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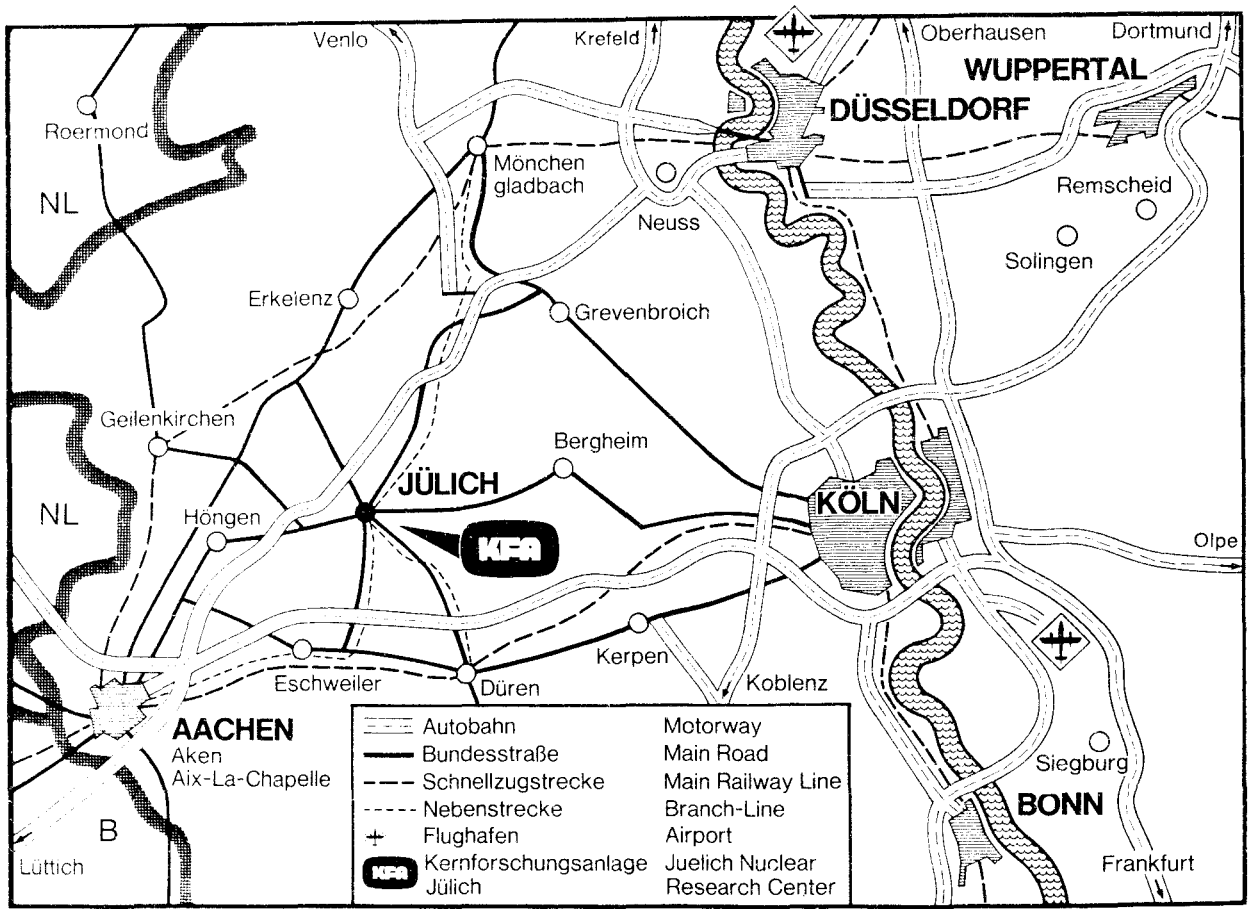

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Hennig Stieve
Ronald Backbier
Mechthilde Bruns
Helga Gaube
Birgit Steinbeck

Institut für Neurobiologie
der Kernforschungsanlage Jülich GmbH
Postfach 1913, D-5170 Jülich 1

Key words
comparison of extracellular and intracellular recordings; effect of ouabain treatment and potassium depolarization.

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C o n t e n t s

1. Abstract

In retinular cells of *Astacus* and *Limulus* the following electrical characteristics of the visual cell membrane were measured by a single intracellular microelectrode: (1) the pre-stimulus membrane potential, pMP; (2) the light-induced receptor potential, ReP; (3) the input resistance of the measuring circuit in the dark; R_d, which is the sum of the resistances of cell membrane, visual cell and recording electrode; and (4) its light-induced change ΔR, which can be attributed mainly to a change in membrane resistance.

Results

1) During the light response both membrane resistance and membrane potential change with a similar but not identical time course. After reaching a maximum value the amplitude of the ReP decreases faster than ΔR. This difference is greater at high light intensity of the stimulus than at low intensity.

2) A method is described to record simultaneously intracellularly from one retinular cell and extracellularly from the whole isolated retina of *Astacus*. The ratio of the extracellularly recorded value over the intracellularly recorded one (e/i) was determined for different parameters of RePs recorded under various experimental conditions.
The ratio e/i for the absolute amplitude values (voltages) of the ReP is ca. 0.05. The ratio e/i for the time parameters of the ReP is close to 1.
The intensity dependence of extra- and intracellularly recorded responses is fairly similar.
The effect of high external potassium concentration on extra- and intracellularly recorded RePs is also similar, except for the fact that the maximal amplitude h_{max} of the intracellularly recorded response goes down to zero whereas the extracellularly recorded response remains at a small constant value.
The reversibility to injuring experimental manipulations (potassium depolarization, ouabain poisoning) is much better for extracellular measurements.

3) Poisoning of *Limulus* retinular cells by ouabain leads to a transient increase of pMP and ReP, followed by a decrease, which is faster for the ReP ($t_{1/2}$ 34 $\pm$ 4.3 min) than for pMP ($t_{1/2}$ 64 $\pm$ 17 min). Inexcitability occurs when the pMP is still ca. 40% of its reference value. The dark resistance R_d is slightly increased under the influence of ouabain. The changes are not reversible, in contrast to extracellular recordings in the *Astacus* retina.

4) Due to high external potassium (without external sodium) in *Limulus* retinular cells the pMP reverses polarity (to +20 mV). The ReP decreases, but even after depolarization of the pMP beyond zero there is still a positive going ReP measurable for ca. 10 min. The dark resistance decreases to ca. 50%. All changes are reversible.

5) The effect of high external potassium in *Astacus* retinular cells is generally similar to that described for *Limulus*. Basically the same applies to the effect of high external potassium in the presence of external sodium (10% of normal concentration).
Parallel extracellular recordings under the conditions of high external potassium and decreased external sodium show that the response does not decrease to zero as in the intracellular recordings, but remains at a steady low value.

Interpretation

1) The differences between the observed changes of extra- and intracellularly recorded RePs can be understood on the basis of this consideration: While intracellularly one measures the membrane potential and its change of an individual retinular cell one records extracellularly the mass response of many cells, to which to individual cells contribute differently according to their position and orientation.
Extracellularly recorded RePs and their relative changes are good measures for the changes of the intracellularly recorded response as long as the resistances involved and their light-induced changes are not much altered in relation to each other. Experimental treatment influencing the membrane resistance and the shunt resistance differently leads to changes of the attenuation of the extracellularly recorded response as compared to the intracellular recording. This is true for variation of the temperature of experiment or other treatments influencing the membrane conductance.
The better reversibility of extracellularly recorded responses after drastical experimental treatments like potassium depolarization or ouabain poisoning is due to the fact that unpenetrated cells are less injured and therefore more resistant to strains. The fact that the extracellularly recorded response does not go down to zero under the conditions just described indicates that the test solution does not reach all cells of a retina equally well.

2) The difference in the time course of the intracellularly recorded ReP and the membrane resistance change (and the intensity dependence of this difference) is caused by the fact that the ReP is not linearly dependent on the membrane resistance. The reasons for this are that there exist at least two types of ion-specific channels (light- and dark channels) in the visual cell membrane. Moreover suggests the delayed increase in the membrane resistance during the repolarization of the ReP possibly a delayed increase in potassium permeability (additionally to the closing of light channels), which could be induced by the light stimulus or by the membrane potential drop.
Electrogenic pump activity and temporal partial exhaustion of ionic gradients may contribute to the difference between ReP and membrane resistance change in the repolarizing phase only to a minor extent.

3) The excitability of retinular cells poisoned by ouabain is lost when the membrane potential is still at 40% of its original value, because the ability of the membrane to undergo conductance changes is destroyed. At this time the ionic gradients are not exhausted. A certain membrane potential and possibly a functional sodium/potassium transport mechanism is necessary to enable the membrane to undergo conductance changes.

4) The fact that even after reversal of the measured overall-pre-stimulus membrane potential during potassium depolarization a positive going ReP can still be recorded for several minutes is due to the existence of an inward directed gradient of positive ions (most probably sodium ions), at least in certain areas of the cell membrane undergoing conductance change, which have not yet been sufficiently reached by the test saline.

5) The inactivation of the light channels caused by depolarization does not depend on the presence of extracellular sodium and seems to be a direct effect of the membrane potential.

6) Probably the membrane potential is necessary to create and sustain an ordered structure of membrane constituents of dipole character, which seems to be essential for the ability for conductance changes. Possible dipolar membrane constituents could be ionic channel forming molecules (probably not rhodopsin) or the sodium/potassium ATPase; the latter, hypothetical, assumption could explain both the effect of depolarization and that of ouabain.

2. Introduction

It is a well established fact that in the nerve fiber the sodium conductance of the nerve membrane changes during excitation only if the resting membrane potential is sufficiently high. At lower membrane potentials, following depolarization by rising the external potassium concentration from 10 to 25-60 mM in the squid nerve (Cole and Curtis, 1939), the sodium permeability system is inactivated, so that the sodium channels cannot be opened by the electrical stimulus. Hodgkin and Huxley (1952) found that depolarization by 20 mV leads to inactivation of more than 50% of the sodium conductance system of the squid nerve membrane.

One of the important differences between the visual cell membrane and the axon membrane consists in the fact that the visual cell membrane is generally not electrically excitable - except for the fact that a small component of the receptor potential can be elicited by an electrical depolarizing step (Purple and Dodge, 1965; Millecchia and Mauro, 1969).

In the arthropod photoreceptor cell a change of the membrane conductance as response to the light stimulus is the cause for the voltage signal at the cell membrane, the receptor potential (Fuortes, 1959; Stieve et al., 1971).
The question whether the permeability change of the visual cell membrane as response to light depends on the membrane potential has not yet been investigated. We wanted to study this question in order to interpret the results of earlier experiments dealing with ouabain poisoning of visual cells (Stieve, 1973, 1974).

The experiments reported here are meant to contribute to the understanding of (1) whether and how the permeability change of the visual cell membrane in response to light depends on the membrane potential when the visual cell membrane is depolarized either by the action of ouabain or by high external potassium concentration and (2) how the permeability change is influenced by repolarizing the depolarized cell by means of passing

electrical current through the extracellular electrode. These experiments were carried out with visual cells of the lateral eye of the horseshoe crab *Limulus polyphemus* and visual cells of the crayfish *Astacus leptodactylus*.

Additionally intra- and extracellular measurements were directly compared in the eyes of *Astacus* (extracellular recording from the whole preparation and simultaneous intracellular recording from one retinular cell of the same preparation). In this way height and time course of the receptor potentials obtained by both methods at the same time from the same preparation could be compared under varying experimental conditions (e.g. intensity dependence, increase of external potassium concentration, etc.).

It seemed important to check whether and how much extracellular measurements differ from those obtained by intracellular recordings, because extracellular recording is much easier to obtain in the crayfish retina than intracellular measurement, while only the latter yields information on the response of a single retinular cell and the membrane potential in the dark. Extracellularly obtained findings are better reproducible from experiment to experiment and scatter less since they always represent an average response of a mass of light-sensitive cells. Moreover straining experimental conditions do not seem to affect extracellular recordings as much as intracellular measurements (Stieve, 1973).
Since for these reasons extracellular recording is more often used by us, a basic comparison between the two methods seemed desirable.

3.1 Material and methods

3.1.1 Material and preparation

The eyes of adult horseshoe crabs (*Limulus polyphemus*) with a carapax width of ca. 15-20 cm and of adult crayfishes (*Astacus leptodactylus*) were used in the experiments. The horseshoe crabs were shipped from the Marine Biological Laboratory, Woods Hole, Mass., USA. The crayfishes were bought from a local provision dealer. All animals were kept in the laboratory aquarium at 15°C for several weeks before they were used for the experiments.

The lateral eyes of the *Limuli* were excised using a small electrical circular saw. A 1-2 mm thick slice was cut by razor blade from the eye in direction of the longest diameter of the eye or the eye was cut in two halves. The slice or one of the halves was placed in a perspex test chamber during the experiment with the cut surface facing upward. The chamber which had a volume of ca. 3 ml, was permanently perfused throughout the experiment.
The single cells concerned in the intracellular measurements in the case of *Limulus* were retinular cells and not eccentric cells.

The retina of the crayfish was excised as described elsewhere (Stieve et al., 1971). From this retina, sucked to a peace of millipore filter (type Millipore AAWPO 1300), about 1-2 mm thick slices were cut parallelly to the long axes of the retinulas. The slice of the retina with the piece of millipore filter attached was placed in a plexiglas vessel (Fig. 1) in which it was permanently superfused by streaming saline throughout the experiment. The volume of the experimental chamber was less than 1 ml and the fresh solution was applied directly on the surface of the retina slice.

3.1.2 Salines

The ionic composition of all salines used in the experiments is given in Table 1. The physiological saline in which the preparation was kept was artificial seawater in the case of *Limulus* and van Harreveld's solution (1936) in the case of *Astacus* (Table 1).
Ouabain was added in a concentration of 1 mM/l to the test solution. In the solution with increased external potassium a corresponding amount of sodium was omitted to keep the osmotic pressure constant. The pH of all solutions was between 7.5 and 8.0.

3.1.3 Perfusion

The chamber was perfused with a flow rate of ca. 0.5 ml/min. The perfusion of the experimental chamber could be switched from one solution to another. In the experiments described here the switch-cock was in a distance of ca. 60 cm from the experimental chamber. In the two different experimental chambers it took a different amount of time from the moment of switching until the old solution was replaced by the new one:
In the *Limulus* experiments (Fig. 2) it took about 30 min until about 99% of the first solution was replaced by the second one; ca. 10 min after the switch the new solution was recognizable (ca. 5% in the neighborhood of the preparation), after 10 min 30-50% of the solution was replaced.
In the experiments with *Astacus* ca. 0.5 min after the switch the new

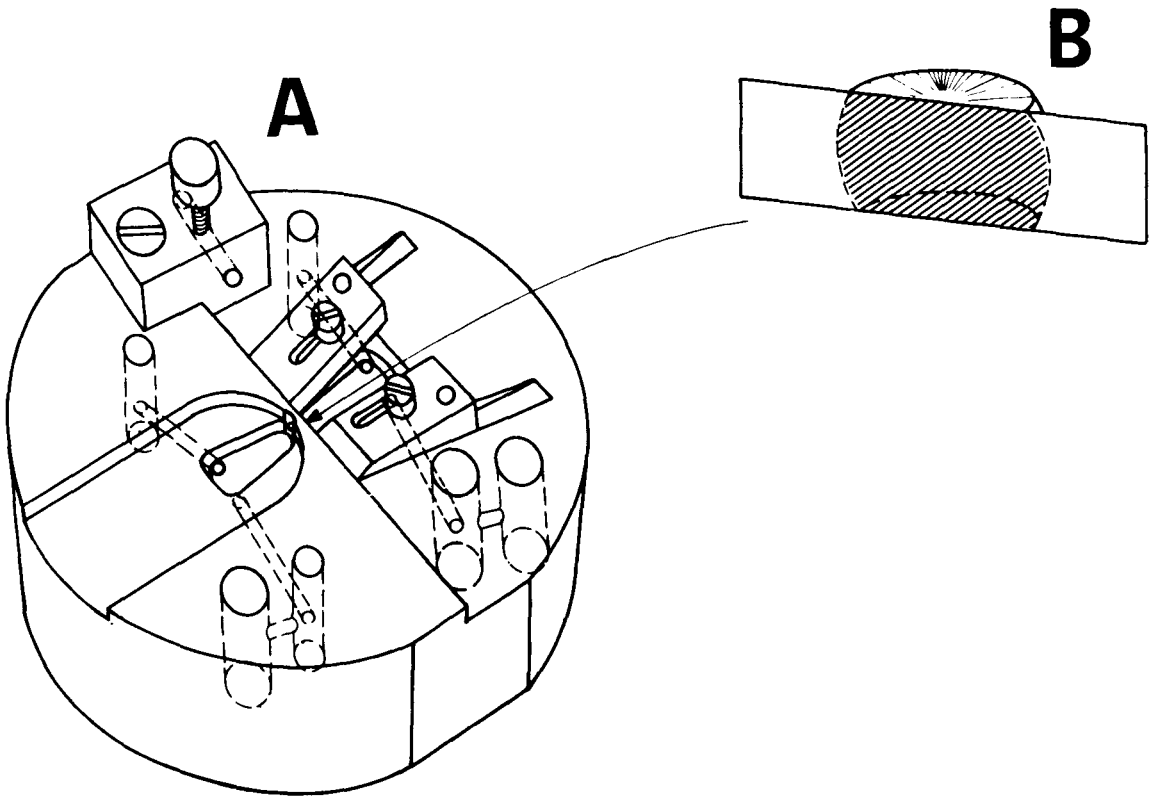

Fig. 1

A: Plexiglass test vessel for *Astacus* retina slices

B: retina slice sucked to a piece of millipore filter

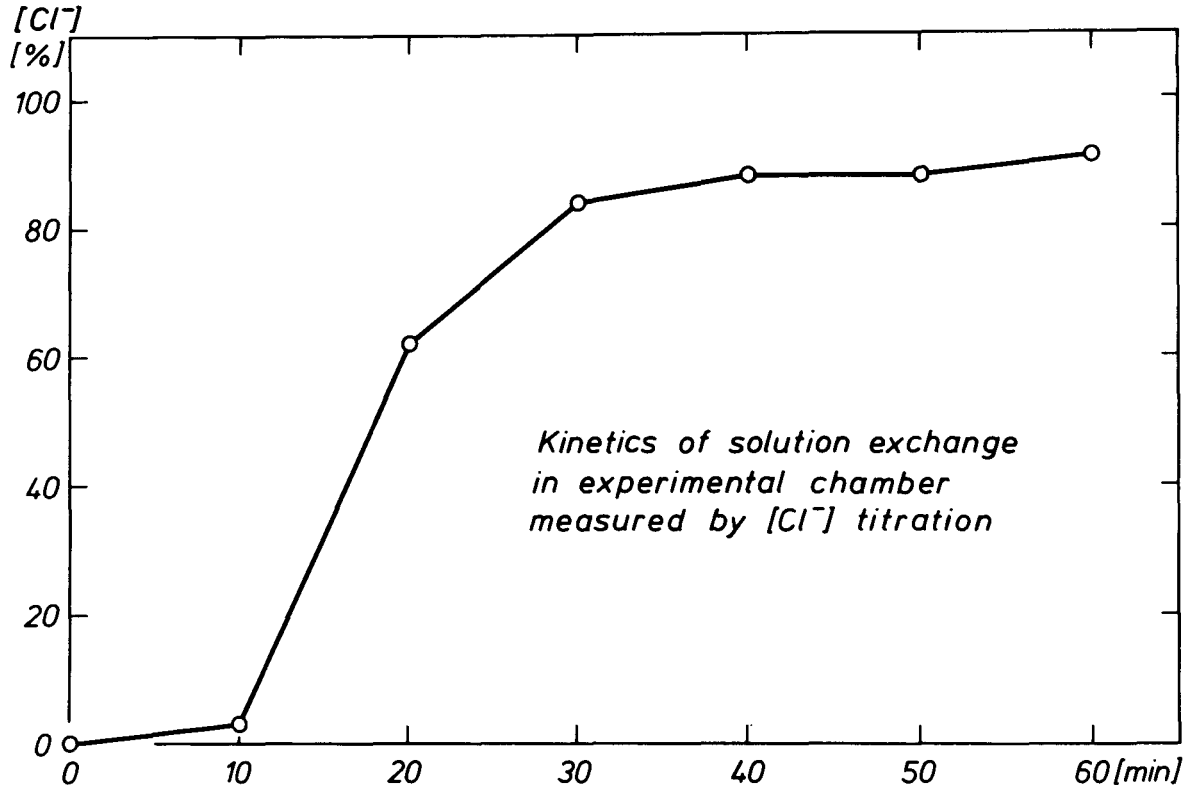

Fig. 2

Kinetics of solution exchange in experimental chamber used for *Limulus* preparations, measured by Cl^- titration [%] vs. time [min].

solution was recognizable at the retina (ca. 5%), and 5 min after the switch more than 90% of the solution was replaced.
In some experiments it took more time until the action of the changes of the extracellular solution developed recognizably as change in the response. This is mainly due to the length of the diffusion way in the tissue (depending on the position of the penetrated cell in the eye, covering by glial cells, etc.).

The temperature of the experimental chambers was kept constant at about 15°C.

3.1.4 Electrical measuring and recording system

In the intracellular experiments the visual cell was penetrated by a glass microelectrode, filled with 0.5 m KCl solution. The indifferent extracellular electrode (a silver chloride electrode) was placed elsewhere in the vessel. The resistance of the microelectrodes was between 10 and 100 MΩ in the *Limulus* experiments and between 30 and 50 MΩ in the *Astacus* experiments.

For the extracellular measurements two chlorided silver wires were in contact with the distal and proximal side of the slice of the retina. The resistance of these electrodes was 1-2 KΩ. The level of the saline in the vessel in which the slice of the retina was located was kept very low in order to make the shunt as small as possible. The input resistance of the measuring circuit, i.e. the resistance between the two extracellular electrodes across the slice of the retina, was 10-100 KΩ.

The electronic recording circuit used allowed DC compensation, current injection through the microelectrode, and compensated current pulses through the measuring circuit in order to measure the input impedance of the measuring circuit (cell membrane, cell and electrode in series). The pre-amplifier was designed by A. Schroth (1972). The time constant of the measuring circuit of the amplifier including the microelectrode was about 50 µs. The data were FM recorded on magnetic analogue tape (Ampex FR 1300) and, after the experiment, photographed by a camera for evaluation by hand. They were parallelly recorded by a paper recorder (Type Helcoscriptor HE 16, recording speed 2.5 mm/min).
The time resolution of the total system both in the intracellular and in the extracellular case was 1 ms; the measuring accuracy of the voltage measurement in the intracellular recording was 1 mV and in the extracellular recording was 1 mV and in the extracellular recording 0.01 mV.

