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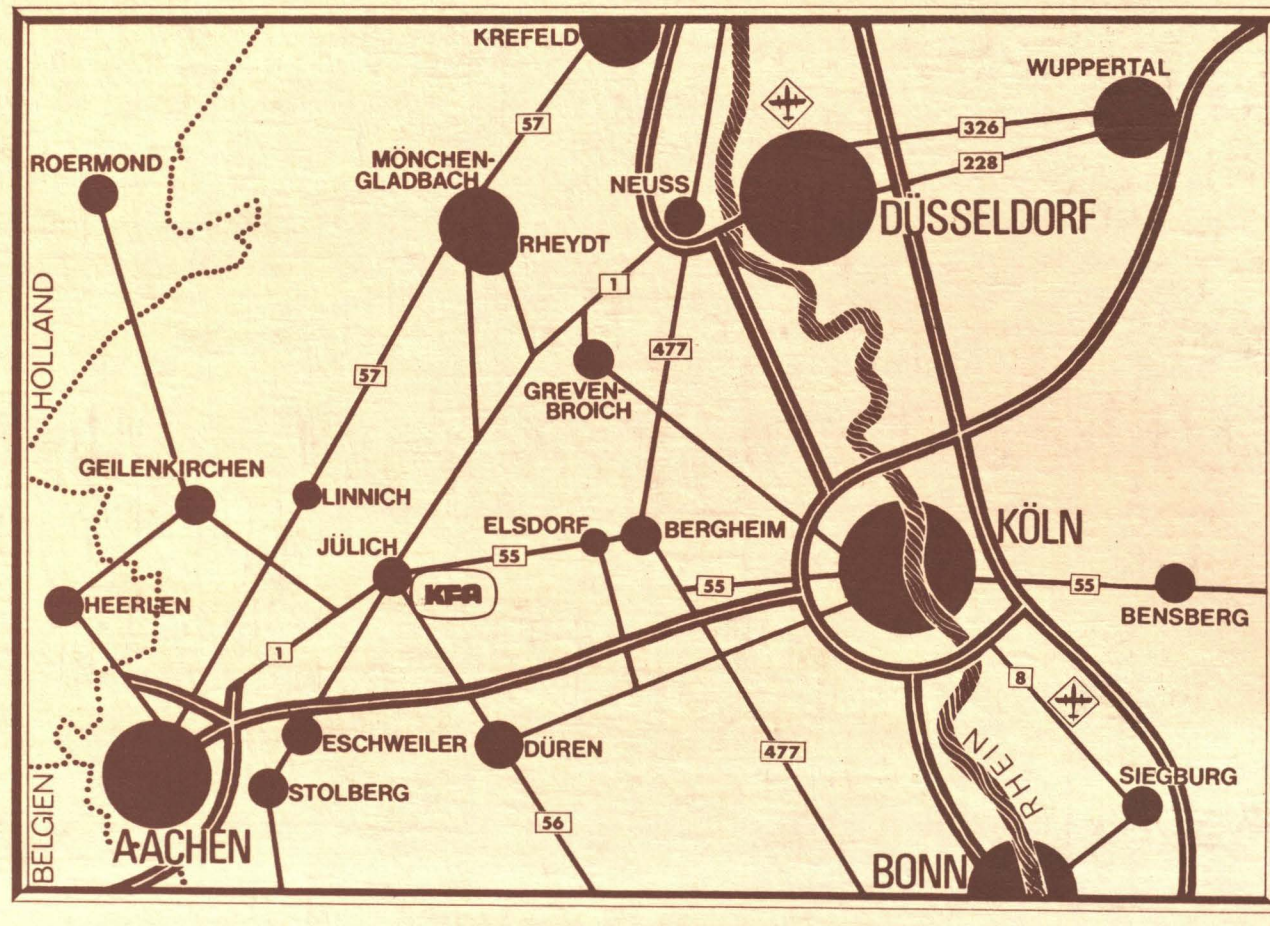

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C o n t e n t s

1. Abstract

The electrical response of the photoreceptor of the excised lateral eye of the horseshoe crab *LIMULUS polyphemus* following a stimulus of white light was intracellularly measured with one microelectrode at room temperature. The pre-stimulus membrane potential (pMP) and the receptor potential (ReP) evoked by test flashes of constant duration (ca 2 000 msec) were recorded in experiments testing (1) the dependence of the flash intensity, (2) the relative change of the resistance of the visual cell membrane on stimulation by flashes of different intensities, (3) the long-term adaptation to light and darkness and (4) the dependence of the stimulus frequency.

1.1 Results

1.1.1 The maximal amplitude (h_{max}) of the ReP (Fig. 2) shows a linear dependence of the stimulus intensity in a small range of low intensities and a log-linear dependence over approximately two logarithmical (base ten) intensity units before it saturates at the highest intensity used (ca 3 000 lx). The plateau-value (h_e) apparently saturates at higher light intensities than h_{max}, and than were used in our experiments. At low light intensities and in the dark discrete slow depolarizing potential fluctuations (bumps) of up to 10 mV were frequently recorded.

1.1.2 The relative changes of the membrane resistance in the course of the RePs evoked by flashes of different light intensities (Table 1) were correlated with, but not proportional to, the light-dependent changes of the amplitudes of the ReP. (Lower light intensity causes smaller amplitudes as well as smaller membrane resistance changes.)

1.1.3 The long-term adaptation to light was measured by applying a sustained background light for 10 min (intensity adjusted to evoke a ReP of at least 80 per cent of the magnitude of the RePs caused by the constant test flashes). 30 seconds

after turning on the light the pre-stimulus membrane potential pMP (Fig. 13) was already depolarized to about half its value in the dark and remained on this level during the light adaptation. Also the peak amplitude h_{max} (Fig. 14) remained on a decreased constant value. The only major change of the RePs evoked by the superimposed test flashes during the sustained background light was the continuous increase of the plateau--value h_e. An interesting effect of the strong background light was the transient hyperpolarization of the after-potential (h_a) after the end of each superimposed test flash. The course of the response was slightly accelerated in the period of light adaptation (shorter values of the peak-amplitude time t_{max}, Fig. 16).

1.1.4 The course of the long-term dark adaptation, measured immediately after the 10 min light adaptation during a dark period of 64 min, showed that the effects of the strong sustained background light were fully reversible. Already 30 seconds after turning off the light the major part of the sensitivity increase had taken place as at this time the value of the pMP was greater than the stationary dark value, and the maximal amplitude h_{max} had returned to about 80 per cent of its reference value.

