-free saline A_2;
La^{+++}: perfusion with saline A_3 containing 1 mM/l La^{+++}.
(JB 110e)

Astacus. However the differences seem not to be significant.

The action of lanthanum together with sodium deficiency (intracellular recording) (Table 6)

The intracellular recordings show that the addition of 1 mM/l La^{+++} to the sodium-free saline leads to a further decrease of the pre-stimulus membrane potential pMP to 10%, and to an increase of the dark resistance to ca. 200%. The amplitudes of the receptor potential are further decreased; h_{max} to 10 to 20%, h_e to 10%, h_a disappears. The shape-quotient h_{max}/h_e rises to 180%. As can be seen in Fig. 9, the decrease of the maximal amplitude of the receptor potential is not parallel to that of the pre-stimulus membrane potential. The latency-period t_{lat} is decreased for short stimuli and increased for long stimuli. The time-to-peak t_{max} increases in both cases, while the decrease-time t_2 is further reduced.

The action of lanthanum together with sodium deficiency (extracellular recording) (Table 7)

After 90 min treatment with La^{+++} the maximal amplitude h_{max} of the receptor potential is ca. 10% of the reference value. The plateau-value h_e is reduced to the same extent, the after-potential h_a still more (to 4%), the ratio h_{max}/h_e is almost unchanged. There is a small decrease of the time-to-peak t_{max} for short stimuli, an increase of t_{max} for long stimuli, a small, but not significant, increase of the decrease-time t_2, and no substantial change of the latency-period t_{lat}.

In the extracellular measurements the effect of lanthanum in sodium-free solution concerns mainly the amplitudes of the receptor potentials. Also in sodium-free saline the effects of lanthanum develop rather slowly: The time $t_{1/2}$ to reduce the height of the response to 50% is 30 $\pm$ 2.7 min (n = 6).

A slight recovery of the changed parameters is indicated after washing out the lanthanum by the normal saline containing sodium (A_1). In the extracellular recordings all parameters recover

somewhat, while in the intracellular recordings only the time-parameters recover partly whereas the amplitude parameters remain decreased, and the dark resistance is even more increased in the normal saline than it was in the presence of lanthanum.

Discussion

According to theoretical assumptions by Lettvin et al. (1964b) lanthanum ions should have a much greater electrostatic attraction for any negative calcium binding site than calcium ions themselves because of their relatively small size and high charge density.

This assumption was supported by many experiments. Blaustein and Goldman (1968) investigated the action of several polyvalent cations on voltage-clamped lobster axons. They found that increased external calcium concentration leads to shifting of the sodium conductance curve in the current-voltage diagram, i.e. a higher extent of depolarization is necessary to obtain a given conductance change. At equimolar concentration the effect of substitute ions for calcium was increasing for a series of cations in this sequence: magnesium, calcium, barium, nickel, cobalt, cadmium, lanthanum, aluminium, ferrum.

La^{+++} ions, which have a rather strong effect, do not abolish the action potential, but make it slower and also somewhat smaller. The data suggest that these ions may occupy the same cation binding sites on the membrane, and that the relative effects on the membrane conductance and rate parameters depend on the relative binding constants of the ions.

Van Breemen and de Weer (1970) found in their investigation of lanthanum inhibition of the calcium efflux from the squid giant axon that 5 mM/l La^{+++} make the axon unexcitable, after the action potentials had become slower and smaller before. This means that lanthanum ions (slowly) affect the sodium conductance increase during excitation.

These effects are not or only slightly reversible, apparently due to the high binding constant of lanthanum ions.

Furthermore the calcium transport through many excitable membranes (in the axon from the interior to the exterior) is restricted by lanthanum.

Mela and Chance (1965) found that the calcium transport in mitochondria is affected by lanthanum ions.

Also the calcium spike (regenerative response) at nerve terminals is abolished when lanthanum ions are applied (Miledi, 1971), i.e. either lanthanum ions compete with calcium ions and replace these at their binding sites, or the binding of lanthanum ions changes the membrane conformation in a way that the calcium conductance change can no longer be produced.
Similar experiments were performed by Geduldig and Junge (1968) who found that in *Aplysia* neurons there exist two types of electrical excitable conductances; one of them is realised by calcium channels, the opening of which is blocked by an agent like cobalt, whereas the other type of channels, for sodium, is not influenced by cobalt. Since lanthanum binds even stronger than cobalt our experiments should indicate whether analogous different types of light channels (letting Na^+ ions pass during excitation, thus producing the receptor potential) exist in the arthropod photoreceptor membrane, besides the dark channels which let preferably potassium pass (Stieve, 1974).
Baker et al. (1969) found that a certain component of the sodium efflux in the squid axon is coupled with a calcium influx; the latter is irreversibly blocked by 0.1 mM/l external La^{+++}.
These results are in favour of strong binding of lanthanum ions to excitable cell membranes. It cannot be washed away or replaced by physiological ions to a reasonable degree - within the duration our experiments - by washing with physiological saline, because of its high binding constant. It is not known for certain whether lanthanum ions penetrate the membrane, however much is in favour of the assumption that this is not the case, or at least only to a minor extent.

Colloidal lanthanum is often used as a marker for extracellular spaces of fixed preparations. Fixed membranes have permeability properties which differ substantially from those of living membranes. As shown by Doggenweiler and Frenk (1965) for various fixed preparations (frog retina and sciatic nerve, lobster and crayfish walking leg nerve, crayfish nerve chord) and Revel and Karnovsky (1967) for mouse heart and liver tissue, lanthanum

does, as a rule, not penetrate fixed membranes, but marks the interstices between cells. This was confirmed by Overton (1967) for localized lanthanum staining of the intestinal brush border of mice, and by Cancilla (1968) who demonstrated the extracellular space of the human Langerhans granule by lanthanum. Perellet and Baumann (1969) showed that lanthanum in the fixed eye preparation of the drone penetrates from the bathing solution into the extracellular space of the microvillous region and fills the entire extracellular space of the rhabdome. In their experiments lanthanum and ferritin could pass through junctional complexes between retinular cells and enter the microvillous area of the drone eye preparation but could not penetrate deeply into the rhabdome under their conditions.