3.1.5 Stimulating light

Two different types of light sources were used:
a xenon high pressure lamp (Osram XBO 900 W) in the experiments labelled JA and a mercury super pressure lamp (Philips CS 100 W/2) in the JB experiments. The white light was filtered by a 5 cm water cuvette for heat absorption. The maximum light intensity used was 20,000 lux, i.e. ca. $3.2 \cdot 10^{17}$ photons $\cdot$ $cm^{-2} \cdot sec^{-1}$ with the xenon lamp and 5,500 lux, i.e. ca. $9 \cdot 10^{16}$ photons $\cdot$ $cm^{-2} \cdot sec^{-1}$ with the mercury lamp. Unless otherwise specified constant maximum light intensity was used for the stimulus. In some cases the intensity of the light stimulus was attenuated by neutral density filters (Bausch & Lomb). The time of exposure was varied by a mechanical photographic shutter which was operated electrically. In the experiments two different stimulus durations were used:

a) short stimuli (shorter than the latency period)
   τ = 10 ms (*Astacus*); τ = 33 or 50 ms (*Limulus*).

b) long stimuli: τ = 1000 ms

Every 10 min (or 5 min) a short stimulus was applied and every 30 min a long stimulus. In a number of experiments the intensity dependence of the light response was measured during the pre-period by applying one stimulus every 2 min; always a stimulus of test intensity in turn with a reference stimulus of full intensity.

3.2 Procedure

All experiments were carried out at 15°C. The experiments lasted at least three hours counted from the first extracellularly recorded receptor potential or, in the case of intracellular measurement, from the successful penetration of the visual cell. During the whole experiment the retina was kept in the dark except for the exposure to the light stimuli.

The experiments started with a pre-period of 60 min during which the preparation was superfused with physiological saline and was allowed to adapt to temperature and to a constant stimulus sequence of one stimulus per 10 min (or per 5 min).

The pre-period was followed by the main-period, in the beginning of which the perfusion was switched from the physiological saline to the test solution. The main-period usually lasted 60-120 min. Generally it was extended as long as to reach a steady state of the effect of the test solution.

In an after-period of at least one hour the preparations were again perfused by the original physiological saline.

3.3 Evaluation

The following quantities and parameters were measured:

3.3.1 Voltages

By means of the intracellular electrode the membrane potential of the visual cell immediately before the light stimulus (the pre-stimulus membrane potential, pMP) was measured. (In our experiments the pMP is identical with the dark potential, DaP, so that the abbreviations pMP and DaP in the figures mean the same.) The voltage signal in response to light (the receptor potential, ReP) was measured with intracellular or extracellular electrodes.

The uncorrected pre-stimulus membrane potential pMP was measured as difference between the initial zero line and the base line before the stimulus. This value was corrected in order to allow for potential drift during the experiments, which amounted up to 20 mV during a 3-4 hours experiment. The corrected dark potential pMP_{corr} was obtained in the

following way: The initial zero potential before penetration of the cell is not identical with the final zero potential at the end of the experiment after withdrawal of the electrode. In order to account for this discrepancy, a linear change of the zero potential during the time course of the experiment was assumed. This assumption is certainly very simplifying. (Since we believe that the main baseline shift probably occurs soon after the penetration of the cell membrane we intend to reduce the error by averaging the initial and the final zero potential in future experiments.)

With the assumption of a linear change of the zero potential at each time of the experiment the corrected dark potential is the actually measured potential in the dark minus the interpolated zero value at the time concerned.

The receptor potentials recorded during the experiments were evaluated concerning their size and shape by measuring the following parameters for intracellular as well as for extracellular measurements:

a) for short stimuli (τ shorter than the latency period):

h_{max} (mV) - the peak amplitude value of the transient of the ReP;

t_{lat} (ms) - the latency period - the time from the beginning of the stimulus until intersection of tangent line in the steepest point of the rising phase of the ReP with the extrapolated level of the pMP;

t_{max} (ms) - the time-to-peak - the time from the beginning of the stimulus until the response maximum is reached;

t_2 (ms) - the time of half decrease, in which the ReP decreases from h_{max} to $h_{max}/2$;

h_n (mV) - the amplitude of the response 500 ms after h_{max}.

b) for long stimuli (τ = 1 s), additionally to h_{max} and t_{max} which were recorded as described for short stimuli:

h_e (mV) - the plateau value, the amplitude at the stimulus end;

h_a (mV) - the amplitude of the response 500 ms after stimulus end;

h_{max}/h_e - the shape quotient of the ReP.

In the following the stimulus duration will be quoted if necessary as a subscript of the respective measured quantity, e.g. $h_{max_6} = h_{max}$ after a stimulus of 6 ms duration. Index i or e refers to intracellular and extracellular recordings.

The changes of the ReP were expressed in per cent of the reference value, the value of the last ReP recorded in the pre-period immediately before the application of the test saline in each experiment).

When the ReP is gradually decreased under the action of the test solution all the measurements of the ReP were determined for potentials where the maximal amplitude h_{max} was 70, 50, 30, and 0 per cent of the reference value. The experimental time, when h_{max} had the value in question (70%,

50%, 30% or 0%) was determined in the individual experiments by drawing the measured data and linear interpolation. The values of the other parameters of the ReP were determined for the same experimental time also by linear interpolation of the measured values when intracellular recordings were used. Also the pMP was determined before each ReP and treated correspondingly. To have a measure for the speed of the action of the applied variation in experimental conditions we measured the half time $t_{1/2}$ needed from the switch until the half effect on h_{max} or pMP was achieved: e.g. if the total change of the pMP (end value in the test solution minus reference value) in an individual experiment was +60 mV, $t_{1/2}$ is the time until the pMP has changed by 30 mV. If h_{max} went down to zero $t_{1/2}$ was the time needed until h_{max} had decreased to 50% of its reference value.

3.3.2 Impedance

Impedance measurements were carried out using current pulses (0.5 - 1.5 nA) passed through the intracellular electrode; usually of 0.5 - 1.5 nA for short stimuli of 20 ms duration and of a frequency of 10 Hz and for long stimuli of 50 ms duration and 5 Hz.
The input impedance of the measuring circuit in the dark R_d, that is the sum of the resistances of the cell membrane, the measuring electrode and the visual cell in series, was measured by the height of the uncompensated current pulses a few seconds before a light stimulus was applied.
The change ΔR of this total impedance of the measuring circuit which can primarily be attributed to a change in the resistance of the cell membrane, was measured at various times of the ReP by the amount of the decompensation of the pulses which were compensated by a bridge circuit for the dark resistance. The change ΔR of this resistance could be measured at various times of the receptor potential:

a) for short stimuli

   ΔR_m (MΩ) at the maximum of the ReP;

   $\Delta R_m/2$ (MΩ) at the time when the receptor potential is decreased to the half value of h_{max} and

   ΔR_n (MΩ) the resistance change 500 ms after h_{max}.

b) for long stimuli, additionally to ΔR_m, which was measured as described for short stimuli:

   ΔR_e (MΩ) at the end of the stimulus;

   ΔR_a (MΩ) the resistance change 500 ms after stimulus end;

   $\Delta R_m/\Delta R_e$ the quotient of the resistance changes at h_{max} and h_e.

The measured resistances were treated similarly as the potential values, i.e. they were normalized with respect to reference values obtained at the same time when the reference receptor potential was recorded.

4. Results

4.1 Visual cells in physiological saline

4.1.1 *Limulus* retinular cell

Observations of the behavior of visual cells in physiological saline were obtained from the pre-periods of all experiments reported here. The results are however reported in detail only from two groups of experiments (5 experiments JB 8-10, JB 41, 42, *Limulus*, EGTA; and 5 experiments JB 46-50, *Limulus*, potassium depolarization). The results are generally similar in the other experiments.

Voltages

At the end of the pre-period the pMP_{corr} is about -40 mV, and under our stimulus conditions the amplitude $h_{max_{33}}$ is about 40 mV. Under the same conditions $h_{max_{1000}}$ is about 40 mV and $h_{e_{1000}}$ about 17 mV. Table 2a shows values for short and long stimuli.

Resistances

Resulting from the penetration of a visual cell the resistance of the measuring circuit increases in the retinular cell of *Limulus* to about the double value. For example in five experiments where cells were penetrated, the resistance of the electrode R_{el} was on the average 15 $\pm$ 4.2 MΩ. On penetration of the cell the input resistance increased to 275 $\pm$ 33%. At the end of the experiments the resistance was 191 $\pm$ 25% before withdrawal of the electrode. After withdrawal from the cell the resistance, which was now only to be attributed to the electrode, dropped to 139 $\pm$ 31% (n = 5; JB 46 - JB 50).

The input resistance of the measuring circuit in the dark R_d was about 30-45 MΩ. To this value contributes the resistance of the electrode R_{el} with estimated 12-15 MΩ. The resistance of the membrane of the visual cell is estimated to be in the range of 5 to 15 MΩ. The light-induced change of that resistance ΔR

is about 7-10 MΩ in the maximum of the ReP (ΔR_m) Under the maximal stimulus conditions used in our experiments R at the end of a 1000 ms stimulus (ΔR_e) was about 4-8 MΩ. The shape--quotient h_{max}/h_e for the receptor potential is considerably greater (2 - 2.35) as compared to the quotient of the resistance changes $\Delta R_{max}/\Delta R_e$ (1.2 - 1.5) (table 2a).
Accordingly for short stimuli (table 2b) the change in resistance follows the course of the receptor potential, but not proportionally.

4.1.1.1 Intensity dependence of membrane resistance change ΔR and receptor potential

In four experiments the dependence of the response amplitude $h_{max_{50}}$ and the resistance drop $\Delta R_{max_{50}}$ was measured as a function of the intensity of the light stimulus (Figs. 3a, b, c and Table 2b). The intensity was varied over three orders of magnitude. The light induced resistance decrease ΔR_m becomes greater with increasing stimulus intensity as does the amplitude of the receptor potential. The dependence of the receptor potential on the stimulus intensity is steeper than that of the resistance change. ΔR_m does not change proportionally with h_{max} (Fig. 3c) over the whole range of h_{max}, but almost so in the range of h_{max} 50% - 100%.

The light-induced resistance change of the membrane lasts much longer after the stimulus than the potential change, at least at high light intensities.
When the potential is decreased to 50% ($h_{max/2}$) after a stimulus of high intensity (lg I = 0), the resistance change is still 2/3 of ΔR_m and remains on this value throughout the receptor potential. After the receptor potential is terminated the resistance change ΔR slowly decreases.
This prolongation of the resistance change as compared to the amplitude decrease in the course of the receptor potential depends on the intensity of the stimulus, in a way that it becomes shorter with decreasing stimulus intensity. At lg I = -1 ΔR_n = 47% of ΔR_m, at log I = -2 ΔR_n is no more measurable, at

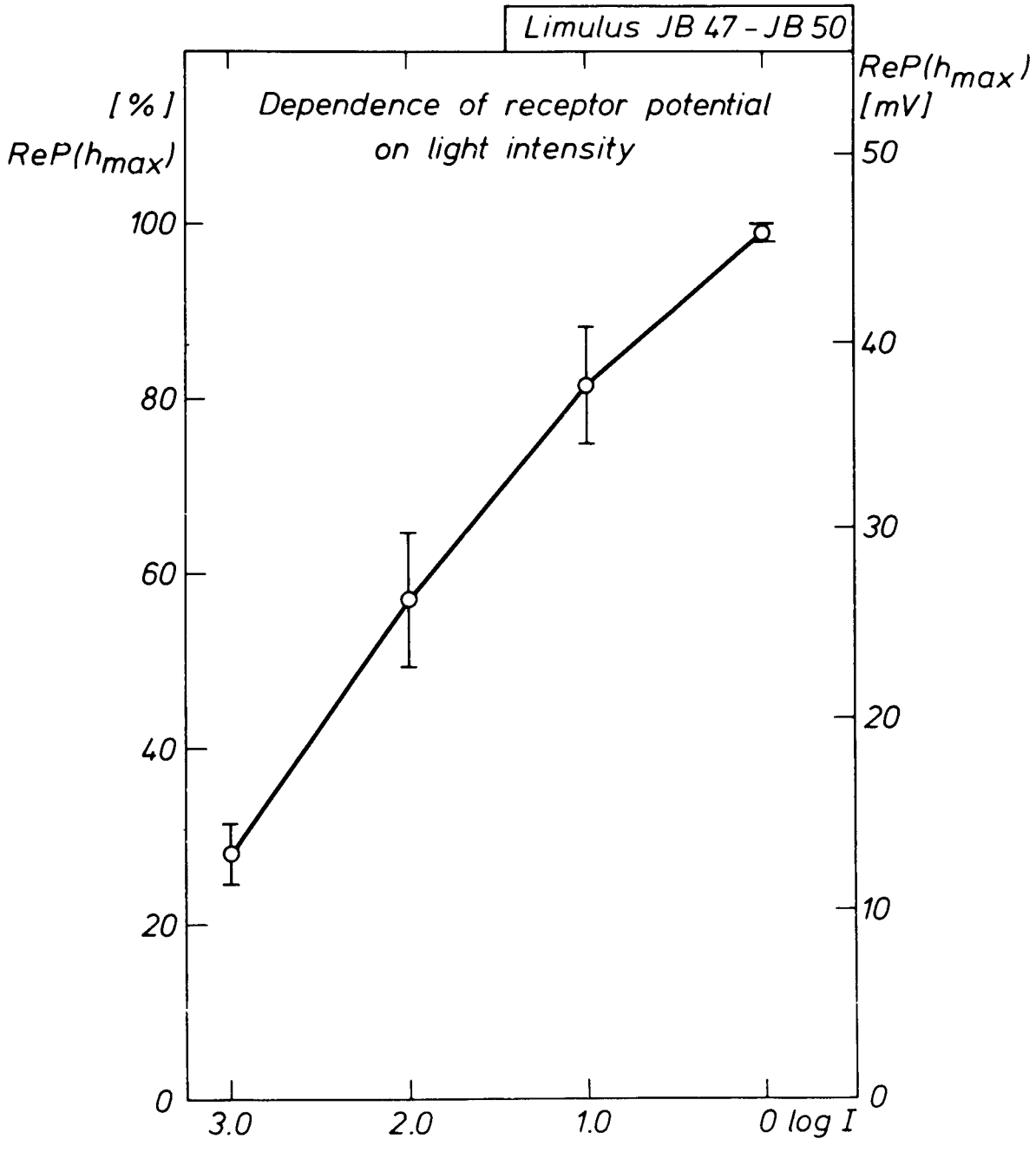

Fig. 3a

Dependence of receptor potentials of *Limulus* retinular cells on light intensity. Maximal intensity of stimulating light ca. 1000 lx, stimulus duration τ = 50 ms. Intracellular measurement of maximal amplitude h_{max} (mV) of ReP. Left scale: h_{max} in % of reference value (last value before first measurement at log I = 0). The horizontal bars indicate the S.E. of the mean.
JB 47-50, *Limulus* retinular cells

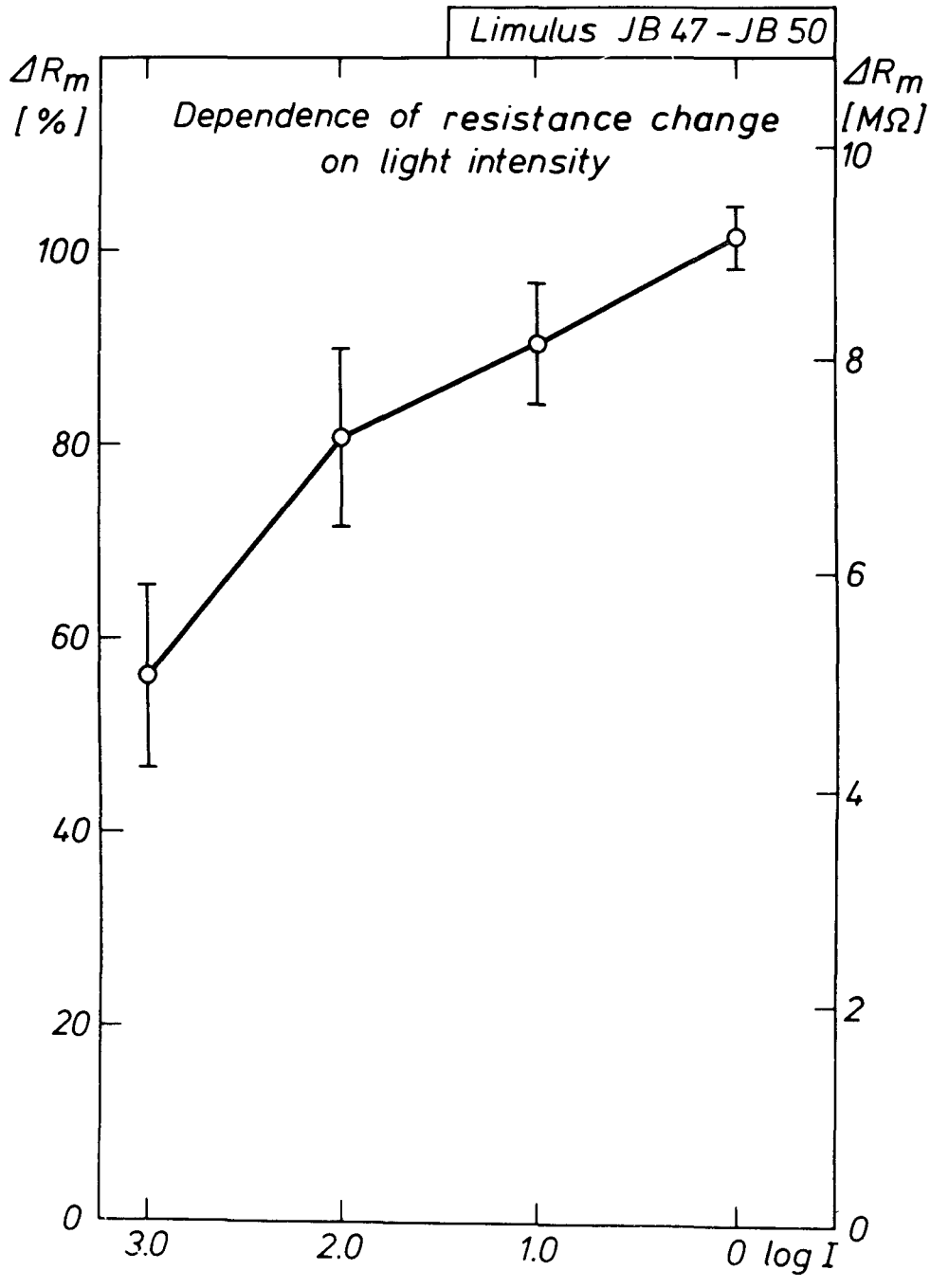

Fig. 3b

Dependence of resistance change on light intensity. Intracellular measurement of membrane resistance change ΔR_m [MΩ] at the time of the maximal amplitude of the ReP. Left scale: ΔR_m in % of reference value. Further details as in Fig. 3a.
JB 47-50, *Limulus* retinular cells

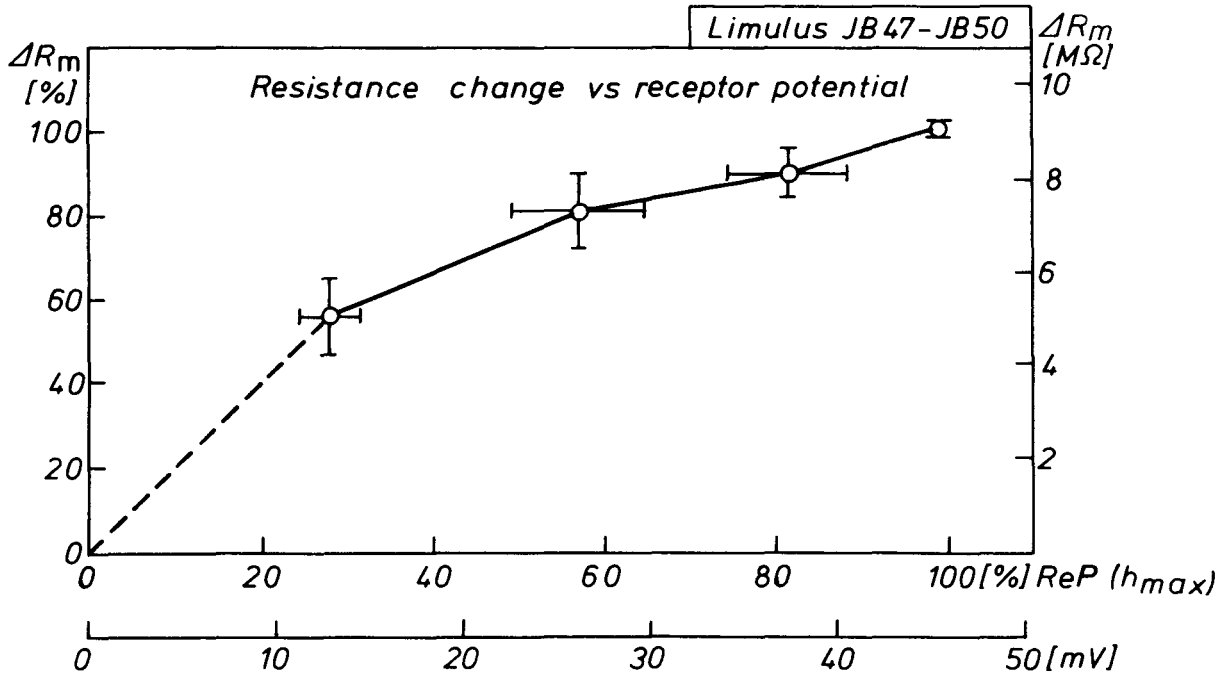

Fig. 3c

Plot of membrane resistance change ΔR_m [MΩ] vs. maximal amplitude h_{max} [%] of ReP. Left scale: ΔR_m in % of reference value. Scale below: h_{max} [mV]. Further details as in Fig. 3a.
JB 47-50, *Limulus* retinular cells

lg I = -3 the resistance change at $h_{max/2}$ is 40% of ΔR_m. At low light intensities the resistance change is almost parallel to the amplitude decrease in the course of the receptor potential.

Generally the measured light-induced resistance drop shows relatively great variation from cell to cell.

4.1.2 *Astacus* retinular cell

Voltages

The pre-stimulus membrane potential of the *Astacus* retinular cell at the end of the pre-period in physiological saline was ca. -50 to -60 mV, the amplitude $h_{max_{10}}$ ca. 30 to 40 mV, $h_{max_{1000}}$ ca. 30 to 45 mV and $h_{e_{1000}}$ ca. 20 to 30 mV (Table 3).

Resistances

In five experiments (Table 3) the electrode resistance R_{el} was 39 $\pm$ 12.4 MΩ. After penetration the input resistance of the measuring circuit increased to 190 $\pm$ 26% (R_d). At the end of the experiment the input resistance was 133 $\pm$ 16% before withdrawal of the electrode and after withdrawal the electrode resistance was 119 $\pm$ 16%.
The total resistance R_d was measured to ca. 70 MΩ at least 40 MΩ of which is the resistance of the electrode R_{el}. The membrane resistance is estimated to be ca. 5-30 MΩ. The light-induced resistance changes are smaller than in *Limulus*. For long stimuli (τ = 1000 ms) ΔR_m is ca. -2 MΩ, ΔR_e ca. -1 MΩ, $\Delta R_m / \Delta R_e$ is 2-3 under maximal stimulus conditions, as compared to h_{max}/h_e = 1.6 under the same conditions (Table 3). The maximal ΔR_m recorded in one experiment was -3.5 MΩ. (In *Limulus* the ratio h_{max}/h_e is greater than $\Delta R_m / \Delta R_e$ but the standard deviation of the measurements of the resistance change is considerable.)