1.1.5 The influence of the stimulus frequency was tested by reducing the flash interval from 8 to 2 min and comparing the values of the pre-stimulus membrane potential and the amplitudes of the RePs recorded during the subsequent periods with different flash intervals (Fig. 20). The gradual increase in light adaptation produced by this stimulus sequence resulted in some experiments in a hyperpolarization (from ca -55 to ca -65 mV) of the pMP and, at the same time, in an increase of the maximal amplitude h_{max} and the plateau-value h_e by about the same quantity.

1.2 Interpretation

1.2.1 The results are in agreement with a general concept explaining the ReP by assuming different ion-specific permeabilities of the visual cell membrane in the dark and during excitation, together with active transport mechanisms keeping the cell excitable by restoring the specific ionic gradients across the membrane (Fig. 21).

1.2.2 A plausible explanation for these different permeabilities lies in the assumption of channels leading through the membrane. In the simplest case two types of channels would be sufficient to explain the data: permanently open "dark channels" preferably permeable for potassium ions, and "light channels" which are opened by light quanta during excitation and let preferably sodium ions pass. This model could be extended to different types of light channels interacting to produce the typical response.

1.2.3 As indicated by the measurement of the overall membrane resistance change the membrane potential in the dark and the ReP are at any time determined by a weighted balance between all specific ion permeabilities. Furthermore exhaustion of ion reservoirs during excitation and active - possibly electrogenic - transport may contribute to the ReP.

1.2.4 Active transport mechanisms, which can be induced by sodium influx into the visual cell, are probably responsible for the second phase of the bi-phasic potential decrease after the end of the stimulus in contrast to the respective monophasic membrane resistance change (Fig. 9).

1.2.5 The hyperpolarization of the membrane potential produced in some cases by raised stimulus frequency is probably caused by electrogenic pump activity, which has a hyperpolarizing effect in *Limulus*. Apparently the occurrence and degree of electrogenic pump mechanisms varies from preparation to preparation.

1.2.6. The adaptation effects recorded 30 seconds after turning on, and off, the background light cannot depend directly on the quantity of bleached rhodopsin as this "pigment adaptation" in *Limulus* is much shorter (ca 500 msec), but are due to "membrane adaptation", i.e. to processes influencing the electro-chemical properties of the visual cell membrane.

1.2.7 Several findings indicate that active transport mechanisms contribute to the observed adaptation phenomena. The fact that the sensitivity does not change during the measured period of light adaptation and is practically fully restored already 30 seconds after turning off the light suggests that active transport mechanisms induced by the background illumination are sufficient to maintain the ionic concentration gradients almost unchanged. At the same time (30 seconds after light off) the pre-stimulus membrane potential is even slightly hyperpolarized, probably due to the activity of a possibly electrogenic pump.
Active transport may also be responsible for the steady increase of the plateau-value during the period of background illumination.

1.2.8 Judging from the plots the parallelity of the changes of membrane potential, plateau-value and after-potential during dark adaptation are influenced by the same processes, probably also active transport mechanisms.

1.2.9 The cause of the hyperpolarizing after-potential during the light adaptation might be a transient adaptative sensitivity increase after the end of each test flash produced as summation effect by the superimposition of flash and background light.

1.2.10 The observed long-term adaptation effects can be interpreted by assuming that the amplification factor of the mechanisms causing the ReP is somehow changed by the effect of the background light.
One possible explanation for the change of the amplification factor could be that the mechanisms responsible for the ReP become effective in the way of a snowball process the extent of which is governed by the state of adaptation. Another

possibility would be that the ReP is determined by co-operative effects, i.e. that perhaps the number of rhodopsin molecules forming a functional unit is altered during adaptation.

2. Introduction

The electrical response of the visual cell of the lateral eye of the horse-shoe crab *LIMULUS polyphemus* following a light stimulus has been investigated in several studies (Hartline et al. 1952, Borsellino et al. 1965, Stieve 1965, Millecchia 1969).

The experiments described in the present paper were carried out with the aim of obtaining comprehensive data of the reaction of the visual cell of the *Limulus* eye to light, as a basis for comparison with earlier single experiments and with experiments in other arthropod species.
For this purpose the membrane potential in the dark and the receptor potential (ReP) of the visual cell were intracellularly recorded by one micro-electrode under varying conditions of stimulation by light.
Besides the dependence of the ReP of intensity and frequency of the light flashes the long-term adaptative effect of a background illumination was tested. In a separate series of experiments the relative change of the resistance of the visual cell membrane during stimulation by light of different intensities was measured.
The results were obtained from a quantitatively representative number of experiments and submitted to statistical procedure.

The RePs recorded during the different series of experiments were evaluated by means of a computer program which was especially developed to measure a number of characteristic parameters describing size and time course of each ReP.

The experiments were carried our partly in the California Institute of Technology in Pasadena, USA and partly in the KFA, Jülich.
The results of a few single experiments out of the series made in Pasadena have already been published (Stieve 1965).

3. Material and Methods

3.1 Preparation

The experiments were carried out with adult horse-shoe crabs of the species *Limulus polyphemus*. The lateral eyes were excised at room temperature and divided into halves in the direction of the longest diameter of the eye. The preparations were kept at 4°C in *Limulus* blood, which had been filtered to remove the coagulated protein, until the experiments began. During the experiments the eye was placed in a lucite test chamber, with the cut surface facing upward. The chamber was filled with a stagnant solution of filtered *Limulus* blood.

3.2 Stimulating Light

White light emitted by two tungsten band lamps (General Electric, Ill. type IBA/T1O/3P-6V), placed in front of the preparation, was used. The two light beams, which had approximately equal intensity, were directed toward the preparation by means of a mirror system. The beams were reflected from above and behind the preparation in such a way that they reached the eye from an unphysiological direction in an angle of light incidence of about 60° to the optical axes of the ommatidia situated at the cut surface of the eye. The diameter of the light spots on the eye was about 2 mm. The stimulus efficiency of the beams could be adjusted within certain limits by varying the position of the light spots.
The maximal light intensity at the eye was about 3 OOO lx (I = 1). It could be attenuated in log (base ten) units by neutral density filters (Bausch & Lomb). A cuvette filled with O.1 n $CuSO_4$ solution was used for heat absorption. The time of exposure was varied by a light chopper using a rotary solenoid.