The only exception known to us from the observation that colloidal lanthanum does not penetrate fixed membranes is reported by White and Walther (1969). They found that in fixed eye preparations of the leech lanthanum can be found, by electron microscopical investigation, to be penetrated ca. 30 nm deep into the cytoplasmic side of the membrane, but only into a special infolded area of the visual cell membrane.

In living tissues also ionic lanthanum is used to investigate the extracellular spaces, as it normally does not penetrate the cell membranes.
(Whittembury and Rawlins, 1971, toad kidney;
Machen, Erlij, and Wooding, 1972, rabbit gallbladder and intestine;
Tisher and Yarger, 1973, renal tubule of rat;
Erlij, Machen, Martinez-Palomo, and Smith, 1972;
Erlij, 1971;
Erlij and Martinez-Palomo, 1972
Ussing, Erlij, and Lassen, 1974;
all dealing with the frog skin.)
It was shown by Erlij and his co-workers that in the frog skin there are "tight junctions" which normally restrict the invasion of lanthanum ions, but which can be opened e.g. by pressure or by hypertonic urea solution.

5.1 The accessibility of the photoreceptor cell membrane in our preparations

Judging from the effect of lanthanum to other excitable cell membranes we expected that after treatment by 1 mM/l La^{+++} from the external solution both preparations would either become soon inexcitable or would be strongly affected concerning their receptor potentials.
The striking difference in the sensitivity of the *Limulus* visual cells to the application of lanthanum as compared to the *Astacus* visual cells could be mainly due to two different reasons: Either the photoreceptor cell membranes of the two preparations are very different in their sensitivity to lanthanum, or lanthanum does not reach the photoreceptor cell membrane in the *Limulus* ommatidium under our experimental conditions.

Limulus

It is very improbable that the visual cell membrane of *Limulus* is actually reached by lanthanum in our experiments, as the observed effects are very small and occur only after a long time (more than 1 h) of application. Fig. 13 shows a scheme of the extracellular spaces (greatly enlarged) in a cross-section of an ommatidium of *Limulus* (after electron microscopical investigations by Fahrenbach, 1969, and Miller, 1957). In the fixed preparation the extracellular spaces have a thickness of ca. 20 nm. As can be seen in the scheme the visual cells in the *Limulus* ommatidium are surrounded by several layers of pigment cells, and the outside of the ommatidium is enclosed in a sheath of fibres from the basilar membrane. It is conceivable that the lanthanum ions cannot traverse from the outside solution through the extracellular spaces to reach the photoreceptor cell membrane.

This may be due to several reasons. According to Fahrenbach (1969) there are various junctional specialisations in the *Limulus* eye, like e.g. spotlike welds between pigment cells, which might render the access to the photoreceptor cell membrane difficult. As described by Erlij and Martinez-Palomo (1972) "tight junctions" in the frog skin prevent the invasion of