Generally the *Astacus* visual cells show the same behavior as

those of *Limulus*. The main differences are: R_d is greater and ΔR_m smaller than in *Limulus*. ΔR_m is about 25% of R_d in *Limulus* and about 3-5% of R_d in *Astacus*. Therefore the measurements of the light-induced resistance changes are less reproducible and reliable in *Astacus* than in *Limulus*, sometimes they are barely measurable with the setup used in our experiments.

4.1.3 Comparison of receptor potentials simultaneously recorded with extracellular and with intracellular electrodes

Astacus

In five experiments receptor potentials were recorded simultaneously both extracellularly from the whole retina and intracellularly from one retinular cell of the same preparation (Figs. 4a and b). Stimulus duration and intensity were varied.

For comparison the ratio of the extracellularly recorded parameters of the ReP over the intracellularly recorded ones was calculated. These ratios are given in Table 6. Tables 4 and 5 show the extracellularly and the intracellularly recorded results. Generally the extracellularly recorded absolute amplitudes (Table 6a) of the RePs are smaller, by a factor of ca. 0.05, than the intracellularly recorded ones. The ratio e/i for $h_{max_{10}}$ is ca. 0.05, for $h_{max_{1000}}$ ca. 0.06, for $h_{e_{1000}}$ ca. 0.05 and for $h_{a_{1000}}$ ca. 0.04. That means that the extracellularly recorded fraction of the receptor potential becomes relatively smaller in the time course of the light response. The e/i ratio of the shape quotients (h_{max}/h_e) is therefore significantly greater than 1, namely 1.5.

The comparison of the absolute values of the time parameters at the highest light intensity shows small but in some cases significant differences (Table 6a). The ratio e/i for t_{lat} is 1.3, slightly longer extracellularly than intracellularly. For $t_{max_{10}}$ and t_2 the ratios e/i are both close to 1. For $t_{max_{1000}}$ e/i is 0.75, extracellularly recorded t_{max} is significantly smaller than intracellularly recorded.

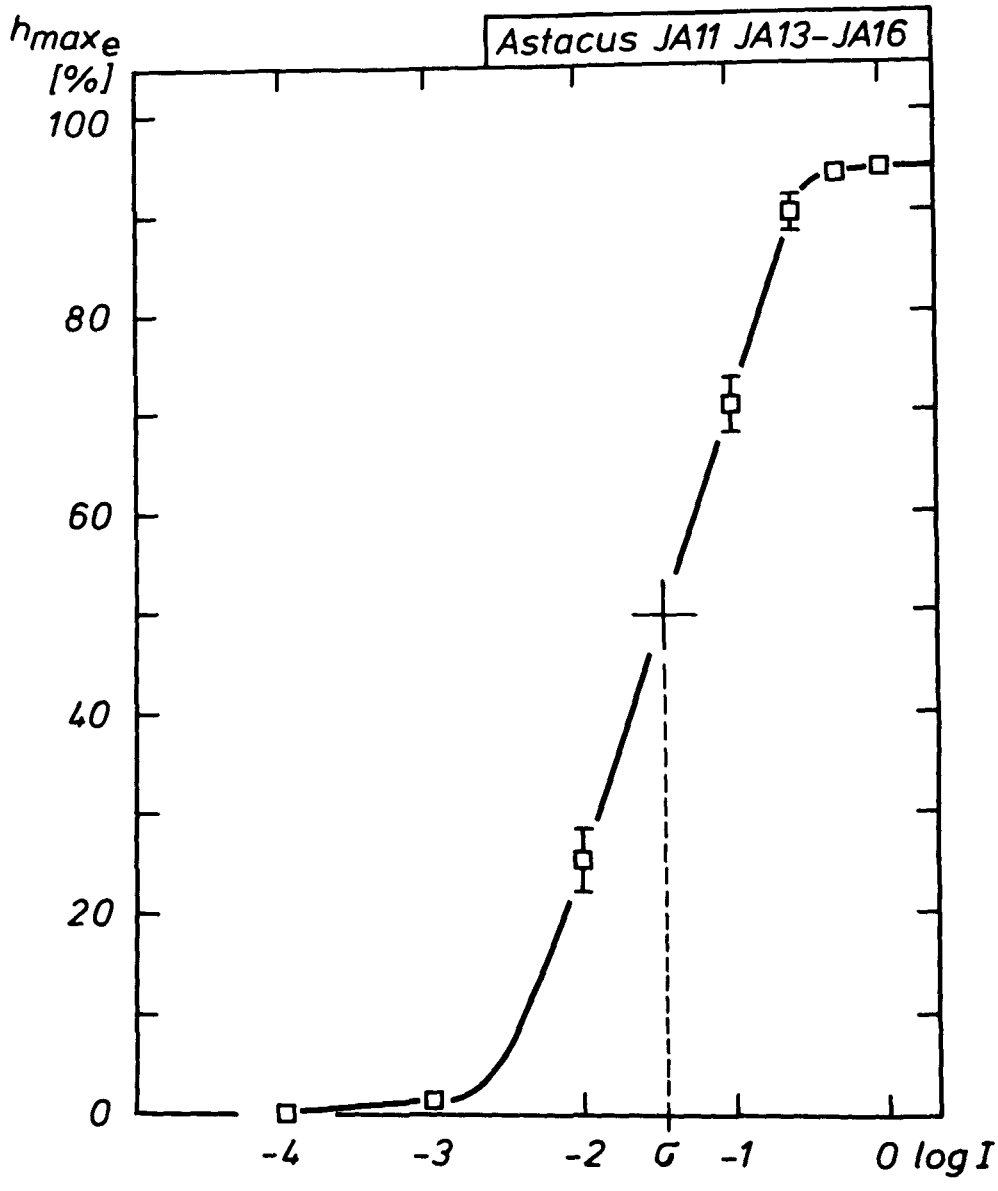

Fig. 4a

Intensity dependence of extracellularly recorded RePs of *Astacus* retinular cells. Maximal intensity of stimulating light ca. 20.000 lx, stimulus duration, τ = 10 ms. Plot of maximal amplitude h_{max} of ReP in % of reference value (1.62 $\pm$ 0.06 mV) recorded before first measurement at log I = 0. Half saturation intensity σ = ca. -1.4 log I . The horizontal bars indicate the S.E. of the mean.
JA 11, JA 13-16, *Astacus* retina slices

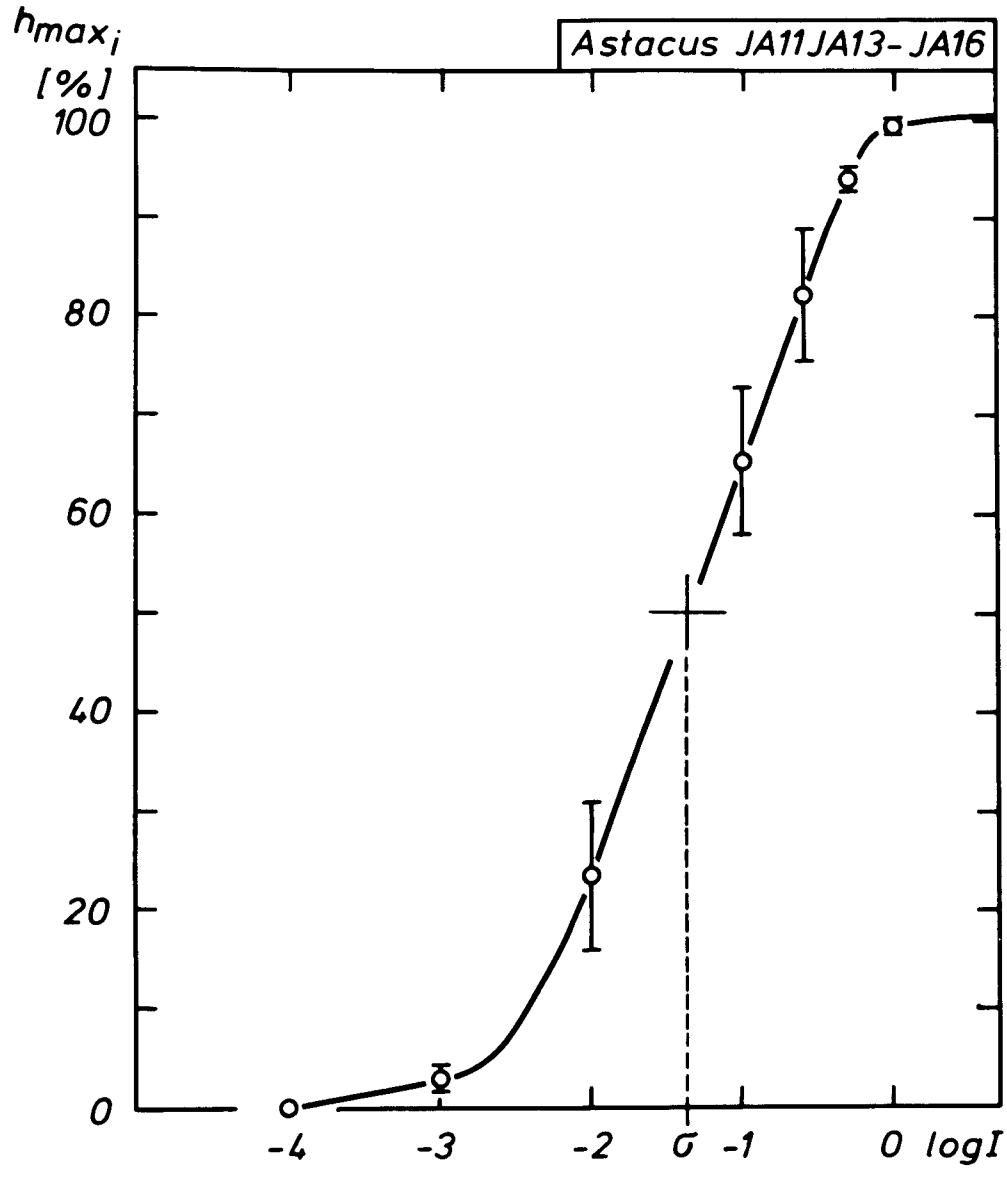

Fig. 4b

Intensity dependence of RePs of *Astacus* retinular cells recorded intracellularly from the same preparations as in Fig. 4a. Plot of maximal amplitude h_{max} of ReP in % of reference value (38 $\pm$ 1.1 mV). Half saturation intensity σ = ca. -1.4 log I. Further details as in Fig. 4a.

JA 11, JA 13-16, *Astacus* retinular cells.

Generally speaking the agreement between extracellularly and intracellularly recorded absolute values is relatively good.

The comparison of the relative values of the parameters of the receptor potentials, standardized with respect to the reference response, and their changes with decreasing light intensity shows (Table 6b): The ratio e/i for $h_{max_{10}}$ does not deviate significantly from 1; the same is true for the ratio e/i for the amplitude values for the long stimulus: the ratios e/i for $h_{max_{1000}}$, $h_{e_{1000}}$ as relative values do not deviate significantly from 1. The ratio e/i for h_{max}/h_e becomes smaller than 1 at light intensities from log I -1 on and lower (0.8 at log I = -5).

The relative ratios of measured time parameters for short stimuli show small deviations from 1 for $t_{lat_{10}}$, but no significant deviations from 1 for $t_{max_{10}}$, $t_{2_{10}}$ and $t_{max_{1000}}$.

Generally the changes of the relative values of the measured parameters of the receptor potentials recorded by extracellular and intracellular electrodes agree fairly well. At lower intensities deviations occur; at least the scatter becomes greater from experiment to experiment.

The relative intensity dependence of extracellularly and intracellularly recorded RePs is almost identical with a half saturation intensity σ -1.4 (log intensity scale) and approximately the same steepness of the log-linear rising phase of the plots (Figs. 4a and b).

The changes of the extracellularly and intracellularly recorded receptor potentials under the influence of depolarization of the visual cell membrane by high external potassium will be compared under 4.3, page 30).

Also under the influence of the ionophore X537A (Stieve and Bruns, unpublished) extracellularly and intracellularly recorded RePs of the same *Astacus* retina show similar changes. After a certain delay the excitability of the retina (measured as h_{max})

decreases with an overall half-time $t_{1/2}$ of 50 min (n = 6) for extracellular and of 65 min (n = 3) for intracellular recording under the influence of X537A. The main difference between the recordings is that the intracellular response on the average almost goes down to zero (2%), whereas the extracellular response remains at a steady value of 5% of the reference value. Both changes are irreversible.

4.2 Ouabain

In earlier experiments we could show by extracellular recording (Stieve et al., 1971) that under the influence of ouabain the excitability of the visual cells of the *Astacus* retina decreases and finally disappears; this happens the faster, the stronger and the more often the cells are stimulated by light. In intracellular recordings in *Limulus* retinular cells we observed that the cell was inexcitable due to ouabain poisoning long before the dark potential had become zero (Stieve, 1973). This is in agreement with the results of Smith et al. (1968) and our own intracellular measurements in the ventral photoreceptor of *Limulus* (Appelhans and Stieve, unpublished).

Inexcitability under the influence of ouabain - which poisons the active sodium and potassium transport across the cell membrane - could primarily have two different reasons:
Either the ionic gradient which causes the receptor potential, the sodium gradient, could be exhausted and/or the light-induced conductance change of the cell membrane could disappear due to direct or indirect action of ouabain.

4.2.1 *Limulus*

In 7 experiments (JA 1 - JA 5; JB 39, JB 45) receptor potential and dark potential were measured under the influence of ouabain. In three experiments (JA 3 - JA 5, JB 39, JB 45) the dark resistance and resistance change caused by light were additionally measured (Table 7 and Figs. 5, 6 and 7, a,b,c).

After the pre-eriod the eye was poisoned with ouabain (1 mM/l

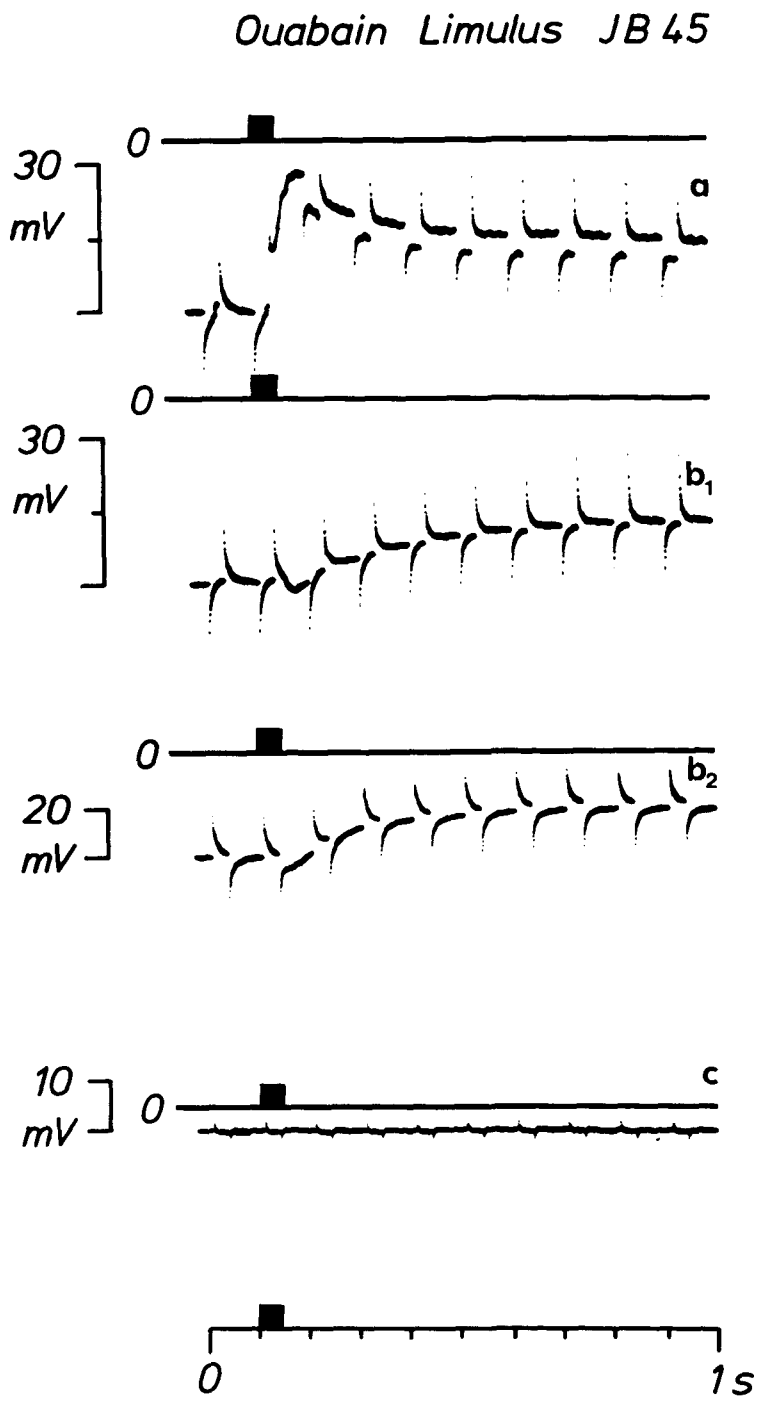

Fig. 5

Intracellularly recorded receptor potentials of a *Limulus* retinular cell and simultaneous measurement of the membrane resistance change ΔR (difference between the two recorded traces of each measurement, i.e. height of the square-wave impulses). Light intensity ca. 5.500 lx, stimulus duration τ ca. 50 ms.
a: in physiological saline; b_1: 40 min in test saline containing 1 mM/l ouabain; b_2: 70 min in Ouabain saline; c: 55 min again in physiological saline.
JB 45, *Limulus* retinular cell

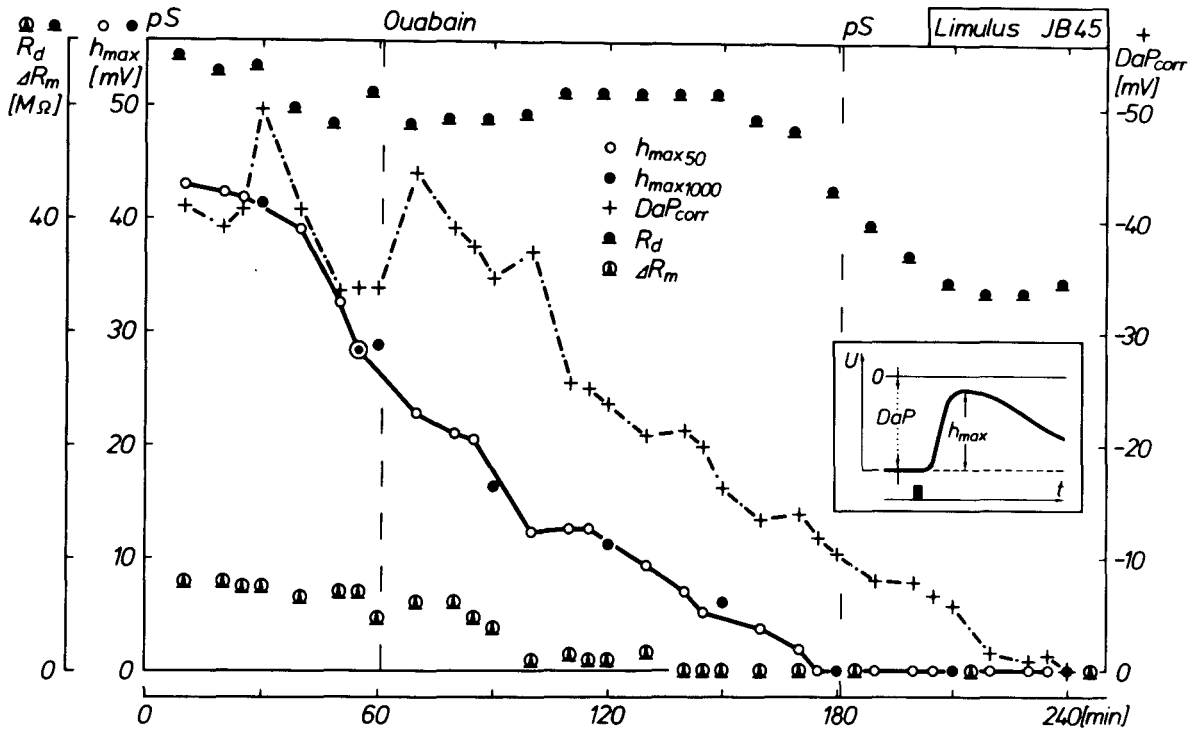

Fig. 6

Time course of the effect of ouabain poisoning on *Limulus* retinular cell. Intracellular measurement of $h_{max_{50}}$ [mV], $h_{max_{1000}}$ [mV], DaP_{corr} [mV] (see 3.3.1 Voltages), R_d [MΩ] and ΔR_m [MΩ] vs. time [min].
pS: perfusion with physiological saline, Ouabain: perfusion with test saline containing 1 mM/l Ouabain. For further details see Fig. 5.