3.3 Measuring Apparatus

Glass microelectrodes (resistance between 2O and 6O MΩ) filled with 3 m KCl were used for the intracellular measurement. The electrodes were directed to the surface of the preparation by mechanical micro-manipulators (Brinkmann). For the final insertion into the visual cell hydraulic micro-manipulators (Wells) were used. For measurement of the membrane resistance and to allow injection of currents through the microelectrode a bridge circuit as described by Frank and Fuortes (1956) was used. A grass stimulator yielded the required currents.

3.4 Recording Apparatus

An Electrometer Amplifier (Medistor Model 35) was used for amplification. A dual beam oscilloscope (Tektronix 5O2 A) recorded dark potential and receptor potential (ReP) on one beam and the zero reference potential and the light stimulus (registered by a photocell) on the other beam. The measurements were parallely recorded by a paper recorder (Varian 622 A). All data were stored by a tape recorder (Data Tape PR 3300 by CEC). The range of the frequency-modulated channels of the tape recorder was O - 1.3 kHz.

3.5 Procedure

All experiments were carried out at room temperature (ca 22°C). The average duration of one experiment was about three hours. Experiments lasting less than one hour were not evaluated.
The test measurements consisted of the intracellular recording of the receptor potential (ReP) across the visual cell membrane by a micro-electrode against an indifferent electrode in the physiological solution.

Immediately before the beginning of the stimulus this measurement yields the pre-stimulus membrane potential, pMP (which is not necessarily identical with the dark potential, e.g. in adaptation experiments where a background illumination is applied).
Before insertion of the microelectrode into the visual cell and after the last measurement of each experiment the zero reference potential was recorded between microelectrode and indifferent electrode in the surrounding medium.
The measuring accuracy of the amplitude measurements was ca 1 mV (1 per cent of the total measuring range); the time measurements were accurate within ca 1 msec.
All experiments began with a pre-period of at least 30 min in the dark after insertion of the microelectrode. During the last 10 min of this pre-period test flashes of maximal light intensity (ca 3 000 lx, $I = 1$, $\log I = 0$) were applied (flash duration τ = ca 2 000 msec, stimulus interval $\Delta\tau$ = ca 2 min).

The test program consisted of:

(1) the measurement of the dependence of the ReP of light flashes of different intensity (intensity dependence),

(2) the measurement of the adaptation to a period of light (measured by applying a long-term background illumination with superimposed test flashes)and a subsequent period of darkness (light and dark adaptation),

(3) the measurement of the dependence of the ReP of increased frequency of the light flashes, which is also a form of moderate adaptation (frequency dependence),

(4) and the measurement of the changes of the resistance of the visual cell membrane during light flashes of different intensities (membrane resistance change).

Generally the intensity dependence, the adaptation to light and darkness and the dependence to the stimulus frequency were tested in the same experiment, provided that the preparation remained in a satisfactory condition. The membrane resistance was measured in experiments with special testing conditions providing injection of currents through the intracellular measuring electrode besides stimulation by light of different intensities. A detailed description of the testing procedure will be given together with the respective results.

3.6 Evaluation

The experimental data were processed using the computer system of the computing center of the California Institute of Technology.
The basic principles of this procedure, under omission of a detailed description, are these:

The analogue tape recordings of all RePs measured in the course of an experiment were transferred into the computer by an analogue - to - digital converter. Each recording was represented by a batch of 3 000 msec duration (an adequate time for the measurement of a ReP evoked by a light flash of 2 000 msec, the stimulus duration used in the experiments).
For every msec of a batch an ordinate (amplitude) value (referred to as numbered data point or sample) was recorded.
Two methods were applied to find the desired parameters of the RePs:

(1) Level values were found by calculating the average of the points of a given period (generally 100 points = 100 msec)

(2) Maxima and points of steepest slope were found from a 3rd order least squares polynomial approximation to the data of a given period where the respective parameter was expected. This second method was used to eliminate the error introduced by noise. The whole region to be examined was extended within relatively wide limits (e.g. 400 points in the case of the maximal amplitude of the ReP) to make sure that the desired parameter occurred in this region. Then a 3rd order least squares polynomial approximation to a selected range of 61 points was made, starting 31 points before the first point of the region to be searched. The selected range was then shifted by one point and the next curve fit was made. In this way the procedure was repeated point by point until the whole region was examined. The absolute maximum of all curve fits was selected and printed as the desired value.

A selected number of RePs were finally plotted by the computer. As all potentials stored on the tape were also photographed for conventional evaluation a comparison with the computer plots was possible. Part of the data were furthermore submitted to statistical evaluation.
The RePs recorded during the experiments were evaluated concerning their size and shape by measuring the following parameters, which were calculated and printed after adjustment and calibration (see insets of Figs. 2 to 4):

pMP (mV) — the pre-stimulus membrane potential or base line (often identical with the membrane potential in the dark) recorded as difference to the zero reference level measured before insertion of the electrode into the visual cell. The value of the pMP was found by averaging the 100 points immediately before the beginning of the stimulus, starting at point 5 of the batch.

h_{max} (mV) — the peak amplitude value of the transient of the ReP. (Maximum of curve fits to 400 points after the beginning of the stimulus at ca point 105 of the batch).

h_e (mV) — the plateau value - the amplitude measured shortly before stimulus end. (Average value of 100 points before end of the stimulus)

h_{max}/h_e — the shape-quotient

h_a (mV) — the after potential. For this parameter the point of the steepest downward slope of the ReP after the end of the stimulus was determined by applying the curve fit procedure described before to the 400 points immediately after the end of the stimulus. The decrease-time between stimulus end and this point of steepest downward slope was measured (t_{end}). The after-potential h_a was recorded at the double value of t_{end}, counted from the end of the stimulus (at this time the fast decrease of the ReP was assumed to be terminated).

t_{lat} (msec) the latent-period - the time from the beginning of the stimulus until intersection of the tangent line in the steepest point (at the time t_{steep}) of the rising phase of the ReP with the extrapolated level of the pMP.

t_{max} (msec) the peak-amplitude-time - the time from stimulus begin until the maximum of the transient is reached. (The time parameters t_{lat} and t_{max} were found by the same curve fits used to determine h_{max}).

All amplitude values of the ReP (h_{max}, h_e, h_a) are related to the level of the respective pre-stimulus membrane potential pMP. In the description of the results the term "increase" is used for these parameters when their difference to the pMP becomes greater in the direction of the zero reference potential, i.e. toward less negative absolute voltage values.
In the case of the pMP which marks the difference to the zero reference potential "increase" denotes a change toward more negative voltage (e.g. from -50 to -60 mV).