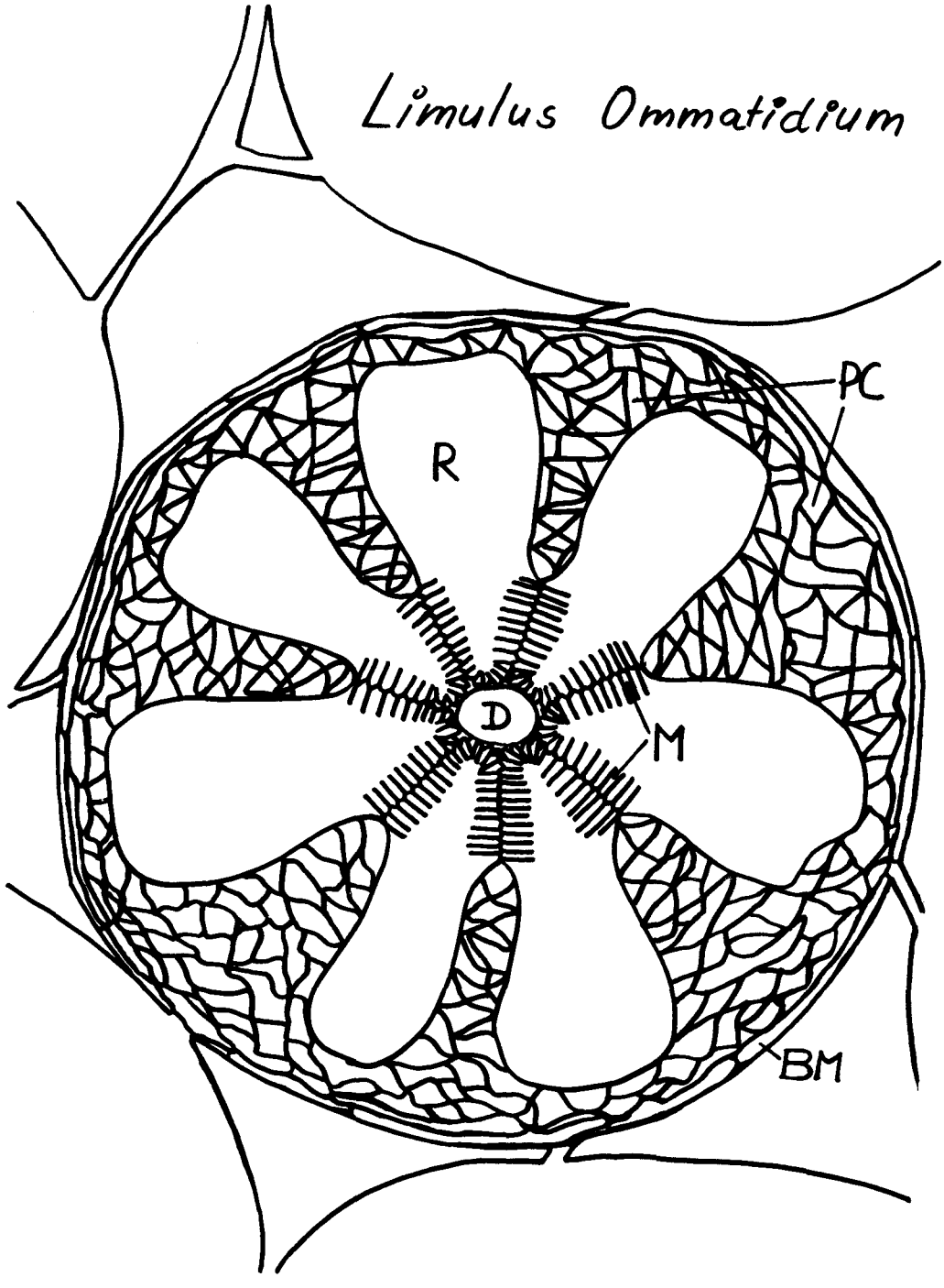

Fig. 13

Scheme of the location of the extracellular clefts in a cross-section of the *Limulus* ommatidium. Extracellular clefts are shown by black lines, greatly enlarged.
D - dendrite of eccentric cell, R - retinula cell, M - microvilli, PC - pigment cell, BM - basilar membrane (drawn after Fahrenbach 1969).

lanthanum ions in vivo. As "tight junctions" have not been found in the areas surrounding the visual cells in the *Limulus* ommatidium, the distance might be too long to be passed by diffusion within a reasonable time. Another possibility could be that part of the lanthanum is precipitated already in the extracellular space, and that the sediment itself blocks the further access. In this connection it should be noted that in the fixed preparation of the drone eye colloidal lanthanum penetrates into the microvillar area (Perrelet and Baumann, 1968). This could mean that the affinity of the extracellular space to lanthanum is greater in the microvillar area than in other areas, so that lanthanum can diffuse more easily, provided that it reaches this area at all.

The basilar membrane seems to be permeable to large molecules such as haemocyanine according to Fahrenbach (1969).
The experimental results concerning the action of lanthanum in the *Limulus* ommatidium can be explained by assuming that the lanthanum ions bind to the outer layer and the outer membranes of the ommatiduum. As a consequence the dark resistance would increase because the binding of lanthanum leads to an increase of the resistances of the outer membranes of the ommatidium. As the effects of lanthanum are not noticeable until ca. 2 h application, and concern mainly the latency-period and the decrease-time, but practically not the maximal amplitude of the receptor potential, this can mean that it takes very long for lanthanum ions to reach the photoreceptor membrane, and that they make the response only slower but not smaller, at least within the duration of our experiments, and that the ionic exchange (passive or active) between external solution and the extracellular space immediately surrounding the visual cells is affected by lanthanum in a way leading to the observed effects.

In our preparation of the *Limulus* ommatidium with attached cornea only those ions can reach the surface of the visual cell from the bathing solution, which can penetrate through the cell membranes of the pigment cells and through the pigment cells themselves, or which can travel through the extracellular spaces.

A number of ions normally present in the intra- and extracellular space, which influence the receptor potential, such as calcium, sodium and potassium ions, and also not ionized substances, obviously reach the membrane of the photoreceptor cell, presumably by crossing the pigment cell layer. Probably lanthanum is not able to use these pathways. The transport mechanism for lanthanum is different from that for potassium ions which are present in high concentration in the cell interior and can pass through many bio-membranes. Sodium and calcium ions can also easily pass through certain bio-membranes, while they can traverse excitable membranes only during excitation, or by means of active transport mechanisms, like e.g. in the frog skin. Lanthanum ions are obviously not easily transportable and furthermore even block the calcium transport and certain kinds of sodium transport. All these reasons suggest the assumption that the spaces immediately surrounding the cell are somehow changed by lanthanum ions.

Astacus

In the *Astacus* retina, at least in our isolated retina preparation where the cornea and the crystalline cones are removed, lanthanum ions can reach the membrane of the visual cells. Fig. 14 shows a scheme of the extracellular spaces (also greatly enlarged) as seen in the longitudinal section through an *Astacus* ommatidium (after Eguchi, 1966, and Krebs, 1972).

Fig. 14 also offers an explanation for the fact that the extracellularly recorded receptor potential is always measured negative at the distal side. If the mechanisms causing the receptor potential (light channels) are restricted to the microvillous area, the distally negative measurement of the receptor potential by extracellular electrodes can be expected when the counter electrode is on the proximal side, since the microvillar membrane seems to have better electrical contact to the distal side of the retina than to the proximal side.