JB 45, *Limulus* retinular cell

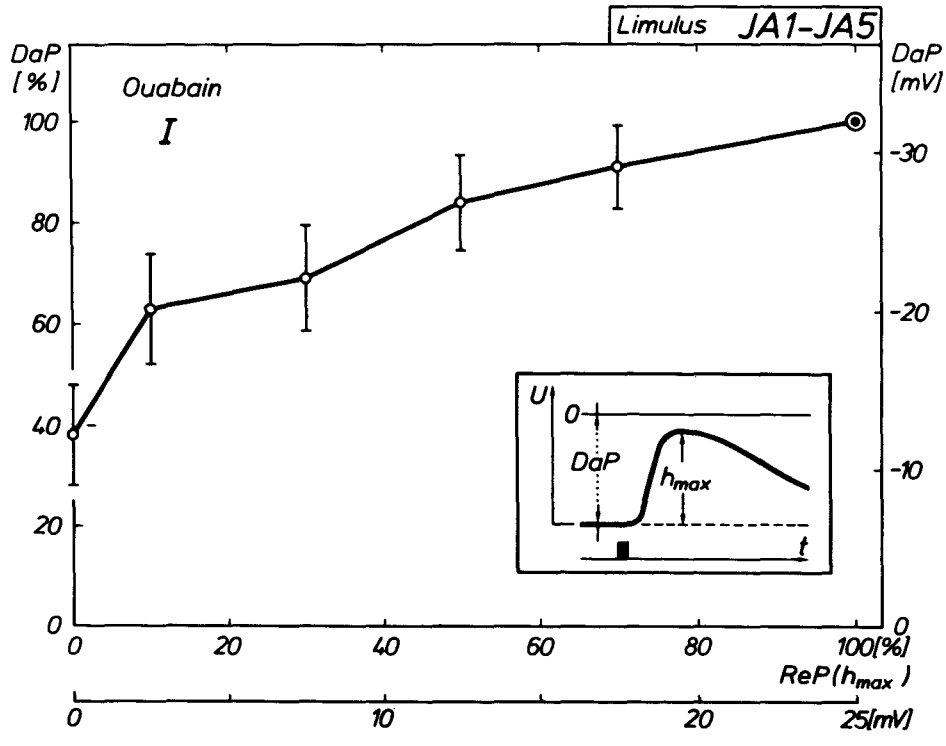

Fig. 7a

Intracellular measurement of pre-stimulus membrane potential pMP = DaP [mV] vs. maximal amplitude h_{max} [% of reference value recorded in physiological saline] of receptor potentials recorded in test saline containing 1 mM/l Ouabain.
Left scale: DaP in % of reference value. Scale below: h_{max} [mV].
Light intensity ca. 20.000 lx, stimulus duration τ ca. 33.3 ms.
The horizontal bars indicate the S.E. of the mean.
JA 1-5, *Limulus* retinular cells

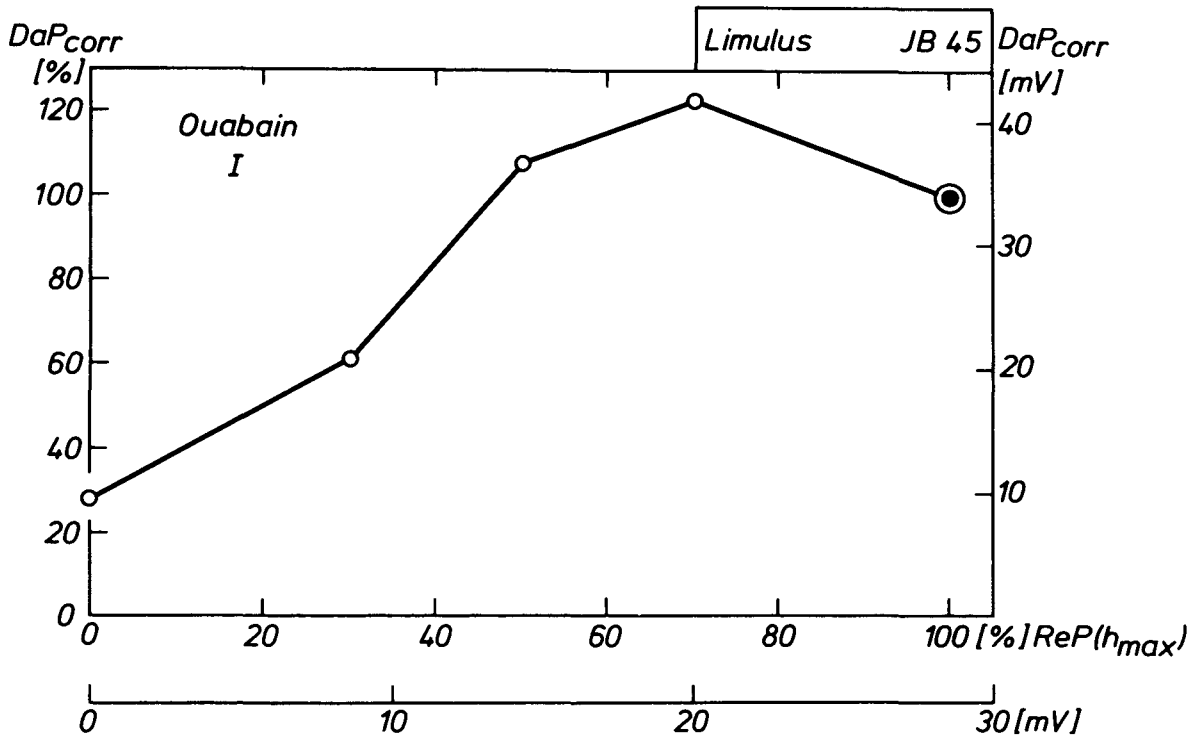

Fig. 7b

Intracellular measurement of pre-stimulus membrane potential pMP = DaP_{corr} [mV] vs. maximal amplitude h_{max} [% of reference value recorded in physiological saline] of receptor potentials recorded in test saline containing 1 mM/l Ouabain.
Left scale: DaP_{corr} in % of reference value.
Scale below: h_{max} [mV].
Light intensity ca. 5.500 lx, stimulus duration τ ca. 50 ms.

JB 45, *Limulus* retinular cell

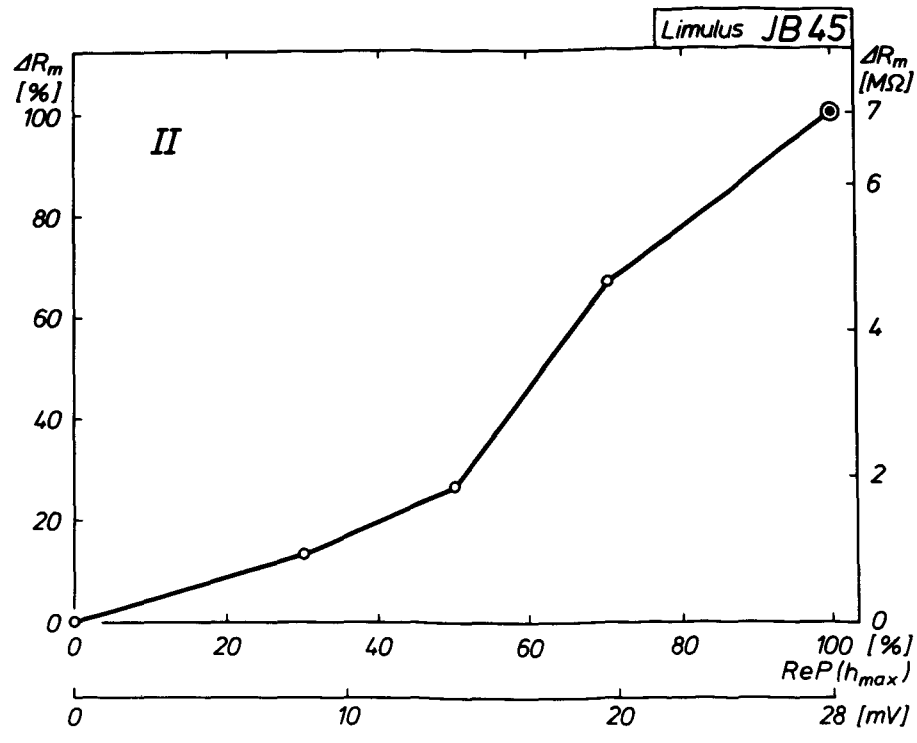

Fig. 7c

Intracellular recording of membrane resistance change ΔR_m [MΩ] vs. maximal amplitude h_{max} [% of reference value recorded in physiological saline] of receptor potentials recorded in test saline containing 1 mM/l Ouabain.
Left scale: ΔR_m in % of reference value.
Scale below: h_{max} [mV]. For further details see Fig. 7b.

JB 45, *Limulus* retinular cell.

added to the saline) for a period of 1-2 hours. Due to the action of the ouabain receptor potential and dark potential showed a transient increase in the first minutes (the receptor potential in most cases and the dark potential always) which was followed by a continuous gradual decrease. The receptor potential decreased more steeply than the dark potential and disappeared long before the dark potential approached zero. The half time $t_{1/2}$ for the decrease of the receptor potential was, on the average, 34 min whereas the half time for the decrease of the pMP was 64 min. Washing out the poison did not lead to a recovery of the cell in contrast to earlier findings with unpenetrated cells of the *Astacus* retina (external electrodes; Stieve et al., 1971). The dark potential decreased further in the after period in which the ouabain was washed out, in most cases the dark potential had already reached zero in the main period and did not recover in the after period.

In Table 7 the parameters of the receptor potential measured when the maximum of the receptor potential had decreased to 50%, 30% and 0% of the reference value (see method) are compared. The values after 1 hour stay in ouabain and at the end of the main-period and the after-period are also listed.

In Table 7 and Fig. 7a it can be seen that the receptor potential is already zero when the dark potential has decreased to ca. 40% of its reference value.

With decreasing maximal amplitude the latency t_{lat} and the time-to-peak t_{max} for short stimuli increase whereas the decrease-time t_2 becomes shorter. For long stimuli the plateau--value h_e decreases slightly less than the maximal amplitude, the shape-quotient h_{max}/h_e decreases. The time-to-peak t_{max} increases as well.

The dark resistance R_d does not change significantly under the action of ouabain. The light-induced resistance drop ΔR_m decreases under the action of ouabain to a value no longer measurable even before the receptor potential has reached zero.

No recovery of the light-induced conductance change could be found. Fig. 7c shows that the decrease in ΔR_m corresponds fairly well with the decrease of the maximal amplitude h_{max} of the ReP. At h_{max} = 50% ΔR_m has decreased to ca. 30% of the reference value.

The curve of ΔR_m versus h_{max} (Fig. 7a and b) shows that the receptor potential is zero when the conductance change is zero. Since the pre-stimulus membrane potential is not zero at this time it is reasonable to assume that the receptor potential disappears because there is no more light-induced conductance change.

Earlier experiments with the ventral photoreceptor of *Limulus* confirm the observation that the receptor potential is zero some time before the membrane potential (Stieve and Appelhans, unpublished).

No intracellular experiments on the action of ouabain on the *Astacus* visual cell were made. In former experiments already published (Stieve et al., 1971) on the action of ouabain on the *Astacus* retina only extracellular electrodes were used. Those experiments are in good agreement with the experiments reported here on *Limulus*, except that under the action of ouabain, when the receptor potential is decreasing, both the latency period t_{lat} as well as the time-to-peak t_{max} decrease in *Astacus* contrary to the intracellular measurements in *Limulus*. The most important difference, however, is that the loss of excitability after poisoning is reversible in the case of extracellular recording, where the receptor potential recovers within 30 min after washing out the poison (Stieve et al., 1971).

4.3 Depolarization by high extracellular potassium concentration

Under the action of ouabain the membrane of the visual cell loses its ability to perform light-induced conductance changes.

It takes a considerable time until the action of ouabain on the dark potential is detectable ($t_{1/2}$ = 64 min). As already mentioned the effect of ouabain could either be an indirect action on the ability for conductance changes via the decreasing of the membrane potential or a more direct action on the conductance change performing system. To answer this question we depolarized the visual cell in a different way, i.e. by increased extracellular potassium concentration. In earlier experiments we had shown that the extracellularly measured receptor potential of crustacean visual cells is still measurable when the visual cell is depolarized by high external potassium concentration (Stieve, 1964; Stieve and Wirth, 1971). By changing the extracellular potassium concentration the membrane potential should be depolarized much faster than under the action of ouabain.

Since (according to a suggestion of Mauro, 1973) the gradient of sodium ions might also be important for the ability of the visual cell membrane to undergo light-induced conductance change we, in the case of *Astacus*, additionally varied the sodium content (no sodium or 1/10 of normal concentration) of the external solution besides increasing the potassium content.

4.3.1 *Limulus* retinular cell

In five experiments (JB 46-50) recorded from a *Limulus* retinular cell by means of an intracellular electrode the influence of high external potassium was investigated. In the test solution (Table 1, L3) all sodium was replaced by potassium while the content of the other ions was unchanged (Table 8; Figs. 8, 9 and 10a and b).

Due to the change of the potassium gradient across the cell membrane by the perfusion of the experimental chamber with high external potassium solution the pre-stimulus membrane potential decreases quickly and reverses polarity (change from minus to plus values in Table 8). During the stay in the test saline the reversed membrane potential is very high (+38% after

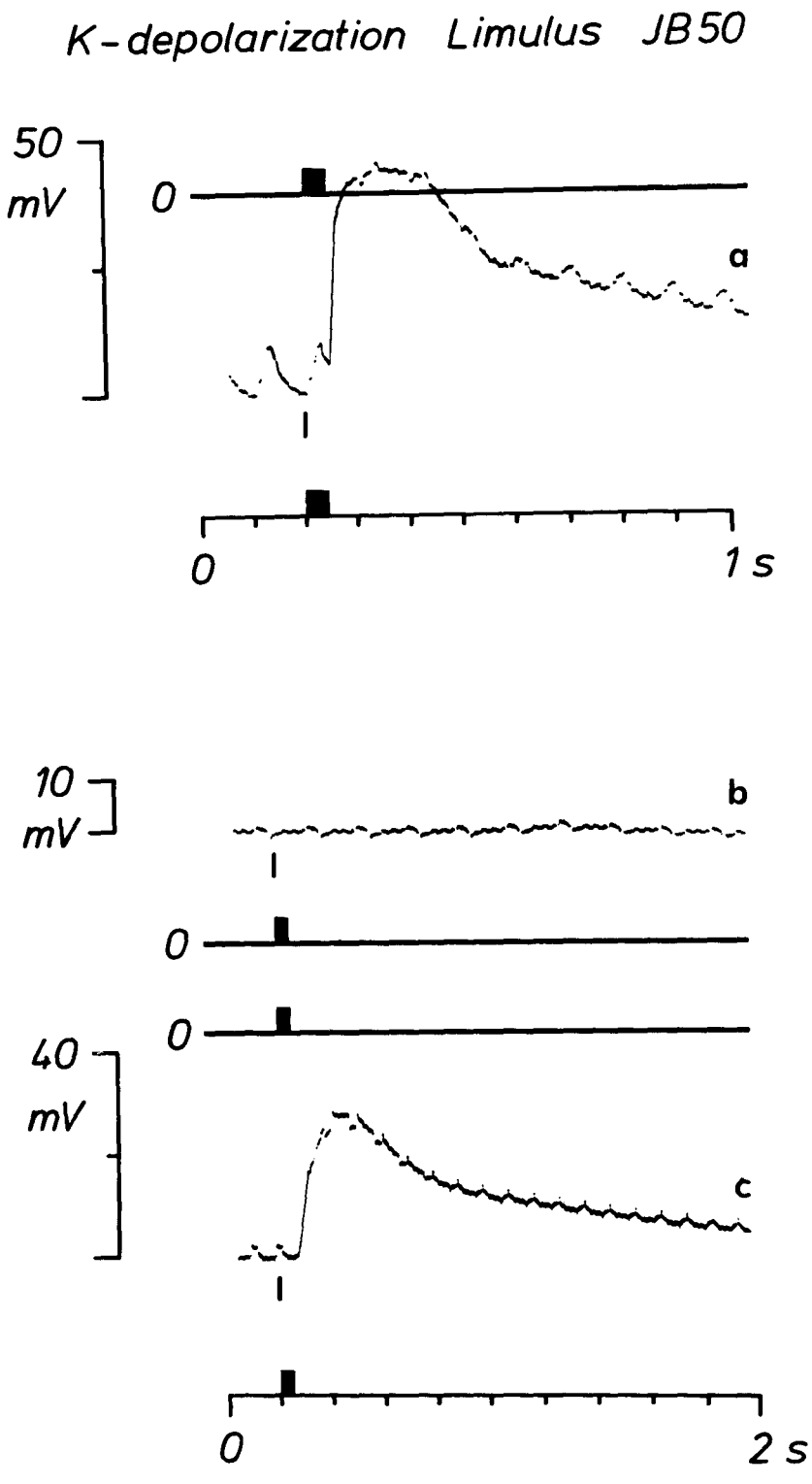

Fig. 8

Intracellularly recorded receptor potentials of a *Limulus* retinular cell and simultaneous measurement of the membrane resistance change ΔR (height of the square-wave impulses). Light intensity ca. 1.000 lx, stimulus duration τ ca. 50 ms.
a: in physiological saline
b: 55 min in test saline containing 498.3 mM/l potassium
c: 55 min again in physiological saline.

JB 50, *Limulus* retinular cell

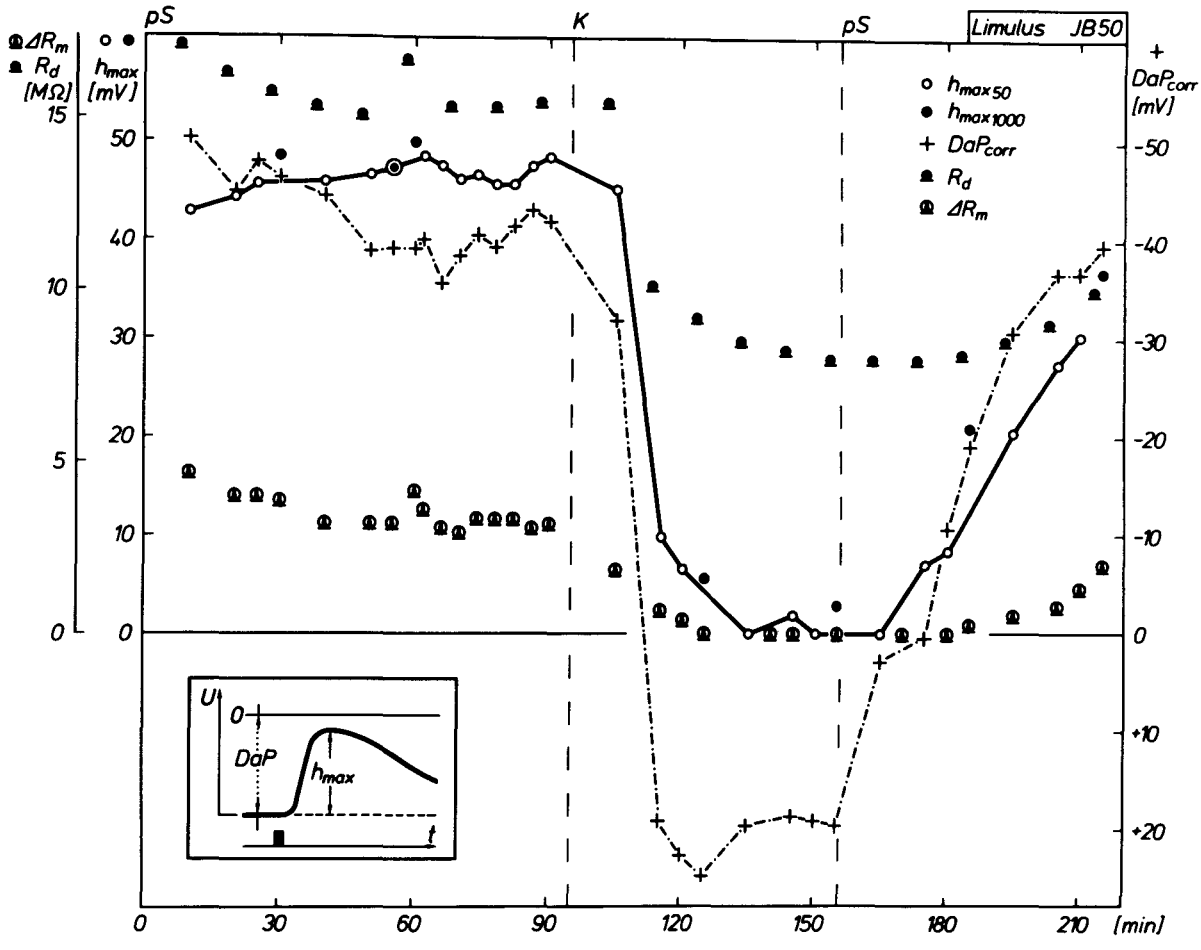

Fig. 9

Time course of the effect of potassium depolarization on *Limulus* retinular cell. Intracellular measurement of $h_{max_{50}}$ [mV], $h_{max_{1000}}$ [mV], DaP_{corr} [mV], R_d [MΩ], and ΔR_m [MΩ] vs. time [min].
pS: perfusion with physiological saline,
K: perfusion with test saline containing 498.3 mM/l potassium.
For further details see Fig. 8.

JB 50, *Limulus* retinular cell

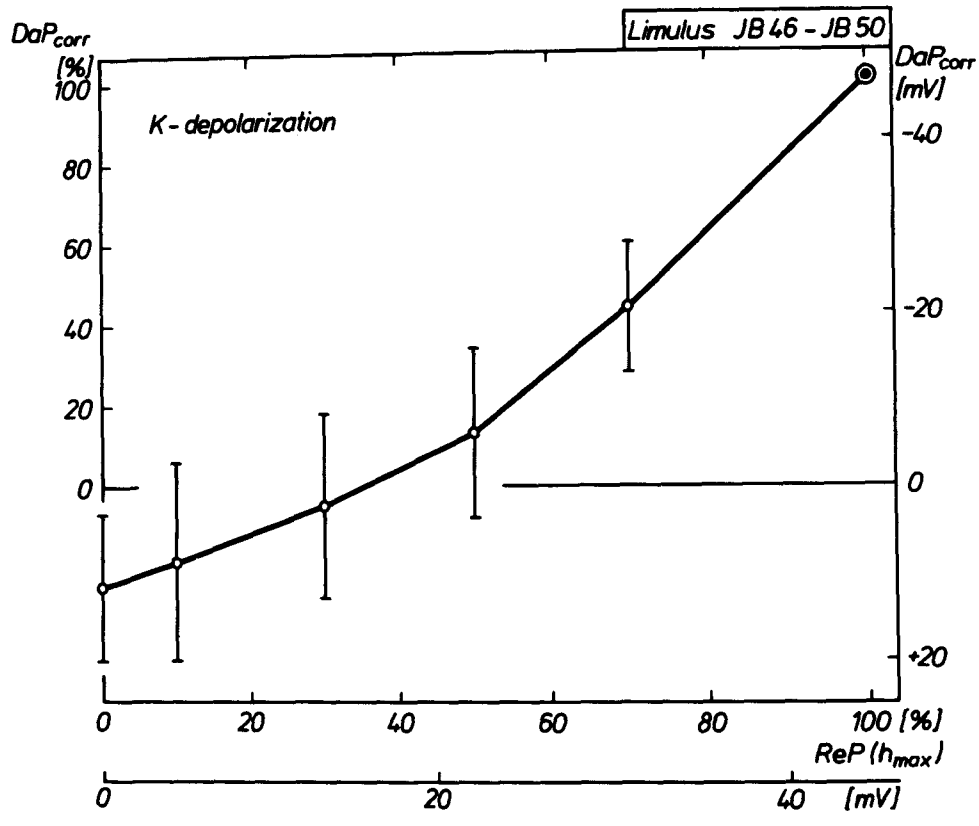

Fig. 10a

Intracellular measurement of pre-stimulus membrane potential pMP = DaP_{corr} [mV] vs. Maximal amplitude h_{max} [% of reference value recorded in physiological saline] of receptor potentials recorded in test saline with 498.3 mM/l potassium.
Left scale: DaP_{corr} in % of reference value. Scale below: h_{max} [mV].
The horizontal bars indicate the S.E. of the mean. For further details see Fig. 8.
JB 46-50, *Limulus* retinular cells

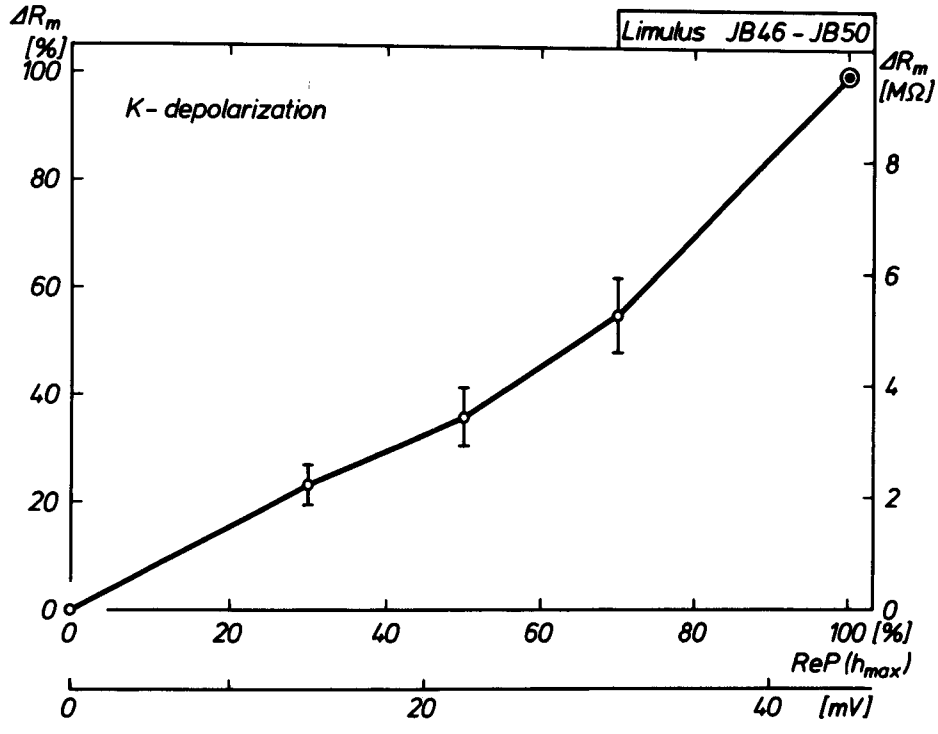

Fig. 10b

Intracellular recording of membrane resistance change ΔR_m [MΩ] vs. maximal amplitude h_{max} [% of reference value recorded in physiological saline] of receptor potentials recorded in test saline with 498.3 mM/l potassium. Left scale: ΔR_m in % of reference value. Scale below: h_{max} [mV]. The horizontal bars indicate the S.E. of the mean. For further details see Fig. 8.
JB 46-50, *Limulus* retinular cells

55 min) and in the following it decreases to a smaller value of about +14% of the reference value. The standard deviations of the pMP in Table 8 are very great but the changes just described are nevertheless significant.