4. Results

4.1 The effect of flashes of various light intensities on the receptor potential

4.1.1 Test program and evaluation

After the pre-period the preparation was subjected to a series of flashes of different light intensities.
Starting from a stimulus of the highest intensity available (ca 3 000 lx, I = 1, log I = 0) the intensity of the subsequent stimuli was decreased in logarithmical units and increased again after the stimulus of the lowest intensity value used (log I = -5). Before each test measurement (with different stimulus intensity) a stimulus of maximal light intensity was applied to cause a ReP which served as reference potential for the following measurement. In other words, the sequence of light intensities in one series was I = log (0, 0) (0, -1) (0, -2) (0, -3) (0, -4) (0, -5) (0, -5) (0, -4) (0, -3) (0, -2) (0, -1) (0, 0), the brackets indicating pairs of reference and test flashes.
The interval $\Delta\tau$ between the flashes was 2 min, the stimulus duration τ was ca 2 000 msec.
Owing to this test program the eye was in a state of moderate light adaptation which was kept almost equal throughout the experiment by the regular reference measurements with stimuli of maximal light intensity.

A typical sequence of RePs recorded after stimuli with intensities decreasing from log I = 0 to log I = -5 is shown in Fig. 1 (HSL 033).

The intensity dependence of the ReP was tested in 10 experiments. Five of them were selected for the plotting as in these cases the curve of the

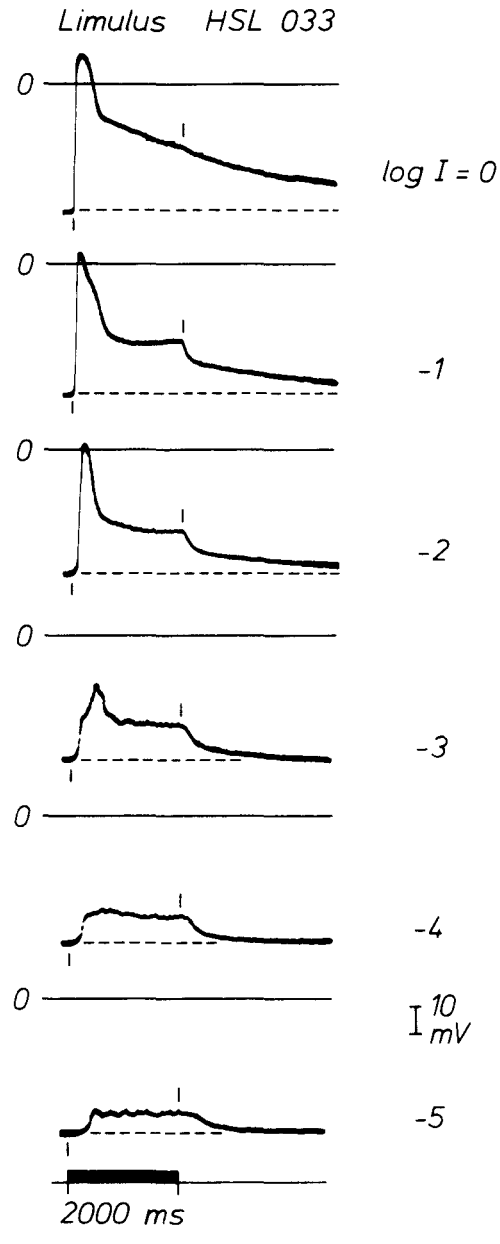

Fig. 1

Receptor potentials recorded at room temperature at different intensities of stimulating light. Thin line: zero reference level.
Maximal stimulus intensity ca 3 000 lx (log I = 0), stimulus duration ca 2 000 msec.
(HSL 033, *Limulus* retinular cell)

transient of the ReP (h_{max}) versus the intensity of the stimulating flash was S-shaped (saturation at the highest intensity) and had a logarithmically proportional rising phase of about the same length (2 intensity units). In the remaining experiments the respective rising phase of the visual cell tested was longer or there was no tendency toward saturation at all. This was probably partly due to unfavourable adjusting of the light spot on the ommatidium and therefore reduced light flash efficiency in these experiments.

The averaged curves of the parameters recorded in the five experiments (HSL 019, 024, 033, 042, 043) are shown in Figs. 2, 3 and 4. These curves were obtained in the following way:
At each light intensity two RePs are recorded, one in the series of decreasing intensity and one in the subsequent series of increasing intensity. The values of each parameter of the two RePs recorded at one intensity were referred to their respective reference value (obtained from the preceding ReP evoked by a flash of maximal intensity) and averaged.

The resulting five single curves for each parameter, however, were not simply averaged for this reason: As the logarithmically proportional phases of the transient, h_{max}, of the RePs did not occur at the same incident light intensities but were shifted with respect to each other, the averaged curve of h_{max} versus the stimulus intensity would have had a much longer rising phase than the curves of each single experiment.

To avoid this error introduction the curves of the transient, h_{max}, were shifted along the abscissa (for convenience by whole intensity units) until superposition of the rising phases was reached. The curves of the other parameters were shifted accordingly by the same amount. By this procedure the effective light intensities could be approximately equallised (i.e. the not directly measurable quantities of light actually reaching the rhabdomere, which had apparently been different in the single experiments although the same incident light intensities had been used). The values of the parameters of the five superimposed curves were averaged and normalised with respect to the mean value calculated for the highest intensity (log I = 0).

As a result of the shifting along the intensity axis the averaged curves are more representative of the results of each single experiment than it would have been the case otherwise.

Instead of plotting these curves directly a procedure was adopted which allowed a comparison of the relative magnitudes of the single parameters. This was done by plotting the absolute value of the original mean value of each parameter at the highest light intensity (which had been set equal to 100 per cent in the normalised curve) and re-calculating the other points of the curve accordingly.

4.1.2 Curve Description

Fig. 2 shows the averaged values of the peak amplitude h_{max}, the plateau-value h_e, and the after-potential h_a versus the stimulus intensity. The measured values of the maximum amplitude h_{max} are represented by the solid circles.