As shown by electron microscopy (Krebs, 1972) the photoreceptor cells are in their distal part in direct contact with greater extracellular spaces (blood lacunes), and are only proximally surrounded by tapetum cells. The distal part of the visual cells is directly accessible to substances solved in the bathing solution. The removal of the cornea and the crystalline cone

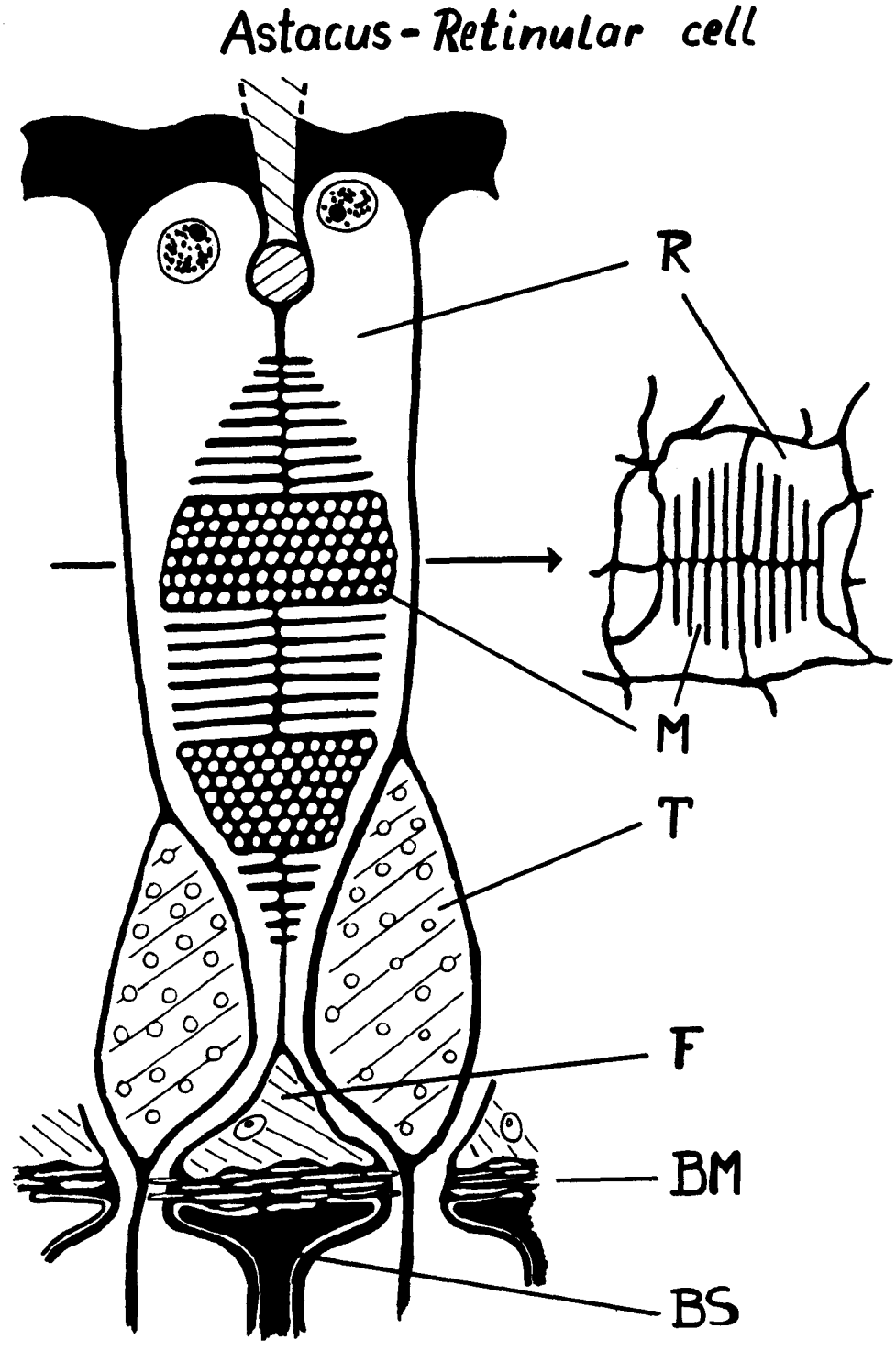

Fig. 14

Scheme of the location of extracellular spaces and clefts in a longitudinal and a cross-section of an *Astacus* photoreceptor.
Black: extracellular volume, extracellular clefts drawn greatly enlarged.
R - retinular cell, M - microvilli, t - tapetum cell, F - foot cell, BM - basilar membrane, BS - blood sinus (drawn after Eguchi 1965 and Krebs 1972).

allows better access to the visual cell membrane, probably also to the small extracellular spaces of the rhabdomeric area.
In the cross-section of the *Astacus* photoreceptor the intra-microvillar space is probably seperated from the remaining extracellular space by junctional complexes (zonulae occludentes) located between the retinula cells close to the rhabdom (Krebs, 1972).
If lanthanum ions were bound to the cell membrane only at the distal ends of the visual cells, and not to the microvillar membrane one would expect a certain increase in dark resistance (which is typical for the binding of polyvalent cations, e.g. Ca^{++} and La^{+++}, to excitable membranes, as described by Blaustein and Goldman, 1968). The loss of excitability could be due to an influence on the dark potential. This seems not to be the case, since the decrease of the dark potential does not occur earlier than the decrease of the receptor potential, but is more or less parallel to it.
Another argument for the assumption that the decrease of the receptor potential is not caused by a decrease of the dark potential is this: When the excitability of the *Astacus* retina is diminished by potassium depolarization (Stieve et al., Jül-Bericht 1976), i.e. by reduction of the pre-stimulus membrane potential, the shape of the receptor potential is changed in a different way (for h_{max} = 30% of reference value: t_{lat} ca. 150%, t_{max} ca. 100%, t_2 ca. 50%) than in the experiments described here (for h_{max} 30% of reference value: t_{lat} ca. 100%, t_{max} ca. 100%, t_2 ca. 130%).
The loss of excitability would be best explained by the assumption that the lanthanum ions reach, and bind to, also those parts of the cell membrane which undergo the light-induced conductance changes. This is most probably the microvillar area (as has been shown for the leech by Fioravanti and Fuortes, 1972).
The accessibility of the external surface of the microvillar membrane in the rhabdomeric area seems to be conceivable according to Fig. 14. The question whether lanthanum ions actually reach that area would also be an object for further histochemical or autoradiographic studies (with an appropriate substitute substance). As shown by autoradiographic investigations, calcium ions reach the rhabdomeric area from the