When the membrane potential is decreased to zero or reversed, the receptor potential and the light-induced permeability changes slowly disappear. When the pMP is already reversed beyond zero a receptor potential can still be measured the maximal amplitude of which is about 30% of the reference value (see Figs. 9 and 10a). Finally the receptor potential and the light-induced permeability change disappear: The retina fails to respond to the light flash. There is a long time lag of more than 10 min between the total depolarization of the cell membrane and the loss of excitability.

The time needed to establish half of the action nevertheless is not different for the pMP and for the amplitude of the receptor potential: On changing the perfusion of the experimental chamber with high potassium solution the half-time $t_{1/2}$ for the change of the pMP is 13 min for h_{max} 15 min.

The observed effects were reversible; after one hour after--period when the test solution was replaced by normal saline, the pre-stimulus membrane potential had come back to (-)50% of its reference value, the receptor potential to about 25%. The restoration of excitability (receptor potential and light--induced conductance change) follows the restoration of the pMP with almost no delay.

In Table 8 the other parameters of the ReP were measured and compared at the times when the maximum of the ReP had decreased to 50%, 30% and 0% of the reference value. All parameters of the receptor potentials were compared at these times and with the potential at the end of the after-period: With decreasing maximal amplitude the latency t_{lat} increases to more than 200%, the time-to-peak t_{max} increases to ca. 150% and the decrease--time t_2 does not change very much. As compared to the changes

of the pMP and the amplitude of the RePs the changes of the time-parameters recover less in the after-period.

Resistances

Under the influence of the depolarization of the cell membrane the dark resistance R_d decreases to about 60% of its reference value. The change of the resistance ΔR_m measured at the time corresponding to the maximum of the ReP decreases under the influence of the changed potassium gradient is still measurable with a value of 17% of the reference value when the pMP is already zero. It becomes immeasurable at the time when h_{max} is zero and the reversed pMP has a value of ca. (+)25% of the reference value. A reasonable recovery of the measured resistances both of R_d and ΔR_m can be seen in the after-period (R_d 67%, ΔR_m 17% of the reference value).

Fig. 10b shows that the decrease in ΔR_m corresponds fairly well with the decrease of h_{max} of the ReP. The conductance changes ΔR_m and the changes of the receptor potential amplitude h_{max} under the influence of potassium depolarization seem to be correlated and have a common zero point. This means that there is no measurable light-induced conductance change when the receptor potential is zero.

When the visual cell was unexcitable as a result of the action of the changed potassium gradient, repolarization of the cell membrane by passing a current of 1 to 2 nA through the intracellular electrode, repolarizing the visual cell membrane by an estimated value of about 10-20 mV for ca. 50 min, did not lead to a recovery. The estimated membrane resistance was ca. 10 MΩ (JB 46-50, 3 experiments 1 nA, 1 experiment 2 nA, n = 5).

4.3.2 *Astacus* retinular cell

4.3.2.1 *Astacus*, potassium depolarization, no sodium

In 5 experiments (JB 52-56) electrical properties of the *Astacus* visual cells were measured intracellularly under the influence of high external potassium in the absence of sodium. Table 9 and Figs. 11-13a and b show the results.

The results are almost identical with those from *Limulus* retinular cells. The pre-stimulus membrane potential decreases and changes polarity under the influence of high external potassium, it goes down to ca. +15 mV with a half-time $t_{1/2}$ of 8 min which is a little bit faster than in the experiments with *Limulus*. The receptor potential decreases also faster (half-time $t_{1/2}$ for h_{max} is 3.3 min). Also in the case of *Astacus* there is a time lag of more than 10 min after reversal of polarity of the membrane potential until the receptor potential totally disappears. The cell becomes inexcitable.

The dark resistance decreases to about 80% under the influence of higher potassium. The measured light-induced resistance change of the cell membrane ΔR_m is small and soon becomes immeasurable. Fig. 13a and b show the same dependence of the receptor potential on the membrane potential and the light-induced resistance change as in the case of the *Limulus* experiments. Also here the changes of the light-induced conductance change and of the receptor potential under the influence of K-depolarization are practically parallel. Comparing the change of the shape of the receptor potential when h_{max} is 50% and 30% of the reference value, the latency t_{lat} increases to about 140%, the time-to-peak t_{max} slightly decreases and the decrease-time t_2 strongly decreases. Here again the changes show only little recovery in the after-period.

In the after-period in artificial seawater minor reversibility occurred, but less as compared to the *Limulus* retinular cell. In two of the five experiments relatively good recovery of R_d could be found; ΔR_m did not show a measurable recovery in all experiments. The determination of the light-induced resistance

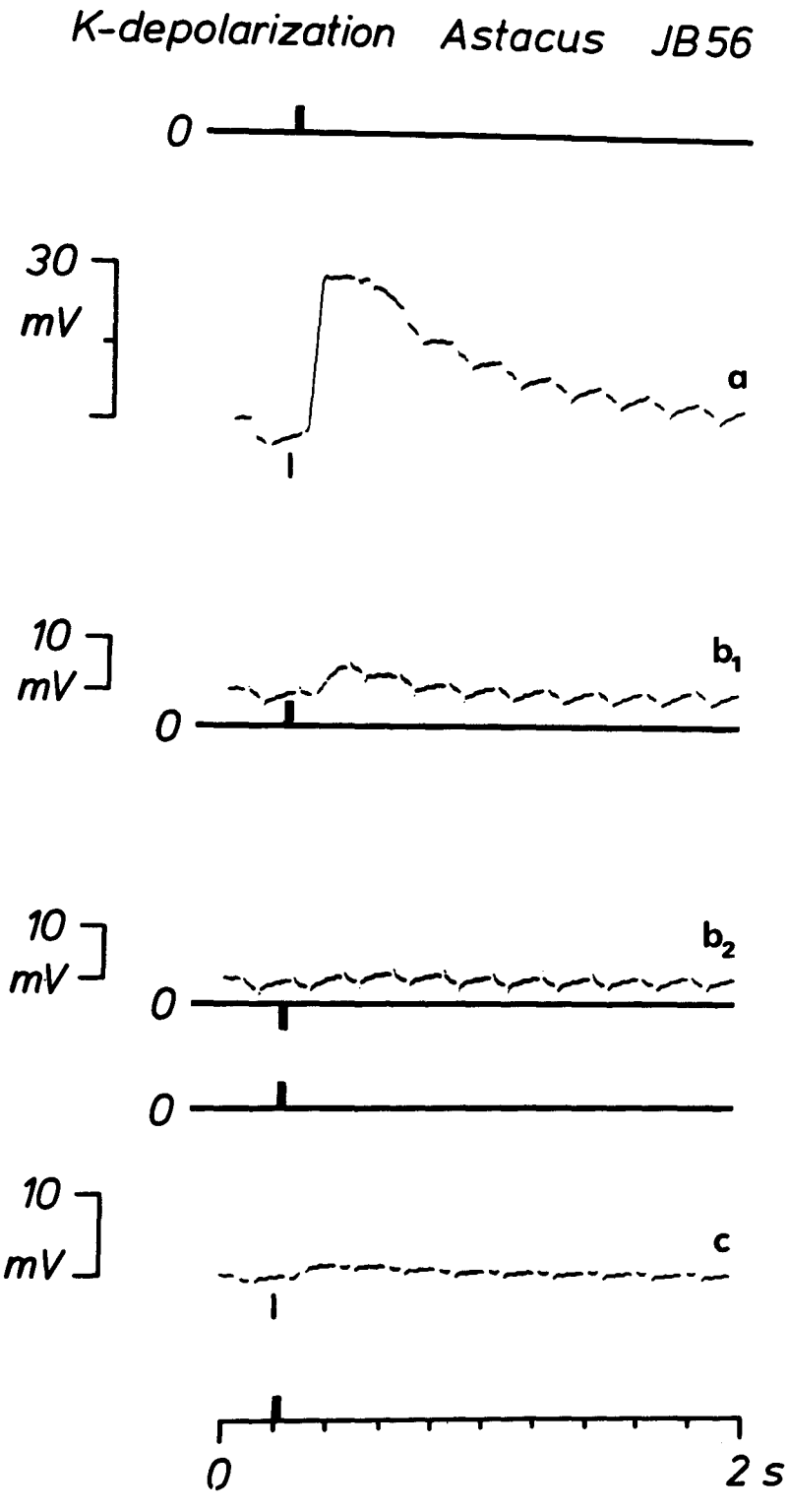

Fig. 11

Intracellularly recorded receptor potentials of an *Astacus* retinular cell and simultaneous measurement of the membrane resistance change ΔR (height of the square-wave impulses). Light intensity ca. 5.500 lx, stimulus duration τ ca. 10 ms.
a: in physiological saline
b_1: 55 min in test saline containing 212.3 mM/l potassium
b_2: 100 min in potassium test saline
c : 55 min again in physiological saline.
JB 56, *Astacus* retinular cell

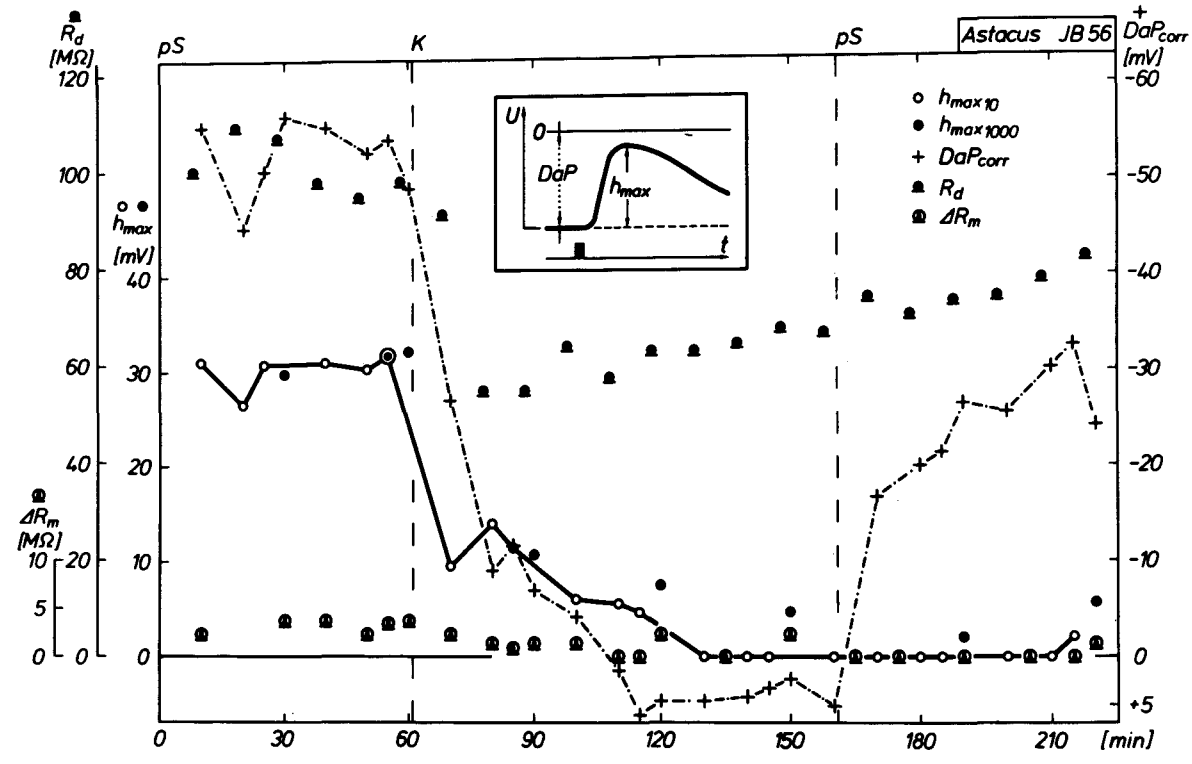

Fig. 12

Time course of the effect of potassium depolarization on *Astacus* retinular cell. Intracellular measurement of $h_{max_{10}}$ [mV], $h_{max_{1000}}$ [mV], DaP_{corr} [mV], R_d [MΩ] and ΔR_m [MΩ] vs. time [min].
pS: perfusion with physiological saline
K: perfusion with test saline containing 212.3 mM/l potassium.
For further details see Fig. 11.
JB 56, *Astacus* retinular cell

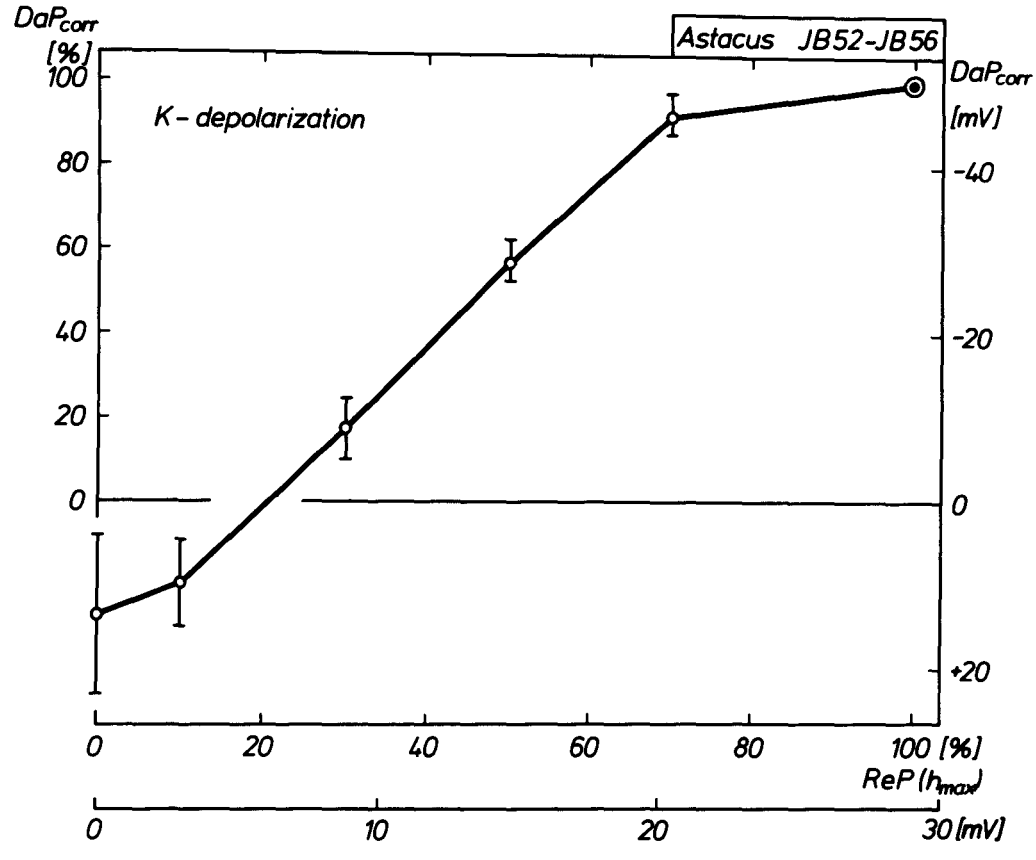

Fig. 13a

Intracellular measurement of pre-stimulus membrane potential pMP = DaP_{corr} [mV] vs. maximal amplitude h_{max} [% of reference value recorded in physiological saline] of receptor potentials recorded in test saline with 212.3 mM/l potassium.
Left scale: DaP_{corr} in % of reference value.
Scale below: h_{max} [mV].
The horizontal bars indicate the S.E. of the mean. For further details see Fig. 11.
JB 52-56, *Astacus* retinular cells

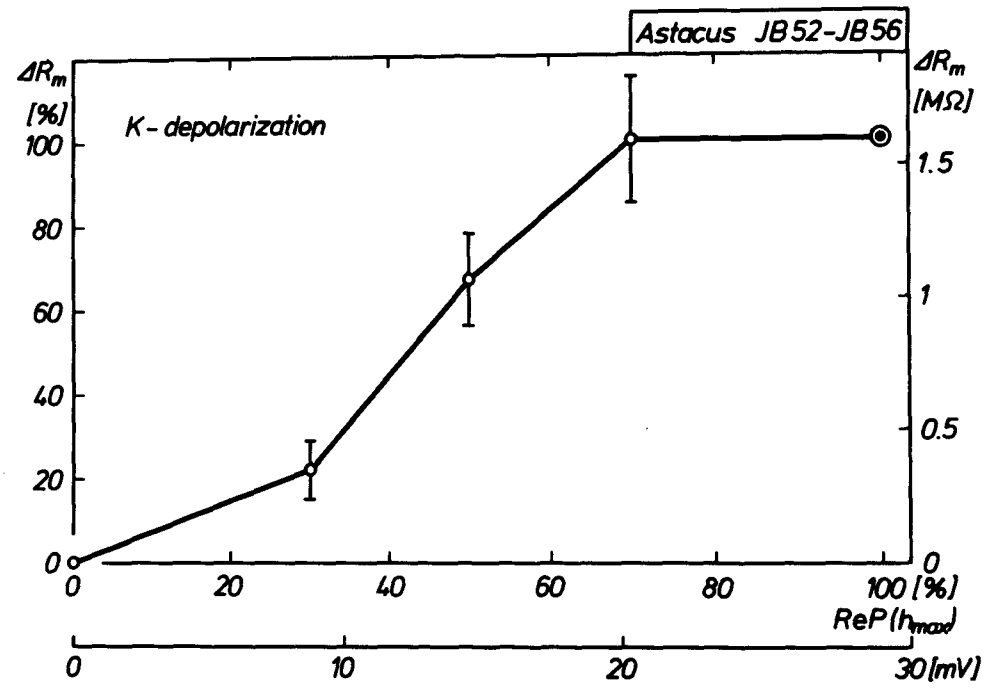

Fig. 13b

Intracellular recording of membrane resistance change ΔR_m [MΩ] vs. maximal amplitude h_{max} [% of reference value recorded in physiological saline] of receptor potentials recorded in test saline with 212.3 mM/l potassium. Left scale: ΔR_m in % of reference value.
Scale below: h_{max} [mV]. The horizontal bars indicate the S.E. of the mean. For further details see Fig. 11.

JB 52-56, *Astacus*

change of the visual cell membrane is much less satisfactory in *Astacus* than in *Limulus*.

In all experiments it was tested whether repolarization of the cell membrane by passing small currents through the intracellular electrode could restore the ability to change the resistance of the cell membrane. The applied current was 1 and 2 nA of ca. 50 min duration. The repolarization of the membrane potential can only be roughly estimated, since the membrane resistance of the retinular cell is not known. If the membrane resistance is assumed to be about 10 MΩ, the membrane is repolarized by about 10 or 20 mV. As in the case of *Limulus* no measurable restoration of the excitability, which had been lost due to potassium depolarization, could be observed while the current was applied.

The extracellular mass response of the whole retina under the influence of high external potassium was measured in earlier experiments (Stieve and Wirth, 1971, *Astacus*; and Stieve, 1964, *Eupagurus*). The changes are similar. During one hour perfusion of the vessel with high potassium solution the excitability was not entirely abolished, the peak amplitude h_{max} remained at 6-8% of its reference value. The reversibility was very good.

4.3.2.2 Potassium depolarization in the presence of low sodium concentration (21 mM/l)

In five experiments potassium depolarization in the presence of 21 mM external sodium (Table 1, A3) was simultaneously recorded in the *Astacus* retina intracellularly with one microelectrode (response of one retinular cell) and by extracellular measurement (mass response of the whole receptor layer) (experiments JA 17 - JA 21, Table 10a and b, Figs. 14-18).