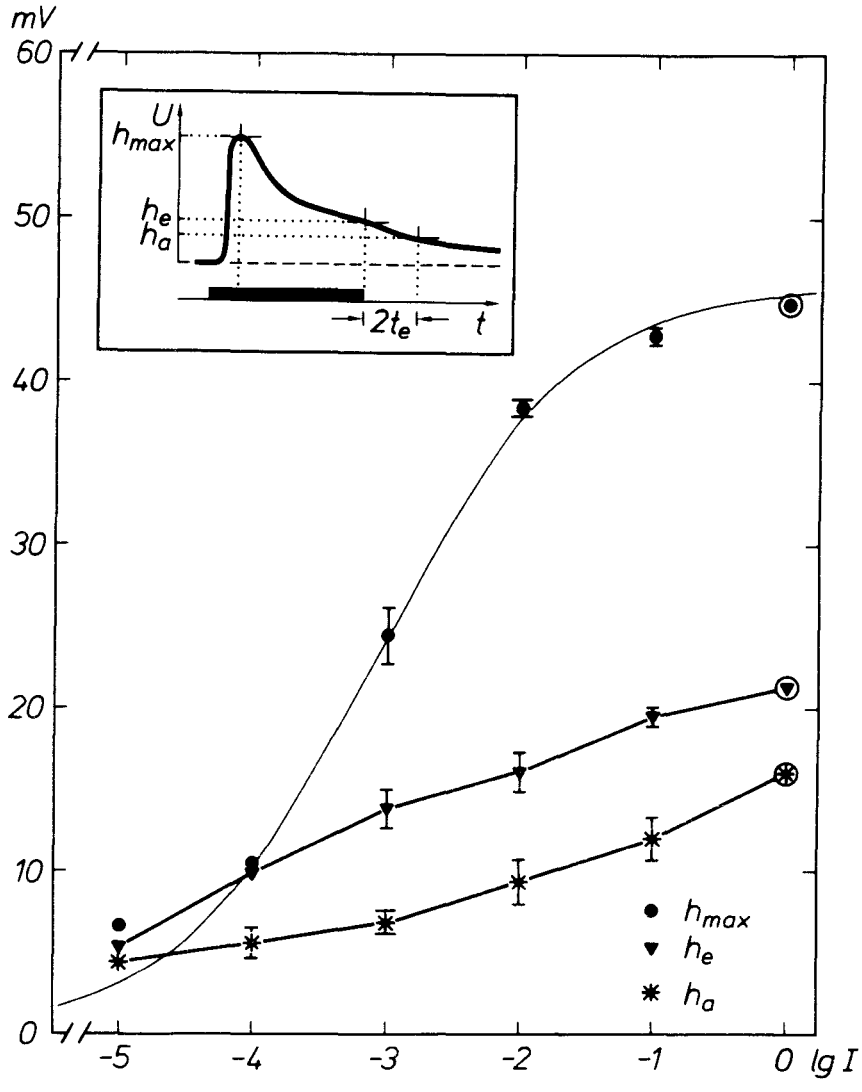

Fig. 2

Averaged values (in mV) of maximal amplitude h_{max}, plateau-value h_e, and after-potential h_a of receptor potentials recorded in five experiments at room temperature, versus intensity of stimulating light. The horizontal bars indicate the standard error of the mean. Maximal stimulus intensity ca 3 000 lx (log I = 0), flash duration ca 2 000 msec. The curves are normalised with respect to the circled values at log I = 0 (average reference values: h_{max} 44.8 mV, h_e 21.4 mV, h_a 16.1 mV).
(HSL 019, 024, 033, 042, 043, *Limulus* retinular cells, except for one eccentric cell in HSL 019)

Note: For explanation of the calculated curve of h_{max} (thin line) see results.

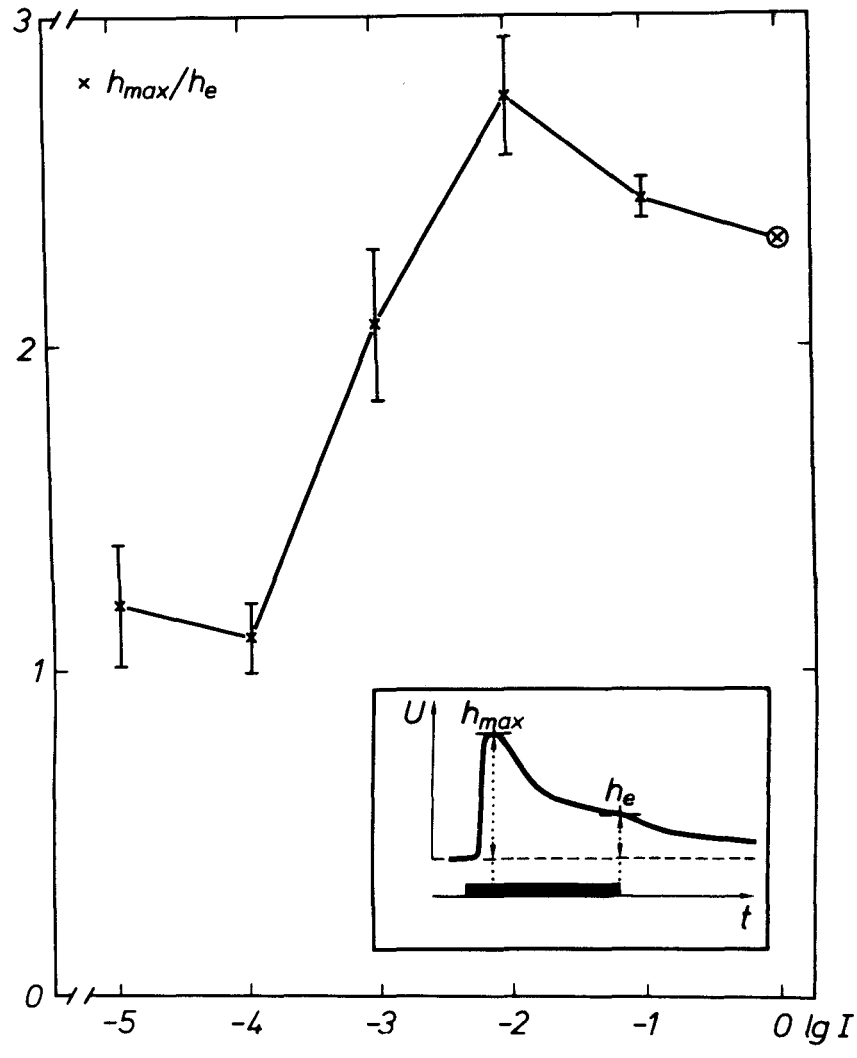

Fig. 3

Averaged values of the shape-quotient h_{max}/h_e (average reference value 2.32), versus intensity of stimulating light. Further details as described in Fig. 2.
(HSL 019, 024, 033, 042, 043, *Limulus*)