external solution, on extracellular pathways or also by crossing the cell membranes (Stieve and Degenkolbe, unpublished). Lanthanum ions are assumed to have only partly the same distribution spaces and transport mechanisms as calcium ions.
One would expect that the dark potential would be influenced prior to the receptor potential by the action of lanthanum ions because the distal parts of the membrane (which are not involved in the light-induced conductance change but produce the dark potential) are reached earlier.
As dark potential and receptor potential, however, were decreased more or less parallelly, this suggests that the influence of La^{+++} ions on the microvillous area (which, according to Krebs, 1972, represents 90% of the total cell surface) is more prominent than the effect on the distal membrane parts.

5.2 The action of lanthanum ions on the properties of the photoreceptor membrane of *Astacus*

Since we believe that the photoreceptor membrane in the *Limulus* ommatidium is not reached by a reasonable amount of lanthanum in our experiments, we cannot discuss the effect of lanthanum in the case of *Limulus*.

In *Astacus* lanthanum binds to the visual cell membrane. Due to that binding the dark resistance is increased, because the dark channels are probably closed or reduced by the lanthanum ions. This could be due to a strong binding to the surface and a consequent change of the surface potential and the potential profile through the cell membrane, as assumed for the nerve membrane by Hille (1968).
An increase of the dark resistance leads to a decrease of the dark potential because the conductance in the dark (which has a selective preference for potassium) is decreased. The membrane loses a considerable fraction of its dark conductance, and consequently of its selectivity to act like a potassium electrode.
Lanthanum also prevents the measurable light-induced conductance change, and consequently the receptor potential, i.e. it prevents the light-induced transient opening of the light

channels. A plausible explanation for this effect could be that lanthanum blocks the conductance changes by somehow binding to the cell membrane, e.g. by occupying membrane surface charges and changing the potential profile through the membrane.

This assumption would fit into the hypothesis of a calcium-sodium binding competition at the visual cell membrane. During light excitation lanthanum in the place of calcium could be bound so strongly that it cannot be replaced by sodium, even though the affinity of the binding sites changes in favour of sodium. Consequently the conductance change cannot take place. The blocking of the light response by lanthanum is an argument in favour of the assumption that in the course of the light-induced opening of the light channels calcium bound to the membrane has to be transiently released.

Another explanation would lie in a sodium flux blockage, like the sodium/calcium exchange in nerve membranes, or a calcium flux with calcium ions reversibly occupying binding sites (Baker 1972).

The shape of the receptor potential is not changed, only the size, but proportionally of all parts of the receptor potential. This possibly indicates that the action of lanthanum is restricted to the areas of the cell membrane which it has actually reached and that the neighbouring areas are not as much affected as in the case of the depolarizing action of e.g. potassium.

The experiments with low external sodium concentration give no indication that there exist two types of light channels (sodium and calcium channels) also in the *Astacus* photoreceptor cell membrane, as described for the *Aplysia* neuron by Geduldig and Junge (1968).

Although this is also no decisive evidence against the existence of such two types of light channels the similar action of lanthanum on the receptor potential in normal and low sodium concentration makes two types of light channels not probable.

The fact that the maximal amplitude of the receptor potentials in normal external sodium concentration does not become zero by the action of lanthanum but remains on a constant low value (extracellular recording) can mean either that lanthanum does not block the membrane completely when it is bound, or that lanthanum does

not reach all parts of the membrane which are responsible for the production of the dark resistance. The former possibility would agree with the results of van Breemen and de Weer (1970) who found that the calcium efflux could not be restricted by more than 87%, but the latter possibility seems to be somewhat more probable because also depolarisation by potassium never leads to a total loss of excitability in the isolated retina of *Astacus* (see Fig. 7).

In the intracellular measurement (Table 5) the decreases of dark potential and receptor potential amount to almost the same values. However the time course is not exactly identical. This contradicts the explanation that the loss of excitability to light is alone due to the diminishing of the dark potential.

The fact that lanthanum decreases both the dark conductivity and the light-induced conductivity change of the visual cell membrane makes it probable that the binding sites for calcium and lanthanum ions are influencing the light channels as well as the dark channels. This is in favour of the assumption of surface charge sites. If binding sites were located at every channel it would not be probable that both types of channels were equally influenced. A simpler assumption would be that unspecific surface charges exist, and that the potential profile over the whole membrane is changed, which in turn influences the channels.

While in our experiments light and dark channels seem to be equally influenced by lanthanum, Vogel (1973) found that lanthanum affects sodium and potassium channels differently in the Ranvier nodal membrane of the toad. In their experiments the normal leakage conductance was reduced - not completely reversible - to 73% in 0.5 mM La^{+++}. The maximal sodium permeability was reduced to 54% while the sodium equilibrium potential was not affected. Their results indicate that 1 mM La^{+++} be equivalent to 55 mM Ca^{++} for this effect. Lanthanum causes the activation (m) and the inactivation term (h) of the sodium permeability to shift in the depolarizing direction. The shift was completely reversible on returning to normal physiological solution. Similarly to calcium lanthanum causes the inactivation term to shift less than the activation term.
From their findings the authors assume that the effective charge density is lower near the potassium channels than near the sodium channels.