The experiments showed similar results as described before for the sodium-free potassium solution: In the <u>intracellularly recorded</u> experiments the dark potential decreased sharply (half time $t_{1/2}$=9 min) and reversed in sign to +5 mV. The receptor

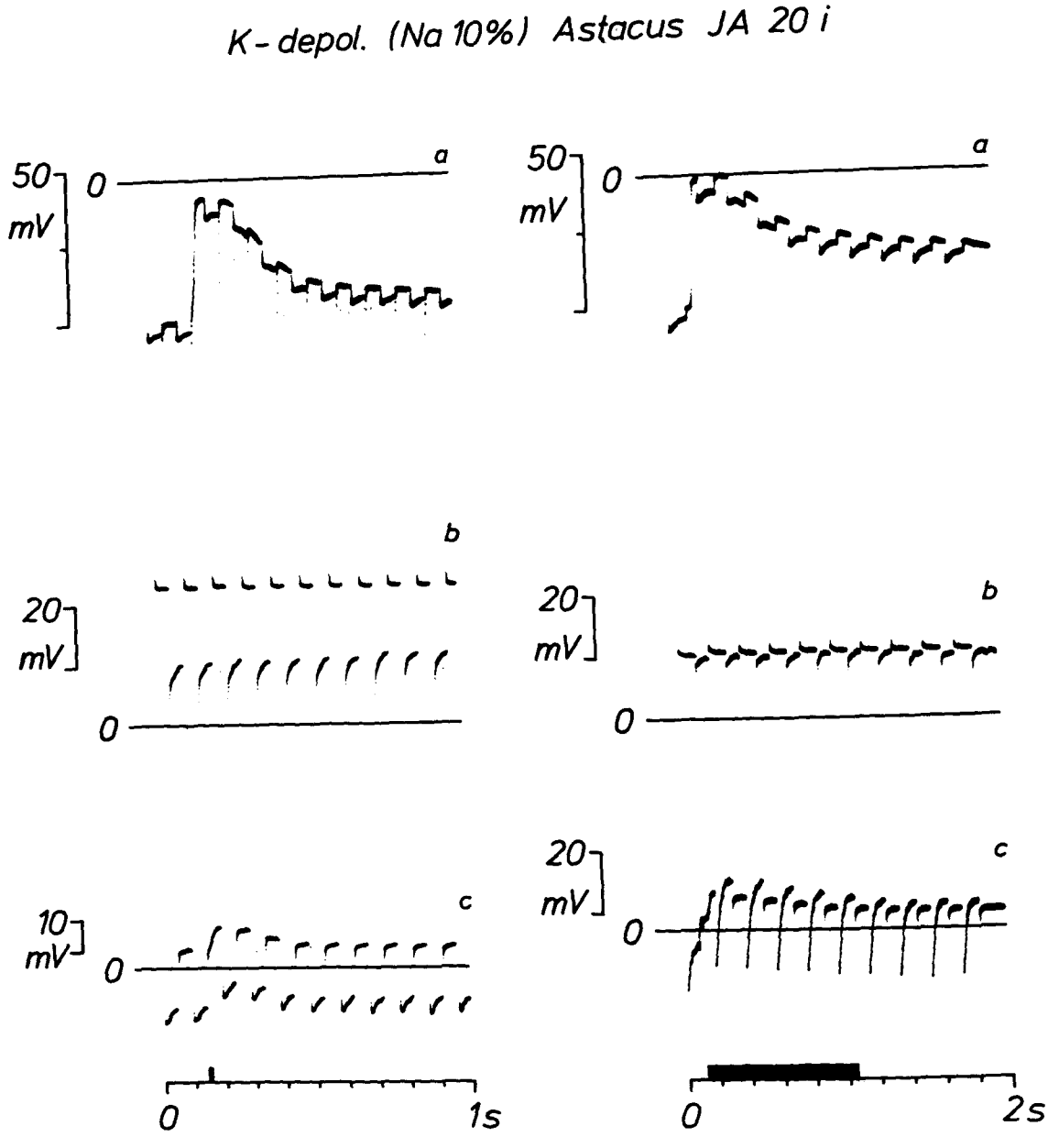

Fig. 14

Intracellularly recorded receptor potentials of an *Astacus* retinular cell and simultaneous measurement of the membrane resistance change ΔR (height of the square-wave impulses).
Light intensity ca. 20.000 lx, stimulus duration τ ca. 10 ms (left column) and 1000 ms (right column).
a: in physiological saline
b: 55 min (left) and 60 min (right in test saline containing 191.6 mM/l potassium and 20.7 mM/l sodium (ca. 10% of normal content)
c: 55 min (left) and 60 min (right) again in physiological saline.

JA 20, *Astacus* retinular cell

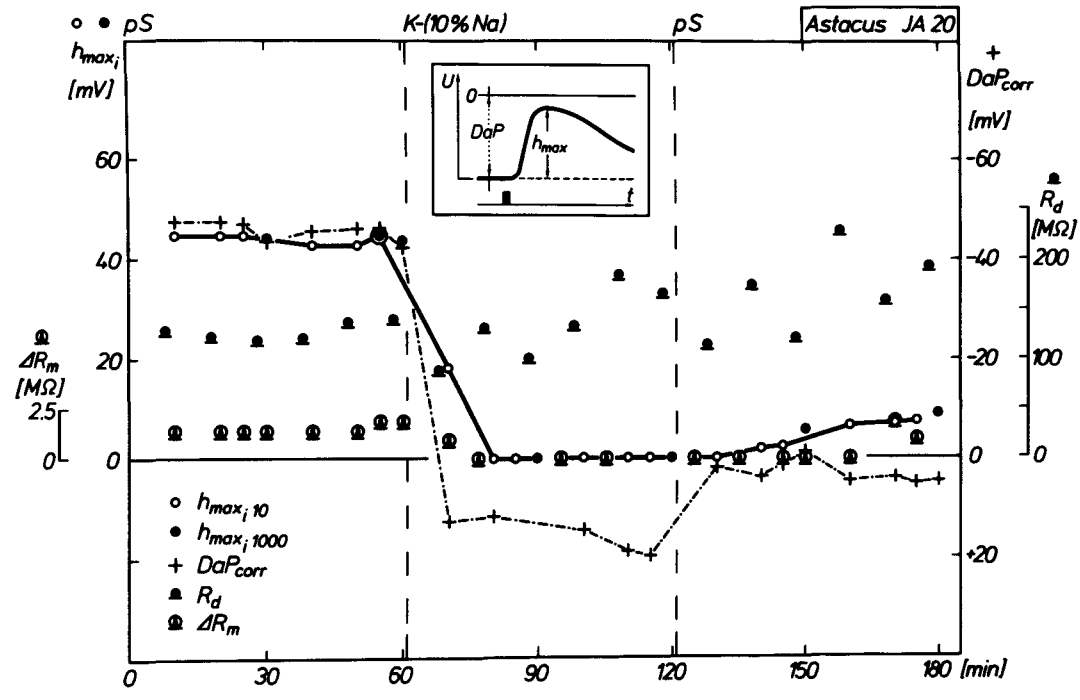

Fig. 15

Time course of the effect of potassium depolarization together with sodium deficiency on *Astacus* retinular cell. Intracellular measurement of $h_{max_{10}}$ [mV], $h_{max_{1000}}$ [mV], DaP_{corr} [mV], R_d [MΩ] and ΔR_m [MΩ] vs. time [min].

pS: perfusion with physiological saline
K (10% Na): perfusion with test saline.
For further details see Fig. 14.

JA 20, *Astacus* retinular cell

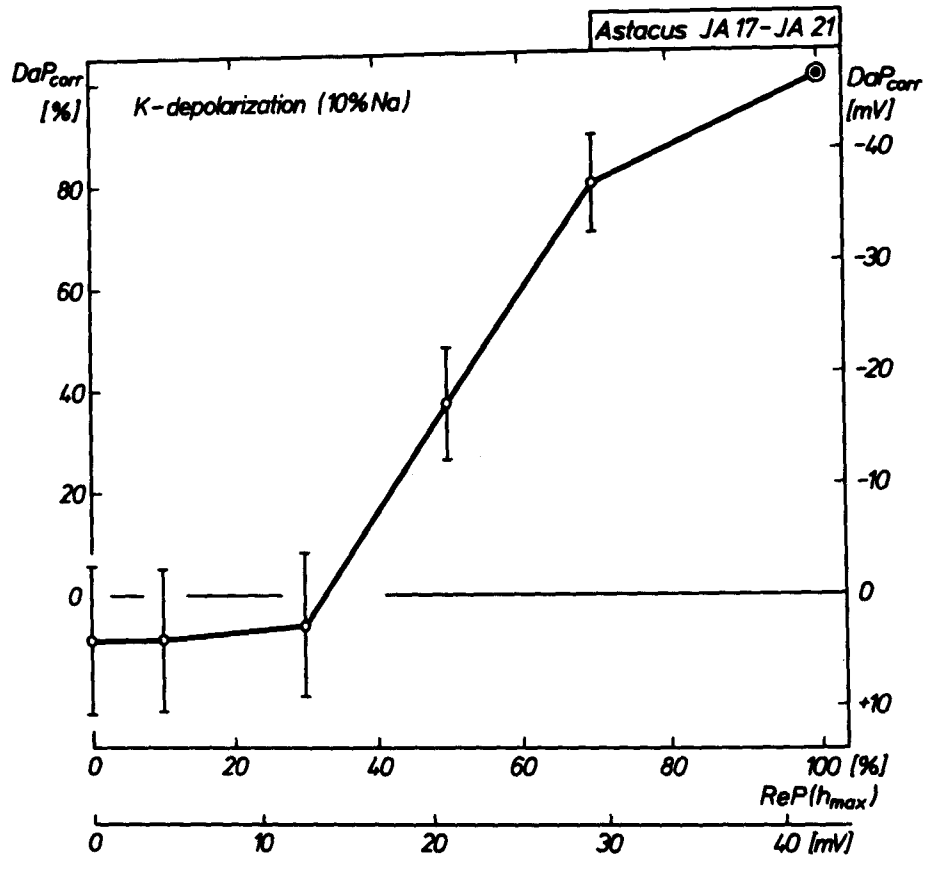

Fig. 16a

Intracellular measurement of pre-stimulus membrane potential pMP = DaP_{corr} [mV] vs. maximal amplitude h_{max} [% of reference value recorded in physiological saline] of receptor potentials recorded in test saline with 191.6 mM potassium and 20.7 mM/l sodium.
Left scale: DaP_{corr} in % of reference value.
Scale below: h_{max} [mV].
The horizontal bars indicate the S.E. of the mean.
For further details see Fig. 14.

JA 17-21, *Astacus* retinular cells

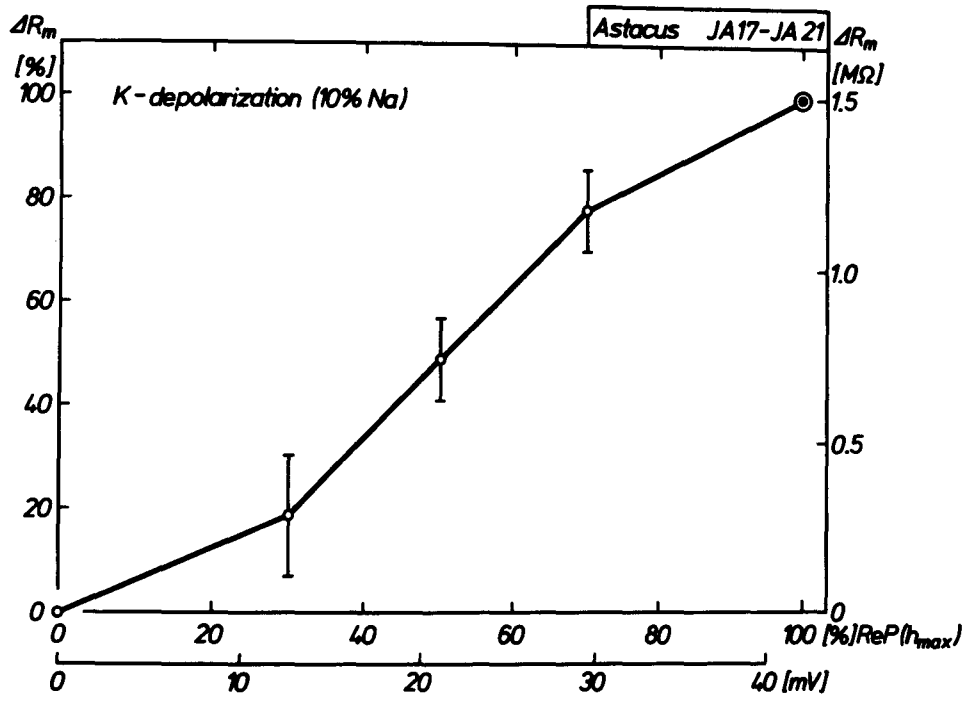

Fig. 16b

Intracellular recording of membrane resistance change ΔR_m [MΩ] vs. maximal amplitude h_{max} [% of reference value recorded in physiological saline] of receptor potential recorded in test saline with 191.6 mM/l potassium and 20.7 mM/l sodium.
Left scale: ΔR_m in % of reference value
Scale below: h_{max} [mV].
The horizontal bars indicate the S.E. of the mean. For further details see Fig. 14.
JA 17-21, *Astacus* retinular cells

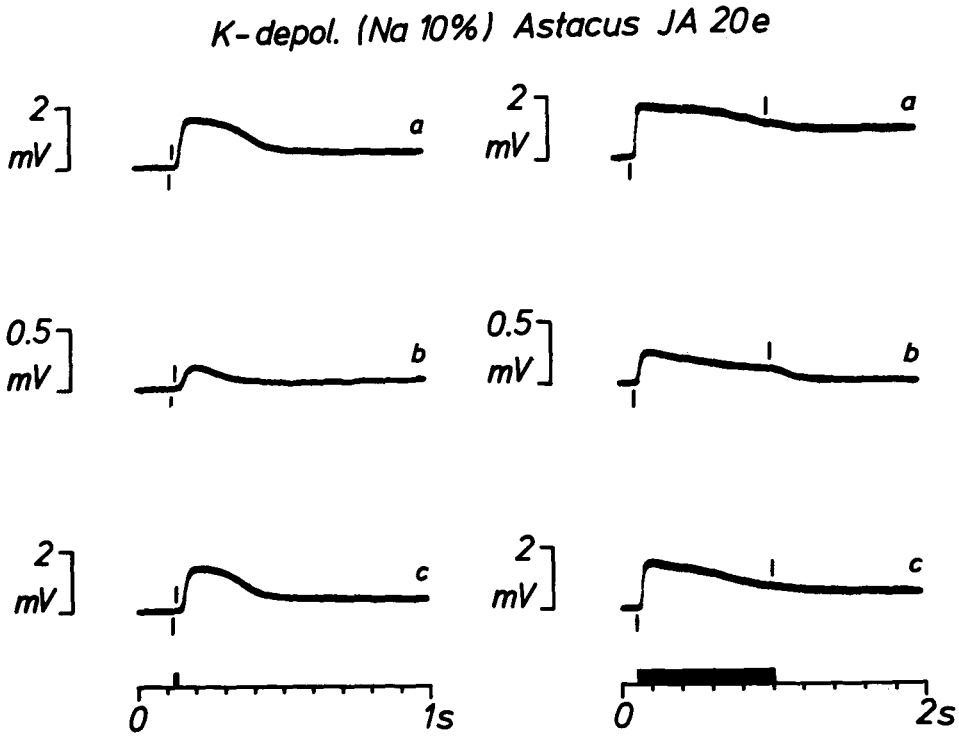

Fig. 17

Extracellularly recorded receptor potentials of an *Astacus* retina slice (same preparation from which the intracellular potentials in Fig. 14 were recorded simultaneously).
Light intensity ca. 20.000 lx, stimulus duration τ ca. 10 ms (left column) and 1000 ms (right column).
a: in physiological saline
b: 55 min (left) and 60 min (right) in test saline containing 191.6 mM/l potassium and 20.7 mM/l sodium
c: 55 min (left) and 60 min (right) again in physiological saline.

JA 20, *Astacus* retina slice

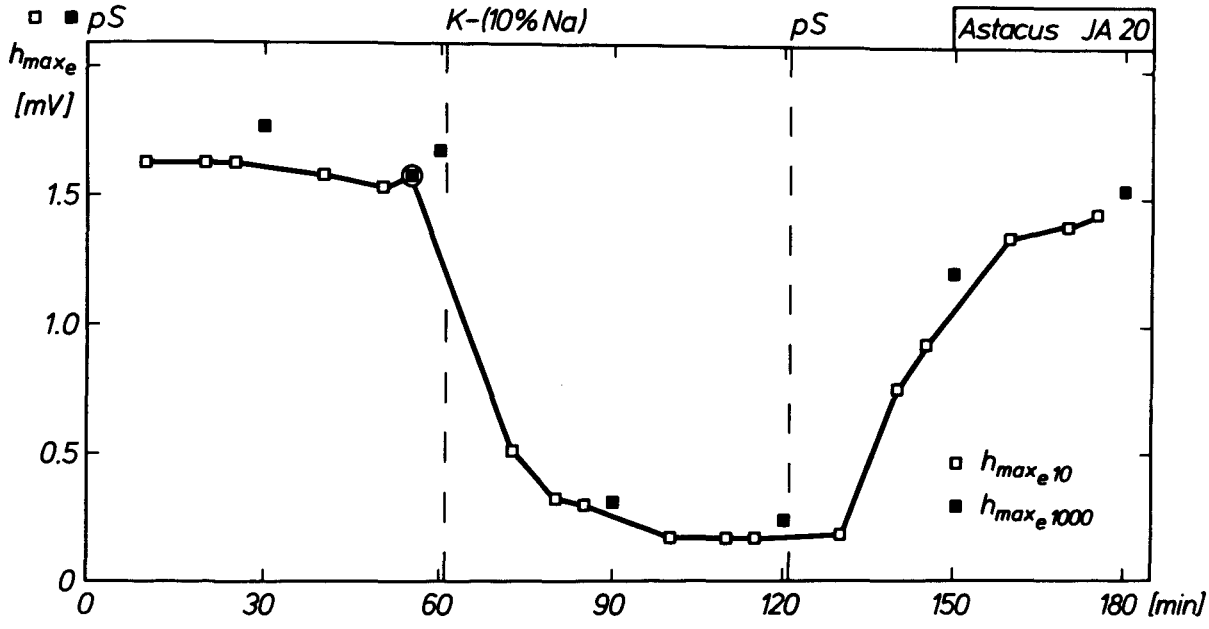

Fig. 18

Time course of the effect of potassium depolarization together with sodium deficiency on *Astacus* retina slice (same preparation from which the time course of the intracellular responses was recorded simultaneously, see Fig. 15). Extracellular measurement of $h_{max_{10}}$ [mV] and $h_{max_{1000}}$ [mV] vs. time [min].
pS: perfusion with physiological saline,
K (10% Na): perfusion with test saline.
For further details see Fig. 17.

JA 20, *Astacus* retina slice

potential disappeared (half time $t_{1/2}$=5 min). Again the total loss of excitability occurred some 10 min following the polarity change of the pre-stimulus membrane potential. Again even when the measured dark potential was reversed, the receptor potential was still measurable as a positive potential change.

The dark resistance R_d did not change significantly which is in contrast to the experiments with depolarization by high external potassium without external sodium. The light-induced conductance change decreased parallel to the receptor potential under the influence of the depolarizing high external potassium. Again ΔR was not very well measurable in these preparations.

Under the influence of high external potassium in the presence of 21 mM sodium with decreasing receptor potential (h_{max} 30%) the latency period t_{lat} was increased, the time-to-peak t_{max} was decreased to 64% and the decrease-time t_2 was even more decreased to 35%. The shape-quotient h_{max}/h_e was strongly increased to over 300% after 60 min in the test saline. (The time parameters t_{max} and t_2 are much more decreased than in the experiments without sodium and the shape-quotient is increased here, whereas without sodium it is decreased.)

Recovery was also observed in these experiments. The dark potential recovered to 15-20 mV and the receptor potential to 10-15 mV. The light-induced resistance change reappeared, t_{max} and t_2 showed recovery while t_{lat} remained unchanged.

The extracellularly recorded receptor potentials of the whole retina showed similar results (Table 10b, Figs. 16 and 17). The receptor potential was strongly decreased to ca. 15% (half time $t_{1/2}$ 8 min for h_{max}) and did not totally disappear. The shape-quotient h_{max}/h_e was strongly increased, the latency--period t_{lat} was increased, t_{max} and t_2 decreased, t_2 not as much as in the intracellular measurements. All changes showed good reversibility, better than in the intracellular recordings.

The main differences between intra- and extracellular measure-

ments are: The decrease of h_{max_e} is smaller than that of h_{max_i}; the reversibility of all measured parameters of the ReP is much better in the extracellular recordings, e.g. the peak amplitude $h_{max_{10}}$ is 83% at the end of the after-period in contrast to 27% in the intracellular measurement. The decrease of t_2 is smaller in the extracellular measurement (80%) as compared to the intracellular recording (35%).

5. Discussion

5.1 Comparison of extracellular and intracellular recording

Since extracellular recording provides a relatively easy possibility of doing a greater number of experiments under rather stable and reproducible conditions, this method is very often applied. For this reasons it is desirable to know how extracellularly recorded receptor potentials differ from intracellularly recorded ones. This information can be obtained from simultaneous intracellular and extracellular recordings from the same preparation under the same conditions.

Besides general agreement of the receptor potentials recorded by intracellular and extracellular electrodes there are some differences: The extracellular recorded receptor potential amplitudes are ca. 5% of the intracellularly recorded ones. The shape-quotient (h_{max}/h_e) is 150% of the intracellular value in the extracellular case. The latency period is longer in the extracellular recording, while the time-to-peak and the decrease--time are slightly shorter.

The main disadvantage of the extracellular recording is of course that one does not get any information on the membrane potential of unstimulated cells, like the pre-stimulus membrane potential pMP.

The intracellular recording measures the membrane potential and its change of one particular cell, integrating the response of different areas of the cell membrane. Effects occurring far away from the recording electrode may contribute less to the measurement than changes in the neighborhood of the tip (Wulff, 1973). So the potential and the signal which is recorded intracellularly depends to a certain degree from the location of the electrode.
Injury of the cell due to the penetration of the electrode may also cause certain partial injury of the responsiveness of areas of the cell membrane. Since the conductance of the intra-

cellular volume of the visual cell is high as compared to the conductance of the cell membrane, the deviations of intracellular responses depending upon the situation of the electrode are not very great. However, for instance the relative size of the spike of the eccentric cell of the *Limulus* ommatidium depends more on the electrode tip situation, since the spike generates in a narrow part of the cell. Wulff (1973) has also shown that the relative size of certain components of the receptor potential of the retinular cell of *Limulus* depends on the situation of the electrode tip.

In extracellular recording the single record is that of an average response from a great number of retinular cells. It shows more the general properties, but less "fine resolution" since not all the responses of the single cells are exactly synchronized and equal in time course. Moreover the record is the measure of the voltage signal caused by extracellular current due to the change in the membrane resistance and membrane potential of the visual cells. The visual cells in the retina have different positions and are oriented differently. This causes different contributions of retinular cells of different retinulas to the recorded mass receptor potential. One important reason for the different contribution to the receptor potential is that the retinulas, because of their different orientation are not stimulated equally strong. The retinular cells oriented in the direction of the incident light contribute most, at least at higher stimulus intensities to the overall response.

Since the extracellular measurements are in effect the measurements of the extracellular current, they are influenced by the change of membrane resistance of the visual cells. The magnitude of the extracellularly measured receptor potential is influenced by the membrane resistance change. Since the decrease in membrane resistance is greater in the maximum of the receptor potential than in the plateau-value, the measured fraction of h_{max} is greater than the measured fraction of h_e. This could account for the greater value of the shape-quotient h_{max}/h_e in

the extracellular recording, and can also be the reason for the shorter value of the decrease-time t_2 when recorded extracellularly.
The resistance change during the light response should also increase the steepness of the initial ascent of the receptor potential. Since the latency-period is determined in our experiments as the intersection of the tangent line of the steppest point of the rise of the receptor potential with the base-line this effect should increase the latency-period t_{lat}. The time-to-peak t_{max} is only shortened significantly for long stimuli. Possibly also the resistance change may be responsible for this effect.

The relative changes of extracellularly and intracellularly recorded receptor potentials are similar when the light intensity is changed.
The relative changes are also similar when the retina is treated with high external potassium except for two important differences: The amplitude of the extracellularly recorded receptor potential does not decrease to zero whereas it does so in the intracellular recorded case. Secondly the reversibility is much better for the extracellular response.

Both these facts are also true for other treatments of the retina. For instance treating the retina with 2 mM/l X537A causes total loss of excitability in most of the intracellular recordings, whereas it causes a diminuition of the receptor potential to ca. 5% in the extracellular recordings (Stieve and Bruns, unpublished, 1975).
Treatment of the retina with calcium-free solution, containing 1 mM/l EDTA or EGTA, causes irreversible loss of excitability when recorded intracellularly but reversible loss of excitability in the extracellular recorded case (Stieve, 1973).
Ouabain treatment also causes reversible loss of extracellularly recorded receptor potentials of the *Astacus* retina (Stieve, Bollmann-Fischer, Braun, 1971) and irreversible loss of excitability in intracellular recordings (see present results, and Stieve, 1973).