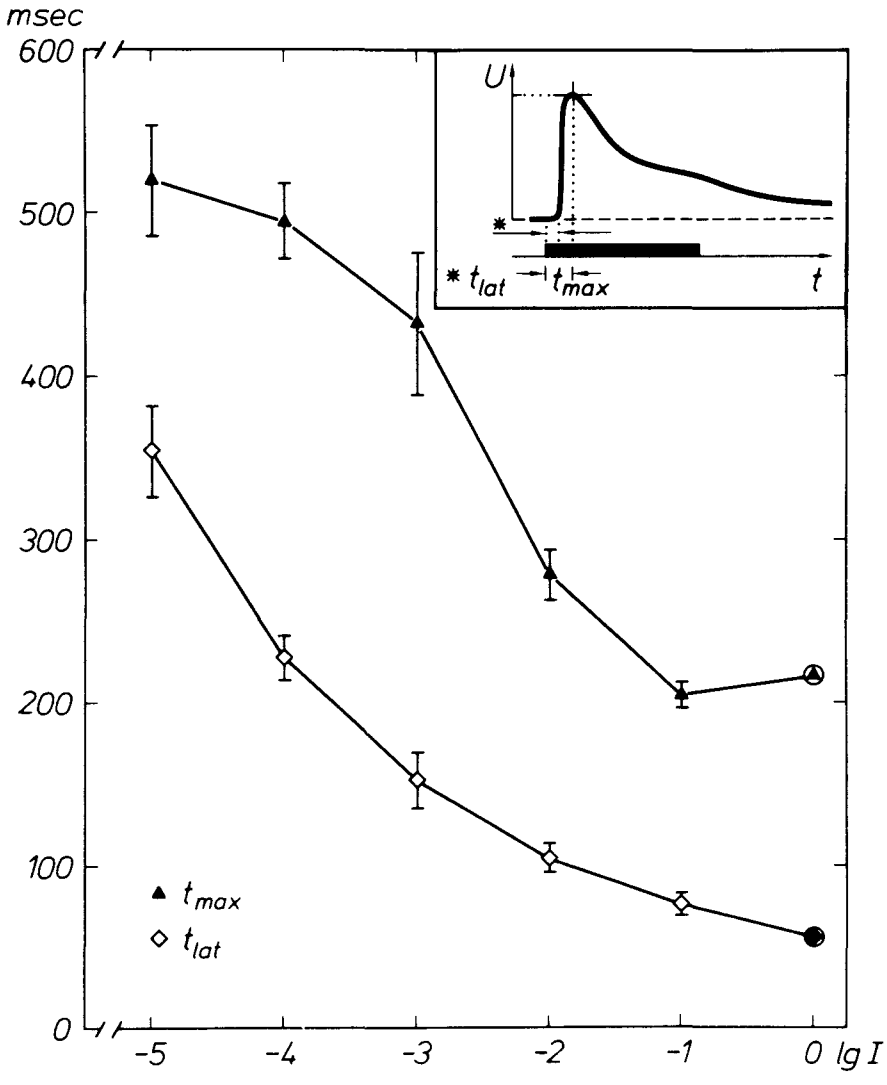

Fig. 4

Averaged values (in msec) of the latent period t_{lat} (average reference value 55.6 msec) and the peak-amplitude-time t_{max} (average reference value 216.0 msec), versus intensity of stimulating light. Further details as described in Fig. 2.
(HSL 019, 024, 033, 042, 043, *Limulus*)

As the intensity dependence of the ReP of the Limulus visual cell is known to be S-shaped the curve of h_{max} was calculated using an equation first introduced by Naka and Rushton (1966) and modified by Pak et al. (1973).

$$V = \frac{V_{max} \cdot I^n}{\sigma^n + I^n}$$

where V is the desired maximum amplitude of the ReP, V_{max} is the maximum amplitude in the state of saturation (assumed to be 46 mV for the averaged curve of our experiments), I is the respective light intensity and σ is the half saturation constant ($8.3 \cdot 10^{-4}$). An estimated value of n = 0.6 seemed to best fit our data.

The fitted curve of the maximum amplitude (shown by the thin line) is in good agreement with the measured values except for the range of low intensities (measured value at log I = -5 ca 6 mV, calculated value ca 3 mV). This divergence is due to slow potential fluctuations (bumps) which at room temperature occur spontaneously even in the dark and have the same magnitude (up to 10 mV) as the small RePs caused by weak light stimuli. Therefore the response curve does not approach zero at zero light intensity.

The plateau-value, h_e, (Fig. 2. medium curve) rises slowly but constantly from an initial value of ca 5 mV at the lowest intensity to ca 20 mV at the highest intensity. The first section (between log I = -5 and -4) of the curve is almost parallel to that of the amplitude of the transient. Apparently the plateau-value does not reach saturation at the highest intensity used in the experiments, i.e. h_e seems to saturate at higher light intensities than h_{max}.

Due to the course of the curves of maximum value and plateau--value (h_{max} saturating earlier than h_e with increasing light intensity) the curve of the shape-quotient h_{max}/h_e (Fig. 3) has a marked maximum value at a medium stimulus intensity (log I = -2).

The after-potential h_a (Fig. 2, lower curve) rises from a value below 5 mV at the lowest intensity to ca 16 mV. The increase becomes steeper towards higher light intensities. There is no saturation tendency in the intensity range studied.

In some rare cases negative after-potentials were recorded. These values were neglected in the averaged curve.

The latent period t_{lat} (Fig. 4, lower curve) becomes generally shorter with increasing stimulus intensity. The longest value at log I = -5 is about 350 msec. The falling tendency is more marked at the low intensities. An asymptotic approach to a minimal value (about 50 msec) seems probable, but is not yet reached at the highest intensity used.
Measurement of the latent period at the lowest intensities was made unreliable by slow potential fluctuations (bumps) occurring at irregular intervals.

The course of the curve of the peak-amplitude-time t_{max} (Fig. 4, upper curve) is roughly speaking, reversely proportional to that of the peak amplitude h_{max}. However the logarithmically proportional (decreasing) phase is shorter for t_{max} (between log I = -3 and -2), than for h_{max} (between log I = -4 and -2). The longest value of t_{max} is slightly above 500 msec at log I = -5. The course of the curve, which is not yet fully saturated suggests that this period would still be exceeded for lower light intensities.
The peak-amplitude-time reaches its minimal value (about 200 msec) at log I = -1 and rises again to a slightly higher value at log I = 0. This minimum at log I = -1 was observed in all experiments.
As compared to Fig. 2 the peak-amplitude-time has its minimal value at a lower light intensity than the one at which the maximal amplitude saturates.

As expected the pre-stimulus membrane potential pMP (not shown in a figure) did not change significantly but remained at a value of about -50 mV during the measurement of the intensity dependence owing to the relatively constant state of adaptation of the eye.