5.3 The influence of sodium deficiency on the membrane characteristics of the visual cell

The changes of the electrical properties of the *Limulus* and *Astacus* photoreceptor cell membrane due to low external sodium concentration consist in a small decrease (to 60-70%) of the dark potential, a decrease of almost the same height of the receptor potential and an increase of the dark resistance. The changes were similar to those observed by Wulff (1973), except for the increase of the dark resistance.
According to Stieve and Wirth (1971) and Wulff et al. (1975) the adaptation sensitivity of photoreceptor cells of *Limulus* and *Astacus* is greatly increased in low external sodium concentration. According to Wulff lowering of the external sodium concentration results in a decrease of the intracellular sodium content.
The observed changes of the receptor potentials in our experiments could be primarily due to a change in the state of adaptation, since our preparations were stimulated at regular intervals and were therefore always slightly light-adapted. Due to the increase in adaptation sensitivity in low sodium solution the decrease of the receptor potential could strongly depend on the number of photons of previous stimulation.

Under normal conditions the dark potential does not depend much on the sodium concentration. The observed decrease of the dark potential in both preparations in the sodium-deficient medium could, to a certain degree, also be due to light adaptation. Under normal conditions light and dark adaptation do not influence the dark potential markedly (Stieve et al., 1975). In this case a possible explanation for the observed change of the dark potential could be the following: It is believed that the intracellular sodium concentration controls the active sodium potassium transport, which can also be electrogenic under certain conditions. In sodium-deficient medium the sodium gradient across the cell membrane necessary for complete dark adaptation can still be sufficiently restored by active transport mechanisms after light-adaptation, despite the low sodium concentration,

but the time needed for complete restoration of the gradient will be markedly longer.

Under normal conditions during light adaptation the (electrogenic) transport is triggered at a specific increased intracellular sodium concentration. As external sodium deficiency causes also low intracellular sodium concentration, a lack of sodium results in retarded triggering of the pump activity. As the potassium transport is coupled with the sodium transport this delayed pump activity also affects the potassium gradient which results in a decrease of the resting potential. Although the gradients will be fully restored even by the reduced pump activity, the process of restoration will be retarded.

Immediately after light adaptation the dark potential should be reduced since the potassium gradient is reduced. Under normal conditions this is conceiled because of the electrogenic activity of the sodium/potassium pump which increases the membrane potential for several minutes during the pumping process. If the electrogenicity or the activity of the pump is reduced in low sodium, the dark potential should be a truer measure of the potassium concentration gradient following a light stimulus than under normal conditions.

The increase of the dark resistance in low external sodium concentration, but normal calcium concentration, could be caused by the binding of relatively more calcium ions to the membrane if one assumes a calcium/sodium binding competition. In this case the influence of calcium would increase and consequently the membrane conductance would become lower.

Final remarks

Lanthanum ions applied from the external solution affect the membrane of the photoreceptor cell strongly by increasing its resistance in the dark and preventing the light-induced resistance change - presumably by blocking the light channels and blocking or reducing the dark channels.
Lanthanum is possibly bound at the cell membrane and replaces calcium at its binding sites. Our results are consistent with the hypothesis (Stieve 1974) that calcium is released from the cell membrane and replaced by sodium in order to open the light channels.
The prerequisite for lanthanum to act on the cell membrane is to reach it.
The accessibility of the photoreceptor membrane is different in the two preparations used. While in the isolated retina of *Astacus* the access to the distal part of the membrane is free, and the access to other membrane parts is only restricted by the narrowness of the extracellular clefts, in *Limulus* (slices of lateral eye) only such substances seem to reach the photoreceptor cell membrane which can traverse the pigment cell layer. The way through the extracellular space seems to be very long, or even inaccessible, at least for the duration of lanthanum application, and is probably somehow blocked, either by physiological structures or by the action of the lanthanum ions themselves. This is most probably also true for the ventral eye of *Limulus*.
The reduction of the receptor potential, without changing of the shape, suggests that the action of lanthanum is restricted more or less to the immediate area where it is bound, and does not much affect other parts of the cell.
In *Astacus* substances like lanthanum, which have to invade the microvillar area to affect the conductance changes, need much longer for their action than those which measurably influence membrane potential and receptor potential at any part of the membrane surface (e.g. in the distal part of the retinula cell), or can pass through the membrane and the whole cell, like potassium.

The results of the experiments with La^{+++} together with sodium deficiency give no indication that there exists more than one type of light channels. Since lanthanum affects both dark and light channels the most plausible assumption is that lanthanum acts via binding to surface charges and changing the potential profile of the membrane.
The experiments also suggest that sodium deficiency affects receptor potential and dark potential by increased adaptation sensitivity, decreased intracellular sodium concentration, and therefore less sensitivity for the control of the activity increase of the active sodium/potassium transport following a light stimulus.

Changing of the extracellular ion concentration leads to changes of the concentration in the space immediately adjacent to the visual cell membrane in a rather complicated ion species-, time-, and space-dependent way, because of the restricted accessibility of the cell membrane, which is certainly very different for lanthanum, calcium, potassium and sodium ions in the two preparations used for our experiments.