That the whole retina does not lose its extracellularly measured excitability in the high potassium solution may have two reasons:

1) In the extracellular recording one records the mass response from the whole retina. Possibly not all visual-cells are reached equally good by the test solution. Some of them may

have contact only to diluted test solution and so can sustain the treatment.

2) Not penetrated cells generally show a less delicate behavior towards changes and strains than injured ones.

When the stimulus intensity, or the extracellular potassium concentration is changed, the extracellularly recorded receptor potential is a fairly good measure for the changes occurring in the single visual cells. This should also be true for changes in the state of adaptation and changes which are caused by other variations of the composition of the extracellular solution as long as the ratio of the extracellular and intracellular resistances and their changes occurring during excitation are not strongly influenced.

Those changes which influence the relative resistances between intracellular and extracellular medium and the resistance of the cell membrane, like e.g. temperature, strongly influence height and shape of the extracellularly recorded receptor potential (Fig. 19). Cooling the preparation can cause a decrease of the amplitude of the extracellularly recorded receptor potential whereas the intracellularly recorded potential does not change or even slightly increases (Stieve, 1963). The extracellular recording is then distorted as compared with the intracellular one. Possibly also the quantitative changes of the amplitudes of the receptor potential under the action of certain pharmaca and substances (like calcium ions) which are known to influence the membrane resistance, may be distorted in extracellular recordings. Since the resistance change is the cause of the receptor potential, the general direction of the influence should be similar in extra- and intracellular recordings; only the degree of the change could be varied.

Under most conditions one can fairly safely assume that the relative changes of the parameters of the receptor potential (both amplitudes and time parameters) due to experimental manipulations, are similar in extracellular and in intracellular

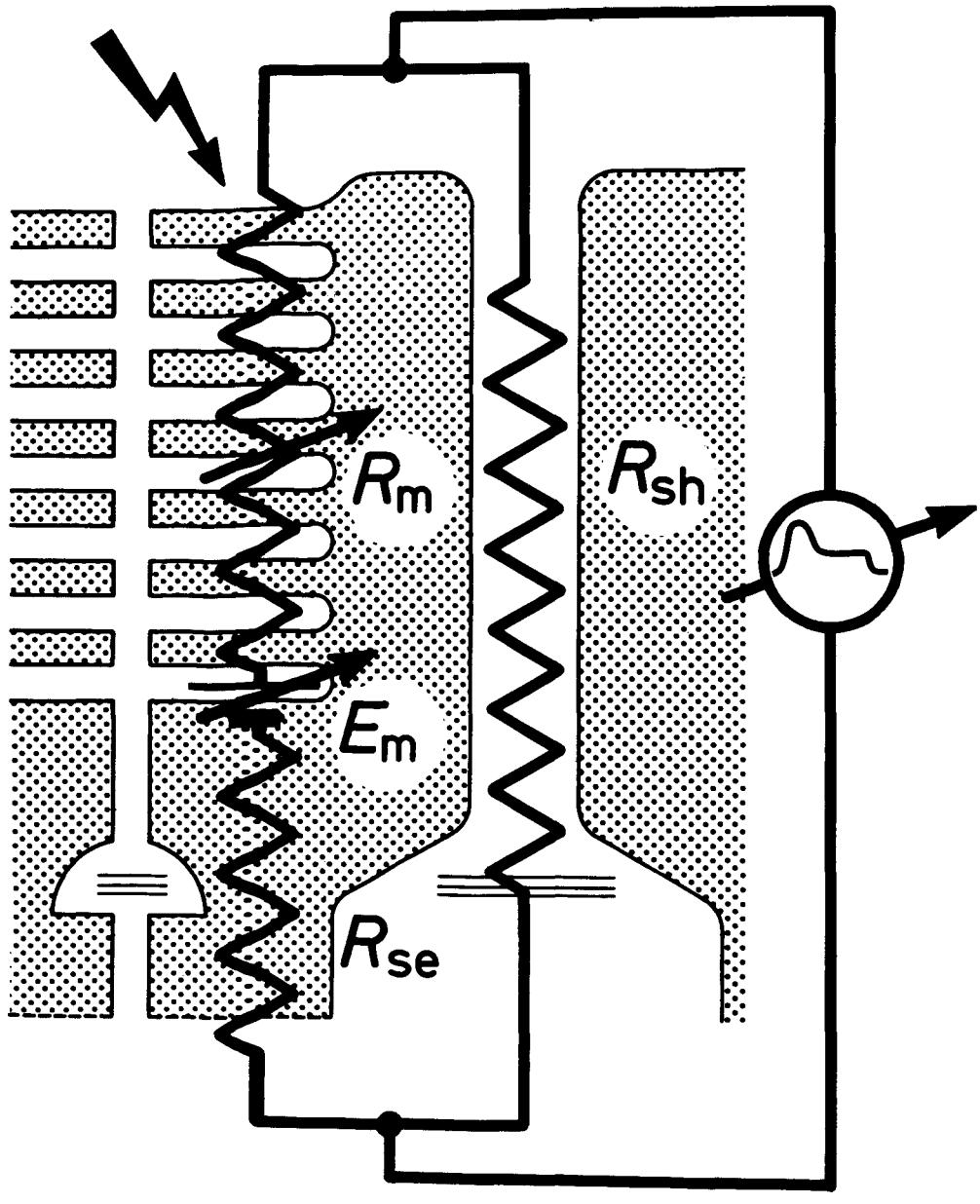

Fig. 19

Extracellular recording conditions in the retina of *Astacus*.
R_m = membrane resistance, E_m = membrane potential; both change in response to the light stimulus (indicated by the flash). R_{se} = serial resistance; R_{sh} = shunt resistance (extracellular). R_{sh} depends differently on temperature than the membrane resistance and its change ΔR_m.

recordings. The influence of the resistances involved (which might be influenced by certain experimental manipulations has to be taken into account when interpreting the observed changes.

5.2 Light-induced resistance change of the visual cell membrane

The membrane potential of the visual cells is determined by the ionic gradient across the cell membrane and by the different membrane permeabilities for different ion species, and under certain conditions by the activity of electrogenic active ion transporting mechanisms (leading to changes of up to ca. 10 mV). The light-induced change in membrane conductance is the cause of the receptor potential. However, the amplitude change in the time course of the receptor potential and the maximal amplitude of the receptor potential h_{max} are both depending on the intensity of the light stimulus not proportionally to the light-induced resistance change. The reasons for this are discussed in some detail elsewhere (Stieve et al., 1976). When the resistance change becomes smaller, the amplitude does not proportionally decrease in the time course of the receptor potential but faster. The same applies to measurements of the intensity dependence: The decrease of the receptor potential amplitude is more marked than that of the resistance change.

This discrepancy can have several different reasons:

1) A light-induced change in conductance for one ion species will normally not cause a proportional change in the membrane potential which is the result of the gradients of several ion species and the respective membrane permeabilities.
2) Also ionic gradients across the cell membrane, and the activity and the inner resistance of the electrogenic pump mechanism can vary during the receptor potential (especially the latter). Kramer (1976) points out arguments which exclude major contribution of exhaustion of ionic gradients.
3) The measured light-induced resistance change in the course of the receptor potential could be a sum effect due to opening and closing of two different species of ionic

channels: First "light channels" preferring sodium are opened and subsequently a delayed increase in potassium conductance enhances a repolarization as in the case of the nerve spike. The opening of the potassium channels could be also light-induced (second type of channels) or membrane potential-induced.

4) There could also be the possibility that ionic current through different parts of the visual cell membrane make the intracellularly measured voltages partially current measurements, and therefore distort the recording of the receptor potential.

It seems probable to us that the complex dependence of the membrane potential on the ionic conductances is responsible for many of the discrepancies between receptor potential and resistance change. Furthermore two types of ion-specific conductance changes fit into the findings.
There are also indications that the potassium permeability increases during the receptor potential since the potassium efflux seems to increase more during the light response than one would expect only by the depolarizing change of the membrane potential (Holt and Brown, 1972; Stieve and Hartung, submitted for publication).

5.3 Ouabain

Smith et al. (1968) report an experiment in which they poisoned a ventral photoreceptor cell of *Limulus* with ouabain and observed that its excitability was lost although the resting potential had decreased only to about 50% of its original value. Our experiments confirm this observation. Since the explanation originally offered by Smith et al., that the change of the electrogenicity of an electrogenic ionic pump would cause the receptor potential, is ruled out (Millecchia, 1969; Mack Brown et al., 1970; Stieve et al., 1971) there exist mainly two different possible explanations:

1) The conductance change of the photoreceptor cell membrane

does not occur when the visual cell is sufficiently poisoned by ouabain or sufficiently depolarized.

2) The gradient of the ion species which determines the receptor potential (mainly Na^+) approaches zero earlier than the gradient which determines the pre-stimulus membrane potential (mainly K^+).

The second explanation at first seemed to us to have a certain probability (Stieve, 1973): The intracellular volume of the photoreceptor cell is very small compared with the extracellular volume (Streaming saline). The height of the pre-stimulus membrane potential is determined mainly by the gradient of potassium ions which is due to a high intracellular and a low extracellular K-concentration. The height of the receptor potential is determined by other cations, mainly Na^+ (see Stieve et al., 1972; Stieve, 1974; Stieve, 1975), which have a high concentration extracellularly and a low one intracellularly. When for instance Na^+ and K^+ are both diffusing along their concentration gradients at about the same rate the Na^+ gradient is much faster decreased than the K^+ gradient, since the low extracellular concentration of K^+ is almost unchanged (great volume streaming solution) in contrast to the intracellular concentration of Na^+ which soon is increased (small volume, stagnant solution). This effect obviously does contribute to the loss of excitability, but only in a secondary way, since our conductance measurements now prove that the receptor potential disappears at the same time as the conductance change does no longer occur (Fig. 7c).

What are the reasons for the loss of ability for light-induced conductance changes. There are again two not necessarily exclusive possible causes:

1) As in the nerve membrane the conductance change of the visual cell membrane can no longer occur when the membrane potential is below a certain value.

2) There is a direct action of ouabain - or of the metabolic energy used for the active transport - on the permeability

changing system of the visual cell membrane. Baumann and Mauro (1973) performed experiments in the photoreceptor of the lateral eye of *Limulus* and the drone photoreceptor where the ability for conductance changes disappears fast when there is no oxygen at the visual cell - by applying a N_2 athmosphere -, whereas the dark potential is still very high and decreases much more slowly. When oxygen is added again the dark potential is regained earlier than the ability for conductance change. These experiments also make a direct need of the cell membrane for metabolic energy probable.

In the ouabain experiments the loss of excitability occurs at much higher membrane potentials than in the case of the potassium depolarization. Either ouabain has a direct action on the permeability system or, since the ouabain depolarization occurs much more slowly than the potassium depolarization, the influence of the decreased membrane potential has sufficient time to develop so that the blockage occurs at high membrane potentials. Since normally smaller depolarizations of up to 50% do not block the excitability of visual cells we think that a more direct influence of ouabain, i.e. not a mediated one via the membrane potential, is more probable. It could be conceivable that the activity, or the intact state, of the sodium/potassium ATPase, is necessary for the membrane to undergo light-induced conductance changes.

In the early phase of the ouabain treatment there is always an increase of the pre-stimulus membrane potential and in most cases of the receptor potential. Probably the early action (or low concentration) of ouabain on the membrane influences the membrane permeability. This effect is not necessarily an action on the ionic pump, but could be a direct action on other components of the membrane, for instance the dark channels. The mechanism of this effect was not investigated further.

5.4 Depolarization by high external potassium concentration

The experiments reported here show that still a small receptor potential (and conductance change) can be measured when the membrane potential is depolarized and even reversed by high external potassium concentration. These observations are in agreement with earlier observations that the retina of the hermit crab and the crayfish (Stieve, 1964; Stieve and Wirth, 1971) still respond in up to 217 mM/l external potassium solutions. In extracellular recordings from the *Astacus* retina there is still a reduced excitability (receptor potentials of 5-15%) measurable (Stieve and Wirth, 1971 and this paper), whereas in intracellular recordings both in *Astacus* and in *Limulus* retinular cells loss of permeability change occurs slowly some 10 min following the total depolarization.

The existence of small but still positive going receptor potentials in visual cells with reversed pre-stimulus membrane potentials indicates that at least in certain areas of the cell membrane in which the light-induced conductance change occurs there is still an inward flux (and therefore gradient) of positive ions, most probably sodium ions, which are not (yet) replaced by the new saline. Also the fact that the extracellular response does not disappear in high external potassium solution is most probably caused by the same effect, a not complete exchange of saline in the small extracellular compartment adjacent to every single visual cell. Some cells will still have a gradient of sodium ions in the normal inward direction. The unpenetrated cells can better resist the strain, and active transport might help too. The intracellularly recorded loss in light-induced conductance change, however, most probably also occurs in the majority of the non-penetrated cells under our experimental conditions.

The experiments indicate that there is a direct influence of the membrane potential on the ability of the cell membrane to undergo light-induced permeability changes, but the action of depolarization develops much slower than in the case of the axon membrane. Probably the membrane potential is necessary to

create and sustain an ordered structure of membrane constituents of dipole character. This order seems to be essential for the ability to produce conductance changes.

The repolarization by passing electrical current through the intracellular electrode did not restore the excitability of the cells. We assume that this is due to the fact that the repolarization was not strong enough; it did not restore the membrane potential to a value close to the resting value, but only by 10 or 20 mV. The fact that restoration of excitability occurs very quickly when the membrane potential is repolarized by superfusion with normal saline shows that the short duration of the current cannot have been decisive.

Mauro (1973) suggested that the loss of the ability for light--induced conductance changes was not due to the depolarization but to the lack of extracellular sodium. It would have been conceivable for instance that the lack of extracellular sodium ions causes a decrease of the intracellular sodium concentration and thus possibly a reduction or a stop of the electrogenic sodium/potassium pump, which in turn causes the disappearance of the light-induced conductance change. This possibility was ruled out by the experiments with high external potassium concentration and 10% of the normal sodium concentration. The presence of 10% sodium did not prevent the loss of excitability after depolarization of the visual cell membrane, although it improved the reversibility and prevented the decrease of the dark resistance due to the high external potassium concentration. We do not yet understand the reason for the different behavior of the dark resistance in the salines with 10% sodium and no sodium at all. Possible causes for this effect might be either activation of the electrogenic pump, as described before, or an influence of the sodium/calcium competition on the membrane resistance.

Also we do not have a plausible explanation why t_{max} is increasing in *Limulus* and decreasing in *Astacus* when the cell membrane is depolarized by high external potassium.

5.5 Final remarks: speculations on the nature of the permeability change

The experiments described here show that the ability of the visual cell membrane to exert light-induced conductance changes depends upon the membrane potential, but the action of the decrease of potential decrease is delayed by several minutes and might possibly be a rather indirect one. The ability for conductance changes could depend on the state of the cell membrane in a way involving the action of the sodium/potassium pump. Blocking this pump by ouabain or indirectly by deprivation of oxygen results, depending in time on the amount of light stimulation, in a loss of the ability for conductance changes. Also depolarization in the presence of oxygen despite the activity of the pumping mechanism, causes a loss of conductance change ability, but stronger depolarizations lasting for a certain time are necessary in this case.

The membrane potential could effect the order or orientation of some membrane constituents with dipole momentum. When this electrical field in the membrane is changed, by lowering or reversal of the membrane potential, the order is no longer sustained in the same way and the molecules can change their orientation. The original order might be necessary for the ability of the membrane to undergo conductance changes.

In the first line those molecules forming the ionic channels in the visual cell membrane could be responsible for the dipole orientation effect. Another possibility would be the rhodopsin molecules. We think it is not very probable that a dis-orientation of the rhodopsin molecules is involved since the early receptor potential, which is believed to be a signal coming from a change in dipole momentum of rhodopsin molecules can be recorded for up to 2 hours without decrement or disruption in totally depolarized visual cells (Dahl, 1976). In artificial lipid bilayer membranes channel forming substandes are known which change their orientation due to the membrane potential. Alamethicin is such a substance a charged part of which is believed to be drawn into the lipid layer of

the membrane by the membrane potential (Böheim, 1972).

The facts that in visual cells poisoned by ouabain the conductance change ceases to occur at much higher membrane potentials than under the influence of high external potassium concentration, and also that oxygen deprivation causes the conductance change to disappear earlier than the membrane potential (Baumann and Mauro, 1973) seem to indicate that also the membrane bound sodium/potassium ATPase (which is blocked specifically by ouabain) seems to be involved in the ability for conductance changes, not only via the membrane potential but via other mechanism. If could be that under normal conditions the pump mechanism feeds also an energy depending process which provides the ability of the cell membrane to undergo conductance changes in response to light stimuli. Under the influence of ouabain or oxygen deprivation the active transport is blocked and therefore the feeding of that mechanism is blocked too. Under the influence of potassium depolarization the pump could be less active or not efficient since the ATPase molecules are not oriented properly. It could also be that a higher energy consumption, which is observed in depolarized nervous cells, leaves less energy supply for the process necessary for the ability for conductivity changes. There are no indications about the possible nature of such a process which could be sustained by the activity or intactness of the sodium/potassium ATPase.

Some earlier observations of ours are in agreement with these experiments and may have the same explanation:

1) In *Limulus* eyes which are in not good condition (and less frequently in *Astacus* preparations), one sometimes finds cells with a fairly high dark potential, but which do not show light-induced conductance changes and receptor potentials. Sometimes the light-induced conductance change is lost shortly after the penetration whereas the pre-stimulus membrane potential stays high. That is to say: Even with normal dark potential the ability for conductance change can be lost.

2) Under certain poor conditions of the preparation of penetrated visual cells the ability to respond can be greatly improved by light stimulation. When the cell is penetrated it responds to the first light flash rather well, but within a few minutes the responses disappear despite a high pre-stimulus membrane potential. When the cell is regularly stimulated, i.e. every 10 s or so, excitability returns and even increases, but when the cell is rarely stimulated it soon becomes inexcitable. Since active sodium/potassium transport is triggered by increased intracellular sodium concentration the improvement of the light-induced conductance change could be due to the activation of the sodium/potassium ATPase feeding some necessary process for the ability for conductance changes. The recovery obtained by frequent stimulation suggests that the activity, and not only the intactness, of the sodium/potassium ATPase may be decisive.

3) The fact that penetrated cells recover much less than unpenetrated cells could also indicate that the conductance changing system is very delicate. At least this could apply for such cases, where the dark potential recovers but not the ability for conductance changes.

Perhaps the light-induced conductance change itself in the visual cell membrane is in some way directly energy dependent.

There are two hypothetical explanations for our findings: There is either the possibility that two separate processes are responsible for the effects described here:

1) The ability of the membrane potential to undergo light-induced conductance changes is membrane potential-dependent.

2) Additionally the conductance changes are in some way more directly depending on the activity of the Na/K-ATPase.

The second explanation would be that in all effects described here (ouabain, potassium depolarization and oxygen-deprivation) the activity of the sodium/potassium ATPase plays the key-role; the depolarization changes the orientation of the ATPase, ouabain inhibits its activity and oxygen deprivation inhibits ts energy supply. The first possibility, two separate dependences, is the less extreme assumption which we therefore prefer.

Table 1

Ionic composition [mM/l] of salines used in the experiments

	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	SO_4^{--}	HCO_3^-	Ouabain
Limulus								
L_1 (physiological saline)	488.3	10.0	10.0	55.0	566.0	30.0	2.3	-
L_2 (ouabain added)	488.3	10.0	10.0	55.0	566.0	30.0	2.3	1.0
L_3 (K^+ added)	-	498.3	10.0	55.0	566.0	30.0	2.3	-
Astacus								
A_1 (physiological saline	207.3	5.0	14.0	3.0	244.0	-	2.3	-
A_2 (K^+ added)	-	212.3	14.0	3.0	244.0	-	2.3	-
A_3 (K^+ added, Na^+ decreased to 10%)	20.7	191.6	14.0	3.0	244.0	-	2.3	-

Table 2a

Average values of corrected pre-stimulus membrane potential, amplitude and time parameters of receptor potential, and dark resistance (R_d) and its change (ΔR) in *Limulus* retinular cells. Intracellular measurement, stimulus intensity ca. 20.000 lx (JA 1-5), and ca. 1.000 lx (JB 46-50), n = 10

1) short stimuli (50 ms for JB 46-50; 33.3 ms for JA 1-5)						
pMP_{corr} [mV]	h_{max} [mV]	t_{lat} [ms]	t_{max} [ms]	t_2 [ms]	R_d [MΩ]	ΔR_m [MΩ]
-40 ± 3.9	35 ± 4.2	40 ± 5.3	203 ± 45	595 ± 176	44 ± 11 (n = 5)	9.5 ± 1.7 (n = 5)

2) long stimuli (1000 ms)							
pMP_{corr} [mV]	h_{max} [mV]	t_{max} [ms]	h_e [mV]	h_a [mV]	h_{max}/h_e	R_d [MΩ]	ΔR_m [MΩ]
-38 ± 3.5	37 ± 4.4	320 ± 80	20 ± 2.3	15 ± 2.0	1.97 ± 0.36	44 ± 11 (n = 5)	7 ± 1.8 (n = 5)

ΔR_e [MΩ]	$\Delta R_m/\Delta R_e$
8 ± 1.2 (n = 5)	1.22 ± 0.083 (n = 5)

Table 2b

Intensity dependence of maximal amplitude h_{max} of intracellularly recorded receptor potentials in *Limulus* retinular cells, and simultaneous recording of membrane resistance changes ΔR_m, $\Delta R_{m/2}$ and ΔR_n. Stimulus intensity ca. 1000 lx, stimulus duration 50 ms. N = 4 (JB 47-50)

	h_{max}	ΔR_m	$\Delta R_{m/2}$	ΔR_n
reference value	46.3 ± 0.8 mV	-9.3 ± 0.7 MΩ	-6.4 ± 0.4 MΩ	-6.7 ± 0.5 MΩ
log I = 0	98.9 ± 0.9 %	101 ± 3.2 %	97 ± 4.3 %	98 ± 5.0 %
log I = -1	82 ± 6.7 %	90 ± 6.2 %	96 ± 7.3 %	47 ± 6.0 %
log I = -2	57 ± 7.8 %	81 ± 9.2 %	87 ± 13 %	6.0 ± 2.1 %
log I = -3	25 ± 3.5 %	56 ± 9.4 %	39 ± 5.7 %	2.0 ± 1.4 %

Table 3

Average values of corrected pre-stimulus membrane potential, amplitude and time parameters of receptor potential, and dark resistance (R_d) in *Astacus* retinular cells. Intracellular measurement. Stimulus intensity ca. 20.000 lx (JA 17-21), and ca. 5500 lx (JB 52-56). n = 10

1) short stimuli (10 ms)						
pMP_{corr} [mV]	h_{max} [mV]	t_{max} [ms]	t_{lat} [ms]	t_2 [ms]	R_d [MΩ]	ΔR_m [MΩ]
-50 ± 4.8	36 ± 4.1	93 ± 6.4	20 ± 2.0	259 ± 59	61 ± 5.4	-1.6 ± 0.05