Generally the curves of the parameters recorded in each of the five single experiments are basically the same as the averaged

curves. Figs. 5 to 7 show the curves of one of the five experiments, HSL 033.

In this case the values were not averaged from two measurements but are single values recorded in the sequence of falling stimulus intensity.

The fitted curve of the maximum amplitude h_{max} (Fig. 5, thin line) was calculated in the same way as for the averaged experiments (V_{max} = 53 mV, $\sigma = 12.5 \cdot 10^{-4}$, n = 0.6).
The curves of the single experiments deviate only insignificantly from the averaged curves.

It is not possible to state exactly for each experiment whether the RePs were recorded from the eccentric cell or from one of the retinular cells. Judging from the size of the spikes which, in some cases, were superimposed on the RePs in one experiment (HSL 019) an eccentric cell was hit because in this case the spikes were relatively large. All the other cells were most probably retinular cells.

The results described above concerning the dependence of the *Limulus* photoreceptor on the intensity of the stimulating light correspond with those obtained by Fuortes (1959) and Fuortes and Hodgkin (1964) in their investigations of the eye of *Limulus*.

The responses recorded in the photoreceptor of *Limulus* are generally larger in size and duration than those of other crustacea.
In intracellular measurements in the visual cells of *Astacus* (the RePs of which are similar to those of *Eupagurus*) the peak amplitude of the saturation response is about 30 mV as compared to ca 50 mV in *Limulus* (Stieve, unpublished). The latent period is generally 10 to 20 msec, sometimes still shorter, while 50 msec seems to be the lowest limit value in *Limulus*. The peak-amplitude time is about 100 msec in *Astacus* as opposed to about the double value in *Limulus*.
The values of the membrane potential in the dark are similar in *Astacus* and *Limulus* (about 60 mV).

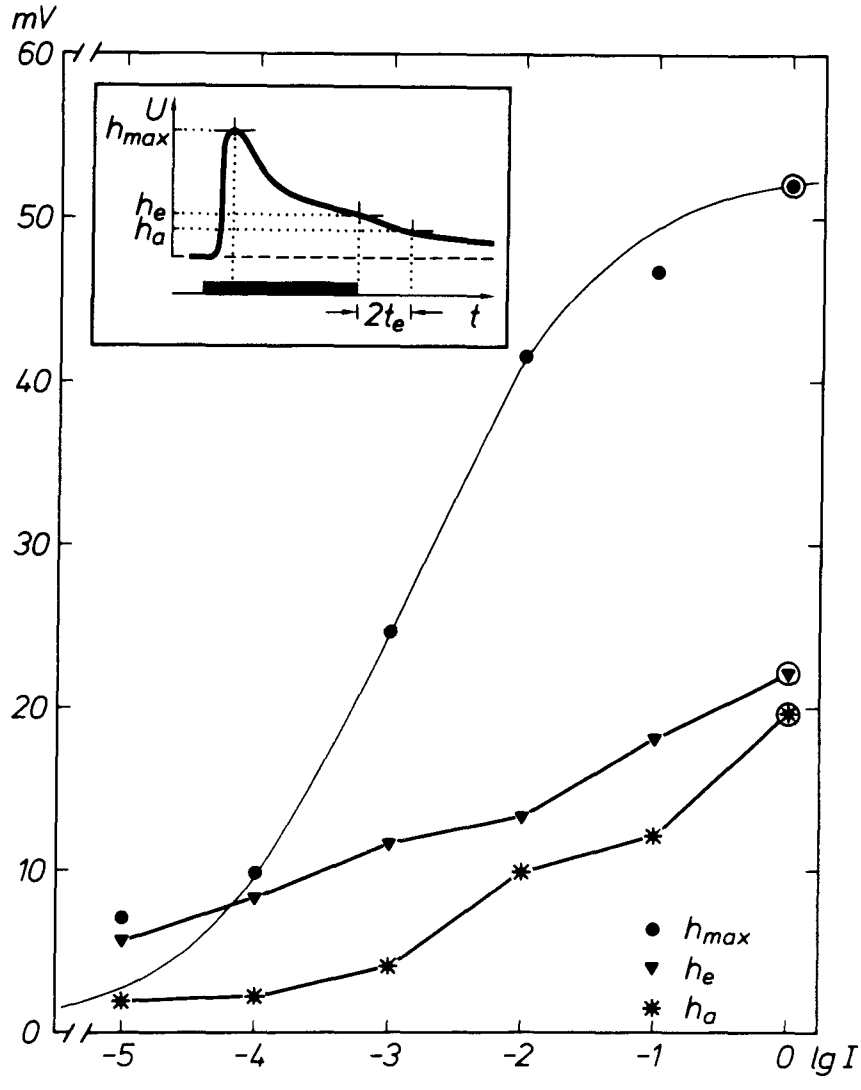

Fig. 5

Values (in mV) of maximal amplitude h_{max}, plateau-value h_e and after--potential h_a of receptor potentials recorded in a single experiment at room temperature, versus intensity of stimulating light. Maximal stimulus intensity ca 3 000 lx (log I = 0), flash duration ca 2 000 msec.
The curves are normalised with respect to the circled values at log I = 0 (reference values: h_{max} 52.0 mV, h_e 22.15 mV, h_a 19.7 mV)
(HSL 033, *Limulus* retinular cell)

Note: For explanation of the calculated curve of h_{max} (thin line) see results.