Table 1 Ionic composition [mM/l] of the salines used

	Na^+	K^+	Ca^{++}	Mg^{++}	La^{+++}	$Tris^+$	Cl^-	SO_4^{--}	HCO_3^-
Limulus									
L_1	488.3	10.0	10.0	55.0	-	-	566.0	30.0	2.3
L_2	-	10.0	10.0	55.0	-	473.3	613.3	-	-
L_3	-	10.0	10.0	55.0	1.0	473.3	610.8	-	-
L_4	470.8	10.0	10.0	55.0	-	5.0	610.8	-	-
L_5	483.8	10.0	-	55.0	1.0	5.0	606.8	-	-
Astacus									
A_1	207.3	5.0	14.0	3.0	-	-	244.0	-	2.3
A_2	-	5.0	14.0	3.0	-	207.3	246.3	-	-
A_3	-	5.0	14.0	3.0	1.0	207.3	246.3	-	-
A_4	202.3	5.0	14.0	3.0	-	5.0	246.3	-	-
A_5	221.3	5.0	-	3.0	1.0	5.0	240.3	-	-

Table 2

Effect of extracellular application of La^{+++} on pre-stimulus membrane potential and receptor potential of *Limulus* retinular cell(intracellular recording) (JB 79-83)

a) Stimulus duration τ ca 50 ms

n = 5	pMP_{corr}	h_{max}	t_{lat}	t_{max}	t_2
a reference value (L_4)	-34 ± 3.9 mV	35 ± 8.8 mV	73 ± 7.2 ms	435 ± 121 ms	1756 ± 630 ms
b_1 55 min La^{+++} (L_5)	77 ± 15 %	85 ± 13 %	116 ± 8.2 %	82 ± 5.8 %	255 ± 153 %
b_2 115 min La^{+++} (L_5)	120 ± 8 %	115 ± 7.8 %	163 ± 12 %	146 ± 15 %	493 ± 341 %
c 55 min in L_4	140 ± 25 %	80 ± 20 %	140 ± 7.1 %	140 ± 34 %	336 ± 196 %

b) Stimulus duration τ ca 1000 ms

n = 5	pMP_{corr}	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e
a reference value (L_4)	-34 ± 3.9 mV	39 ± 7.9 mV	509 ± 137.2 ms	29 ± 5.5 mV	26 ± 4.5 mV	1.3 ± 0.2
b_1 60 min La^{+++} (L_5)	72 ± 15 %	86 ± 11 %	92 ± 17 %	79 ± 15 %	83 ± 19 %	104 ± 8.2 %
b_2 120 min La^{+++} (L_5)	124 ± 12 %	127 ± 16 %	187 ± 66 %	135 ± 5.5 %	148 ± 12 %	93 ± 14 %
c 60 min in L_4	128 ± 28 %	106 ± 37 %	227 ± 92 %	112 ± 35 %	111 ± 41 %	91 ± 4 %

Table 3

Effect of extracellular application of La^{+++} together with sodium deficiency on pre-stimulus membrane potential and receptor potential of *Limulus* retinular cell (intracellular recording) (JB 85-88)

a) Stimulus duration τ ca 50 ms

n = 4	pMP_{corr}	h_{max}	t_{lat}	t_{max}	t_2
a reference value (L_1)	-34 ± 2.1 mV	41 ± 2.2 mV	64 ± 13 ms	281 ± 25.6 ms	1398 ± 354 ms
b 55 min Na^+-free (L_2)	72 ± 12 %	60 ± 5.9 %	111 ± 9 %	83 ± 12 %	71 ± 25 %
c 55 min La^{+++} (L_3)	73 ± 6.4 %	47 ± 2.3 %	188 ± 34 %	82 ± 13 %	27 ± 8 %
e 55 min in L_1	99 ± 31 %	61 ± 24 %	187 ± 28 %	123 ± 17 %	78 ± 45 %

b) Stimulus duration τ ca 1000 ms

n = 4	pMP_{corr}	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e
a reference value (L_1)	-36 ± 2.1 mV	43 ± 1.8 mV	335 ± 37 ms	28 ± 3.0 mV	22 ± 4.7 mV	1.6 ± 0.2
b 60 min Na^+-free (L_2)	66 ± 15 %	55 ± 9.5 %	91 ± 15 %	47 ± 9.5 %	55 ± 12 %	121 ± 11 %
c 60 min La^{+++} (L_3)	74 ± 12 %	57 ± 4.6 %	86 ± 4.8 %	39 ± 5.8 %	45 ± 12 %	159 ± 25 %
e 60 min in L_1	96 ± 32 %	69 ± 26 %	124 ± 19 %	40 ± 12 %	36 ± 10 %	165 ± 47 %

Table 4

Effect of extracellular application of La^{+++} on receptor potential of *Astacus* retina (extracellular recording) (JB 119-123)

a) Stimulus duration τ ca. 10 ms

n = 5	h_{max}	t_{lat}	t_{max}	t_2
a reference value (A_1)	1.4 ± 0.3 mV	17.9 ± 0.9 ms	67 ± 7.0 ms	256 ± 44 ms
b_1 55 min La^{+++} (A_5)	26 ± 5.0 %	106 ± 2.2 %	99 ± 2.2 %	142 ± 30 %
b_2 85 min La^{+++} (A_5)	18 ± 2.8 %	100 ± 19 %	93 ± 11 %	137 ± 28 %
c_1 55 min in A_4	15 ± 4.3 %	120 ± 33 %	122 ± 22 %	91 ± 10 %
c_2 85 min in A_4	12 ± 4.9 %	88 ± 5.2 %	116 ± 22 %	77 ± 11 %

b) Stimulus duration τ ca. 1000 ms

n = 5	h_{max}	t_{lat}	t_{max}	h_e	h_a	h_{max}/h_e
a reference value (A_4)	1.4 ± 0.3 mV	18 ± 1.2 ms	67 ± 4.7 ms	0.6 ± 0.1 mV	0.5 ± 0.1 mV	2.5 ± 0.2
b_1 60 min La^{+++} (A_5)	23 ± 4.7 %	104 ± 15 %	106 ± 11 %	24 ± 3.4 %	26 ± 5.6 %	95 ± 19 %
b_2 60 min La^{+++} (A_5)	17 ± 3.0 %	104 ± 20 %	84 ± 6.0 %	19 ± 4.2 %	23 ± 6.5 %	98 ± 14 %
c_1 60 min in A_4	15 ± 4.2 %	106 ± 18 %	105 ± 9.2 %	11 ± 2.0 %	14 ± 2.9 %	120 ± 22 %
c_2 60 min in A_4	15 ± 4.1 %	112 ± 12 %	112 ± 12 %	12 ± 2.5 %	14 ± 3.6 %	117 ± 24 %