2) long stimuli (1000 ms)								
pMP_{corr} [mV]	h_{max} [mV]	t_{max} [ms]	h_e [mV]	h_a [mV]	h_{max}/h_e	R_d [MΩ]	ΔR_m [MΩ]	ΔR_e [MΩ]
-50 ± 5.0	40 ± 4.4	166 ± 14	25 ± 2.8	19 ± 2.3	1.62 ± 0.066	65 ± 1.9	1.9 ± 0.4	0.8 ± 0.3

$\Delta R_m/\Delta R_e$
2.8 ± 0.73

Table 4a

Intensity dependence of intracellularly recorded pre-stimulus membrane potential (DaP_{corr}) and receptor potential of *Astacus* retinular cells after short stimuli. Stimulus intensity ca. 20.000 lx, stimulus duration τ 10 ms (JA 11, JA 13-16)

	DaP_{corr} (n = 10)	h_{max} (n = 9)	t_{lat} (n = 9)	t_{max} (n = 9)	t_2 (n = 9)
reference value (60 min in physiological saline)	-69 ± 1.3 mV	38 ± 1.1 mV	10.2 ± 0.5 ms	71 ± 1.4 ms	226 ± 23 ms
log I = -0	-100.2 ± 0.4 %	99 ± 1.1 %	106 ± 15 %	100 ± 2.2 %	95 ± 3.8 %
log I = -0.3	-100.2 ± 0.5 %	94 ± 1.3 %	115 ± 13 %	99 ± 3.0 %	78 ± 5.3 %
log I = -0.6	-95 ± 5.5 %	82 ± 6.8 %	141 ± 8.8 %	98 ± 5.2 %	64 ± 6.9 %
log I = -1.0	-100.3 ± 0.6 %	65 ± 7.5 %	190 ± 21 %	101 ± 6.7 %	53 ± 7.6 %
log I = -2.0	-100.2 ± 0.3 %	23 ± 7.5 %	234 ± 18 %	95 ± 13 %	60 ± 18 %
log I = -3.0	-100.0 ± 0.2 %	3 ± 1.4 % (n = 8)	442 ± 25 % (n = 4)	94 ± 5.8 % (n = 4)	-
log I = -4.0	-99.8 ± 0.4 %	0.2 ± 0.2 %	800 % (n = 1)	88 % (n = 1)	-
log I = -5.0	-98.9 ± 0.8 %	0 % (n = 8)	-	-	-

Table 4b

Intensity dependence of extracellularly recorded receptor potential after long stimuli of *Astacus* retina slice (some preparation in which the intracellular responses in Table 4a were recorded simultaneously). Stimulus intensity ca. 20.000 lx, stimulus duration τ 10 ms. JA 11, JA 13-16, n = 10

	h_{max}	t_{lat}	t_{max}	t_2
reference value	1.62 ± 0.06 mV	12.8 ± 0.21 ms	66 ± 9.3 ms	188 ± 10 ms
log I = -0	99.3 ± 0.2 %	106 ± 7.7 %	101 ± 3.0 %	95 ± 3.7 %
log I = -0.3	94 ± 1.1 %	113 ± 6.3 %	106 ± 3.5 %	76 ± 2.1 %
log I = -0.6	90 ± 1.9 %	124 ± 3.5 %	104 ± 2.0 %	67 ± 2.9 %
log I = -1.0	71 ± 2.9 %	134 ± 3.1 %	107 ± 5.1 %	49 ± 2.3 %
log I = -2.0	25 ± 3.2 %	203 ± 9.6 %	111 ± 4.9 %	38 ± 2.7 %
log I = -3.0	1.3 ± 0.6 %	350 ± 38 % (n = 4)	101 ± 13 % (n = 4)	174 % (n = 1)
log I = -4.0	-	-	-	-
log I = -5.0	-	-	-	-

Table 5a

Intensity dependence of intracellularly recorded pre-stimulus membrane potential (DaP_{corr}) and receptor potential of *Astacus* retinular cells (after short stimuli. Stimulus intensity ca. 20.000 lx, stimulus duration τ 1000 ms. JA 11, JA 13-16, n = 8

	DaP_{corr}	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e
reference value	-63 ± 1.9 mV	32 ± 1.6 mV	127 ± 1.9 ms	18 ± 1.0 mV	9.5 ± 0.3 mV	1.85 ± 0.02
log I = -0	-102 ± 1.0 %	100 ± 1.4 %	93 ± 3.9 %	102 ± 4.2 %	104 ± 6.2 %	99 ± 3.5 %
log I = -0.3	-99.0 ± 0.8 %	96 ± 1.3 %	101 ± 3.4 %	108 ± 5.1 %	93 ± 4.9 %	91 ± 2.6 %
log I = -0.6	-99.4 ± 0.6 %	90 ± 2.4 %	104 ± 4.5 %	106 ± 6.7 %	84 ± 5.1 %	75 ± 9.8 %
log I = -1.0	-100.7 ± 0.7 %	77 ± 3.9 %	117 ± 9.5 %	98 ± 9.8 %	53 ± 6.2 %	87 ± 7.0 %
log I = -2.0	-99.6 ± 0.4 %	33 ± 8.2 %	212 ± 45 %	55 ± 12 %	15 ± 4.0 %	62 ± 2.1 %
log I = -3.0	-99.9 ± 0.4 %	8 ± 3.5 %	n = 7 146 ± 34 %	15 ± 7.3 %	2 ± 1.2 %	n = 7 56 ± 3.7 %
log I = -4.0	-100 ± 1.1 %	1.0 ± 0.6 %	n = 2 224 ± 109 %	2 ± 1.3 %	0.3 ± 0.3 %	n = 2 49 ± 0.0 %
log I = -5.0	-98 ± 2.5 %	-	-	-	-	-

Table 5b

Intensity dependence of extracellularly recorded receptor potential after long stimuli of *Astacus* retina slice (same preparation from which the receptor potentials in the Table 5a, and Table 4a and b, were recorded simultaneously). Stimulus intensity ca. 20.000 lx, stimulus duration τ 1000 ms. JA 11, JA 13-16, n = 8

	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e
reference value	1.69 ± 0.07 mV	96 ± 1.9 ms	0.7 ± 0.10 mV	0.35 ± 0.02 mV	2.61 ± 0.07
log I = -0	99.1 ± 0.7 %	95 ± 2.8 %	96 ± 3.6 %	103 ± 16 %	104 ± 3.8 %
log I = -0.3	96 ± 1.7 %	114 ± 9.6 %	108 ± 4.2 %	102 ± 24 %	88 ± 3.1 %
log I = -0.6	91 ± 3.5 %	110 ± 4.2 %	102 ± 4.8 %	72 ± 13 %	86 ± 3.3 %
log I = -1.0	73 ± 3.9 %	123 ± 5.9 %	101 ± 8.5 %	49 ± 3.9 %	71 ± 5.4 %
log I = -2.0	29 ± 3.8 %	177 ± 18 %	58 ± 8.5 %	24 ± 3.2 %	52 ± 3.8 %
log I = -3.0	6 ± 1.7 %	347 ± 47 % (n = 6)	13 ± 4.2 %	8 ± 2.5 %	44 ± 1.9 % (n = 6)
log I = -4.0	0.4 ± 0.3 %	440 % (n = 1)	1.1 ± 0.7 %	0.5 ± 0.4 %	39 ± 1.1 % (n = 2)
log I = -5.0	-	-	-	-	-

Table 6a

Comparison of extracellularly and intracellularly recorded receptor potentials of *Astacus* photoreceptors after short stimuli (ratio e/i). Stimulus intensity ca. 20.000 lx, stimulus duration τ 10 ms. JA 11, JA 13-16, n = 8

	h_{max_e} / h_{max_i}	t_{lat_e} / t_{lat_i}	t_{max_e} / t_{max_i}	t_{2_e} / t_{2_i}
reference value	0.049 ± 0.008	1.3 ± 0.16 (n = 7)	0.96 ± 0.047	0.9 ± 0.11
log I = 0	1.000 ± 0.0073	1.2 ± 0.25	1.02 ± 0.047	1.02 ± 0.035
log I = -0.3	1.00 ± 0.012	1.01 ± 0.09	1.03 ± 0.028	1.05 ± 0.087
log I = -0.6	1.2 ± 0.17	0.90 ± 0.047	1.08 ± 0.063	1.2 ± 0.15
log I = -1.0	1.2 ± 0.19	0.77 ± 0.073	1.05 ± 0.070	1.2 ± 0.21
log I = -2.0	1.1 ± 0.1 (n = 7)	1.07 ± 0.087	1.07 ± 0.082	0.9 ± 0.26
log I = -3.0	0.6 ± 0.16 (n = 3)	0.83 ± 0.087 (n = 3)	1.1 ± 0.12 (n = 3)	-
log I = -4.0	-	-	-	-
log I = -5.0	-	-	-	-

Table 6b

Comparison of extracellularly and intracellularly recorded receptor potentials of *Astacus* photoreceptors after long stimuli (ratio e/i). Stimulus intensity ca. 20.000 lx, stimulus duration 1000 ms. JA 11, JA 13-16, n = 8

	h_{max_e} / h_{max_i}	t_{max_e} / t_{max_i}	h_{e_i} / h_{e_i}	h_{a_e} / h_{a_i}	h_{max}/h_{e_e} / h_{max}/h_{e_i}
reference value	0.06 ± 0.010	0.74 ± 0.035	0.049 ± 0.009	0.04 ± 0.010	1.5 ± 0.17
log I = 0	0.99 ± 0.013	1.03 ± 0.06	0.94 ± 0.028	0.9 ± 0.13	1.05 ± 0.057
log I = -0.3	0.99 ± 0.014	1.13 ± 0.095	1.01 ± 0.053	1.1 ± 0.19	0.98 ± 0.042
log I = -0.6	1.00 ± 0.033	1.08 ± 0.056	0.98 ± 0.053	0.9 ± 0.18	1.00 ± 0.035
log I = -1.0	0.95 ± 0.036	1.10 ± 0.07	1.04 ± 0.046	1.0 ± 0.13	0.84 ± 0.061
log I = -2.0	0.94 ± 0.063	1.0 ± 0.17	1.2 ± 0.10	1.5 ± 0.29 (n = 7)	0.82 ± 0.049
log I = -3.0	1.4 ± 0.27 (n = 6)	1.2 ± 0.18 (n = 6)	1.7 ± 0.45 (n = 6)	2.2 ± 0.30	0.82 ± 0.065 (n = 6)
log I = -4.0	-	-	-	-	-
log I = -5.0	-	-	-	-	-

Table 7

Dependence of intracellularly recorded pre-stimulus membrane potential (pMP_{corr}), receptor potential (both JA 1-5) and membrane resistance (JB 39, JB 45) of *Limulus* retinular cells on 1 mM/l ouabain added to the physiological saline. Stimulus intensity ca. 20.000 lx (JA 1-5) and ca. 5500 lx (JB 39, JB 45)

1) τ = 33.3 ms

	pMP_{corr} (n = 7)	h_{max} (n = 7)	t_{lat} (n = 7)	t_{max} (n = 7)	t_2 (n = 7)	R_d (n = 2)	ΔR_m (n = 2)
reference value in physiological saline	-32 ± 4.2 mV	25 ± 4.9 mV	43 ± 8.7 mV	239 ± 91 ms	209 ± 23 ms	36 ± 12 MΩ	7.7 ± 0.64 MΩ
values in test saline at h_{max} 50 %	(-)84 ± 9.6 %	50 %	174 ± 33 %	182 ± 49 %	48 ± 4.4 %	63 ± 24 %	48 ± 21 %
h_{max} 30 %	(-)64 ± 10 %	30 %	210 ± 31 %	188 ± 40 %	49 ± 3.3 %	99 ± 6.1 %	24 ± 9.2 %
h_{max} 0 %	(-)39 ± 10 %	-	-	-	-	94 ± 3.3 %	-
55 min in test saline	(-)62 ± 16 %	43 ± 20 %	194 ± 59 %	128 ± 26 %	50 %	-	-
last value in test saline	-38 ± 8.1 %	(-)1 ± 1.8 % (n = 5)	-	-	-	-	-
55 min in physiological saline	33 ± 13 %	-	-	-	-	134 ± 115 %	-

2) τ = 1000 ms

	pMP_{corr} (n = 7)	h_{max} (n = 7)	t_{max} (n = 7)	h_e (n = 7)	h_a (n = 7)	h_{max}/h_e (n = 7)	R_d (n = 2)	ΔR_m (n = 2)	ΔR_e (n = 2)
reference value in physiological saline	-29 ± 3.2 mV	27 ± 5.6 mV	451 ± 142 ms	15 ± 1.8 mV	12 ± 2.2 mV	2.2 ± 0.76	37 ± 14 MΩ	7 ± 1.8 MΩ	5.5 ± 0.78 MΩ
60 min in test saline	57 ± 20 %	21 ± 12 %	202 ± 129 %	23 ± 11 %	24 ± 11 %	71 ± 18 %	-	-	-
last value in test saline	38 ± 11 %	5 ± 17 %	221 ± 184 %	15 ± 12 %	-	-	-	-	-
60 min in physiological saline	27 ± 2.2 %	-	-	-	-	-	-	-	-

The half-time of the depolarisation is $t_{1/2}$ = 34 ± 4.3 min for h_{max} and $t_{1/2}$ = 80 ± 34 min for the pMP. The signs in brackets indicate the polarity of the pMP.

Table 8

Dependence of intracellularly recorded pre-stimulus membrane potential (pMP_{corr}), receptor potential and membrane resistance of *Limulus* retinular cells on 498.3 mM/l potassium added to the physiological saline. Stimulus intensity ca. 1000 lx. JB 46-50, n = 5

1) τ = 50 ms

	pMP_{corr}	h_{max}	t_{lat}	t_{max}	t_2	R_d	ΔR_m
reference value in physiological saline	47 ± 4.3 mV	45 ± 2.0 mV	38 ± 7.4 ms	165 ± 8.4 ms	749 ± 209 ms	44 ± 11 MΩ	-10 ± 1.7 MΩ
values in test saline at h_{max} 50 %	(-) 13 ± 21 %	50 %	215 ± 33 %	131 ± 19 %	116 ± 19 %	77 ± 2.5 %	36 ± 5.5 %
h_{max} 30 %	(+) 5 ± 23 %	30 %	279 ± 71 %	147 ± 23 %	98 ± 18 %	68 ± 2.7 %	20 ± 3.3 %
h_{max} 0 %	(+) 25 ± 19 %	-	-	-	-	60 ± 2.2 %	-
55 min in test saline	(+) 38 ± 21 %	2 ± 1.4 %	570 ± 431 %	221 ± 133 %	131 ± 119 % (n = 2)	55 ± 3.4 %	-
last value in test saline	(+) 14 ± 16 %	-	-	-	-	-	-
55 min in physiological saline	(-) 55 ± 28 %	23 ± 14 %	180 ± 100 %	135 ± 51 %	63 % (n = 1)	67 ± 4.6 %	17 ± 11 %

2) τ = 1000 ms

	pMP_{corr}	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e	R_d	ΔR_m	ΔR_e
reference value in physiological saline	-46.0 ± 0.30 mV	47 ± 2.0 mV	188 ± 11 ms	26 ± 1.6 mV	18 ± 2.8 mV	1.79 ± 0.11	44 ± 11 MΩ	10 ± 1.6 MΩ	8.4 ± 1.4 MΩ
60 min in test saline	(+) 29 ± 18 %	4 ± 1.8 %	224 ± 91 %	4 ± 1.6 %	4 ± 2.4 %	112 ± 28 %	-	-	-
last value in test saline	(+) 14 ± 21 %	5 ± 2.0 %	301 ± 108%	5 ± 2.5 %	5 ± 4.0 %	-	-	-	-
60 min in physiological saline	(-) 10 ± 42 %	29 ± 17 %	347 ± 152%	42 ± 24 %	53 ± 30 %	67 ± 3.9%	-	-	-

The half-time of the depolarization is $t_{1/2}$ = 15 ± 1.0 min for h_{max} and $t_{1/2}$ = 13 ± 1.3 min for the pMP. The signs in brackets indicate the polarity of the pMP.

Table 9

Dependence of intracellularly recorded pre-stimulus membrane potential (pMP_{corr}), receptor potential and membrane resistance of *Astacus* retinular cells on 212.3 mM/l potassium added to the physiological saline. Stimulus intensity ca. 5500 lx. JB 52-56, n = 5

1) τ = 10 ms

	pMP_{corr}	h_{max}	t_{lat}	t_{max}	t_2	R_d	ΔR_m
reference value in physiological saline	-51 ± 6.4 mV	29 ± 5.1 mV	23 ± 2.3 ms	87 ± 7.9 ms	201 ± 27 ms	56 ± 17 MΩ	-1.6 ± 0.6 MΩ
values in test saline at							
h_{max} 50 %	(-) 59 ± 4.7 %	50 %	125 ± 4.0 %	87 ± 5.1 %	64 ± 3.6 %	86 ± 12 %	-67 ± 11 %
h_{max} 30 %	(-) 18 ± 7.6 %	30 %	144 ± 8.1 %	93 ± 17 %	50 ± 4.3 %	78 ± 11 %	-22 ± 7.0 %
h_{max} 0 %	(+) 27 ± 19 %	-	-	-	-	79 ± 11 %	-
55 min in test saline	(+) 27 ± 13 %	3 ± 3.0 %	-	-	-	-	-
last value in test saline	(+) 23 ± 11 %	-	-	-	-	-	-
55 min in physiological saline	(+) 11 ± 25 %	11 ± 2.0 %	151 ± 23 %	85 ± 14 %	71 ± 9.3 %	78 ± 5.4 %	-

2) τ = 1000 ms

	pMP_{corr}	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e	R_d	ΔR_m	ΔR_e
reference value in physiological saline	-50 ± 7.6 mV	32 ± 5.3 mV	172 ± 21 ms	21 ± 3.6 mV	14 ± 1.9 mV	1.5 ± 0.1	63 ± 21 MΩ	1.6 ± 0.6 MΩ	0.9 ± 0.4 MΩ
60 min in test saline	(+) 28 ± 16 %	5 ± 4.6 %	46 %	6 ± 5.7 %	-	81 %	-	-	-
last value in test saline	(+) 22 ± 2.2 %	0.2 ± 0.2 %	50 %	1.6 ± 1.6 %	-	-	-	-	-
60 min in physiological saline	(-) 6 ± 11 %	16 ± 2.9 %	63 ± 7.6 %	9 ± 5.7 %	3 ± 2.0 %	298 ± 102 %	-	-	-

The half-time of the depolarization is $t_{1/2}$ = 3.3 ± 0.49 min for h_{max} and t_1 = 8 ± 1.5 min for the pMP. The signs in brackets indicate the polarity of the pMP.

Table 10a

Dependence of intracellularly recorded pre-stimulus membrane potential (pMP_{corr}), receptor potential and membrane resistance of *Astacus* retinular cells on 191.6 mM/l potassium and 10% of the normal sodium content in the physiological saline. Stimulus intensity ca. 20.000 lx. JA 17-21, n = 5

1) τ = 10 ms

	pMP_{corr}	h_{max}	t_{lat}	t_{max}	t_2	R_d	ΔR_m
reference value in physiological saline	-49 ± 7.8 mV	42 ± 5.4 mV	16 ± 2.3 ms	98 ± 10 ms	316 ± 109 ms	66 ± 19 MΩ	-1.5 ± 0.6 MΩ
values in test saline at							
h_{max} 50 %	(-) 34 ± 11 %	50 %	112 ± 2.8 %	89 ± 9.3 %	60 ± 6.6 %	104 ± 21 %	-49 ± 8.0 %
h_{max} 30 %	(+) 6 ± 14 %	30 %	115 ± 6.4 %	64 ± 4.2 %	35 ± 9.9 %	112 ± 29 %	-19 ± 11 %
h_{max} 0 %	(+) 9 ± 14 %	-	-	-	-	107 ± 26 %	-
55 min in test saline	(+) 18 ± 16 %	-	-	-	-	-	-
last value in test saline	(+) 19 ± 17 %	-	-	-	-	-	-
55 min in physiological saline	(-) 20 ± 30 %	27 ± 7.1 %	113 ± 7.3 %	96 ± 11 %	69 ± 13 %	99 ± 14 %	-13 ± 13 %

2) τ = 1000 ms

	pMP_{corr}	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e	R_d	ΔR_m	ΔR_e
reference value in physiological saline	-50 ± 7.4 mV	47 ± 5.3 mV	160 ± 20 ms	29 ± 4.1 mV	24 ± 3.2 mV	1.7 ± 0.06	67 ± 19 MΩ	-2.2 ± 0.5 MΩ	+0.3 ± 0.5 MΩ
60 min in test saline	(+) 34 ± 20 %	2 ± 1.5 %	76 ± 13 %	1.0 ± 0.7 %	0.3 ± 0.3 %	314 ± 180 %	-	-	-
last value in test saline	(+) 34 ± 20 %	3 ± 1.7 %	67 ± 22 % (n = 2)	0.5 ± 0.24 %	-	-	-	-	-
60 min in physiological saline	(-) 15 ± 24 %	30 ± 9.2 %	79 ± 19 %	20 ± 8.2 %	15 ± 6.0 %	199 ± 44 %	-	-	-

The half-time of the depolarization is $t_{1/2}$ = 5.3 ± 0.58 min for h_{max} and $t_{1/2}$ = 9 ± 3.0 min for the pMP. The signs in brackets indicate the polarity of the pMP.

Table 10b

Dependence of extracellularly recorded receptor potentials of an *Astacus* retina slice (same preparation as in Table 10a) on 191.6 mM/l potassium and 10% of the normal sodium content in the physiological saline. Stimulus intensity ca. 20.000 lx. JA 17-21, n = 5

1) τ = 10 ms

	h_{max}	t_{lat}	t_{max}	t_2
reference value in physiological saline	1.4 ± 0.25 mV	18 ± 1.4 ms	111 ± 21 ms	253 ± 18 ms
values in test saline at				
h_{max} 50 %	50 %	128 ± 6.8 %	84 ± 9.2 %	87 ± 6.2 %
h_{max} 30 %	30 %	130 ± 7.8 %	78 ± 9.9 %	80 ± 4.8 %
55 min in test saline	14 ± 1.3 %	139 ± 9.7 %	79 ± 12 %	68 ± 7.1 %
55 min in physiological saline	83 ± 5.1 %	112 ± 7.2 %	95 ± 11 %	80 ± 2.4 %

2) τ = 1000 ms

	h_{max}	t_{max}	h_e	h_a	h_{max}/h_e
reference value in physiological saline	1.6 ± 0.28 mV	160 ± 60 ms	1.0 ± 0.18 mV	0.7 ± 0.15 mV	1.58 ± 0.04
60 min in test saline	17 ± 1.1 %	99 ± 25 %	12 ± 1.9 %	0.6 ± 0.6 %	160 ± 26 %
60 min in physiological saline	82 ± 3.7 %	86 ± 17 %	68 ± 3.6 %	69 ± 7.9 %	125 ± 7.7 %

The half-time of the depolarization is $t_{1/2}$ = 8 ± 3.2 min for h_{max}.

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