Table 5

Effect of extracellular application of La^{+++} on pre-stimulus membrane potential and receptor potential of *Astacus* retina (simultaneous intra- and extracellular recording) (JB 134)

a) stimulus duration τ ca. 10 msec, intracellular recording							extracellular recording		
n = 1		pMP	h_{max}	t_{max}	t_2	h_{max}	t_{max}	t_2	
a	reference value (A_4)	-65.5 mV	30.0 mV	127.9 ms	383.7 ms	2.8 mV	104.7 ms	267.4 ms	
b_1	25 min La^{+++} (A_5)	92.2 %	74.4 %	109.1 %	72.7 %	60.8 %	100.0 %	113.0 %	
b_2	55 min La^{+++} (A_5)	80.5 %	60.5 %	118.2 %	75.8 %	27.5 %	88.9 %	130.4 %	
b_3	85 min La^{+++} (A_5)	63.1 %	49.6 %	136.4 %	124.2 %	18.3 %	77.8 %	169.6 %	

b) stimulus duration τ ca. 1000 msec, intracellular recording							extracellular recording			
n = 1		pMP	h_{max}	t_{max}	h_e	h_a	h_{max}	t_{max}	h_e	h_a
a	reference value(A_4)	-65.7 mV	32.1 mV	162.8 ms	7.4 mV	7.4 mV	3.0 mV	104.7 ms	0.96 mV	0.84 mV
b_1	30 min La^{+++}(A_5)	90.1 %	69.6 %	114.3 %	75.0 %	87.5 %	53.9 %	88.9 %	59.5 %	58.3 %
b_2	30 min La^{+++}(A_5)	78.1 %	57.3 %	121.4 %	68.8 %	78.1 %	25.0 %	77.8 %	31.0 %	30.6 %
b_3	90 min La^{+++}(A_5)	57.9 %	46.4 %	142.9 %	34.4 %	50.0 %	15.6 %	55.6 %	26.2 %	27.8 %

Table 6

Effect of extracellular application of La^{+++} together with sodium deficiency on pre-stimulus membrane potential, receptor potential, and dark resistance of *Astacus* retina (intracellular recording) (JB 113)

a) Stimulus duration τ ca. 10 ms

n = 1		pMP_{corr}	h_{max}	t_{lat}	t_{max}	t_2	R_d
a	reference value (A_1)	-70 mV	24 mV	26 ms	116 ms	221 ms	181 Mol
b	55 min low Na^+ (A_2)	73 %	70 %	150 %	90 %	63 %	115 %
c	55 min La^{+++} (A_3)	10 %	8 %	91 %	120 %	32 %	195 %
d	55 min in A_1	12 %	12 %	182 %	150 %	63 %	256 %

b) Stimulus duration τ ca. 1000 ms

n = 1		pMP_{corr}	h_{max}	t_{lat}	t_{max}	h_e	h_a	h_{max}/h_e
a	reference value (A_1)	-70 mV	28 mV	24 ms	349 ms	20 mV	15 mV	1.4
b	60 min low Na^+ (A_2)	70 %	78 %	157 %	47 %	50 %	19 %	157 %
c	60 min La^{+++} (A_3)	12 %	17 %	181 %	53 %	10 %	0 %	179 %
d	60 min in A_1	11 %	23 %	176 %	77 %	23 %	9 %	100 %

Table 7

Effect of extracellular application of La^{+++} together with sodium deficiency on receptor potential of *Astacus* retina (extracellular recording) (JB 108-113)

a) Stimulus duration τ ca. 10 ms

n = 6	h_{max}	t_{lat}	t_{max}	t_2
a reference value (A_1)	1.8 ± 0.2 mV	31 ± 2.3 ms	140 ± 11.6 ms	154 ± 11.6 ms
b 55 min Na^+-free (A_2)	37 ± 6.1 %	104 ± 2.5 %	90 ± 7.9 %	64 ± 8.5 %
c_1 55 min La^{+++} (A_3)	14 ± 2.3 %	105 ± 6.2 %	82 ± 5.0 %	76 ± 12 %
c_2 85 min La^{+++} (A_3)	8 ± 1.2 %	109 ± 11 %	74 ± 2.7 %	89 ± 12 %
e 55 min in A_1	14 ± 3.4 %	116 ± 14 %	111 ± 7.4 %	103 ± 11 %

b) Stimulus duration τ ca. 1000 ms

n = 6	h_{max}	t_{lat}	t_{max}	h_e	h_a	h_{max}/h_e
a reference value (A_1)	2.4 ± 0.3 mV	30 ± 2.1 ms	226 ± 58 ms	1.4 ± 0.1 mV	0.9 ± 0.1 mV	1.8 ± 0.1
b 60 min Na^+-free (A_2)	54 ± 8.1 %	112 ± 5.6 %	87 ± 8.2 %	29 ± 5.2 %	16 ± 3.7 %	225 ± 12 %
c_1 60 min La^{+++} (A_3)	22 ± 4.2 %	106 ± 7.2 %	101 ± 10 %	16 ± 3.1 %	8 ± 3.7 %	143 ± 15 %
c_2 90 min La^{+++} (A_3)	12 ± 3.1 %	102 ± 13 %	108 ± 6.8 %	10 ± 2.5 %	4 ± 1.1 %	157 ± 29 %
e 60 min in A_1	23 ± 6.7 %	113 ± 9.0 %	149 ± 29 %	28 ± 2.5 %	21 ± 5.2 %	83 ± 11 %

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