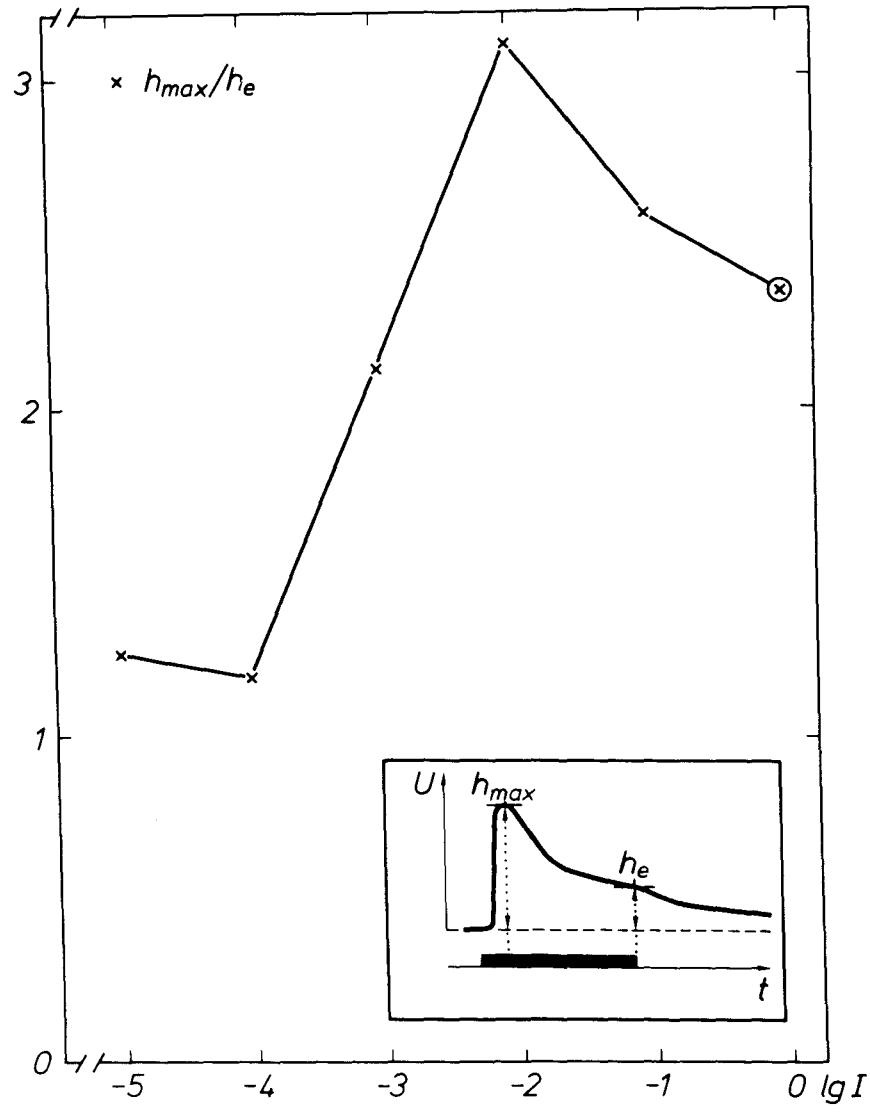

Fig. 6

Values of the shape-quotient h_{max}/h_e (reference value 2.35), versus intensity of stimulating light. Further details as described in Fig. 5. (HSL 033, *Limulus* retinular cell)

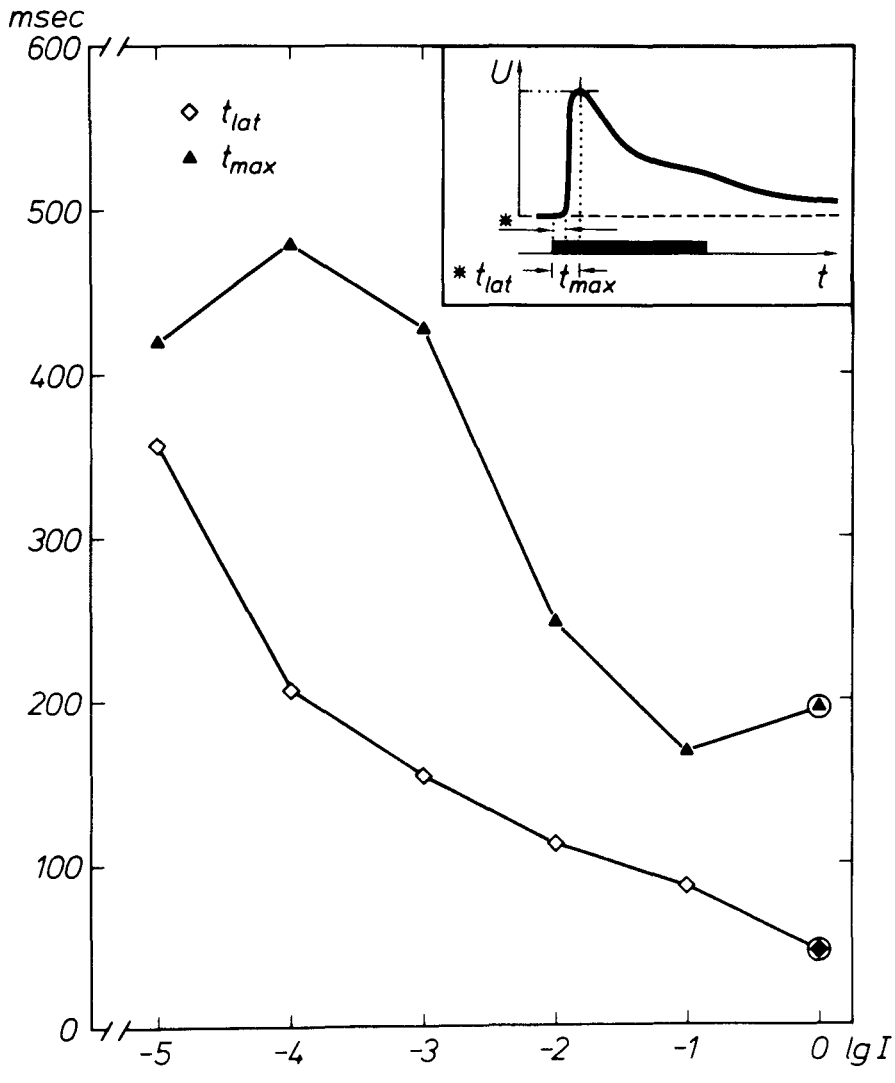

Fig. 7

Values (in msec) of the latent period t_{lat} (reference value 46.0 msec) and the peak-amplitude-time t_{max} (reference value 195.0 msec) versus intensity of the stimulating light.
Further details as described in Fig. 5.
(HSL 033, *Limulus* retinular cell)

4.2 Change of the membrane resistance during excitation by stimuli of different light intensities

4.2.1 Method, program, and evaluation

The change of the membrane resistance in the course of RePs evoked by stimuli of different light intensities was measured in a separate series of experiments in which currents were injected into the visual cells by means of a bridge circuit similar to that described by Frank and Fuortes (1956).

The principle of the determination of the membrane resistance has been described in an earlier publication (Stieve 1965). If two RePs are recorded sucessively under identical conditions except that in one case a polarizing current is injected through the measuring electrode they will differ from each other owing to the changed resistance of the visual cell membrane caused by the excitation. The total resistance occurring in the measuring circuit (Fig. 8) is the sum of electrode (and cell) resistance (R_E) and the resistance of the membrane (R_M).
Since R_E can be assumed to be approximately constant, a rough estimate for the change of R_M is obtained when the curve of the ReP recorded during polarization is subtracted, point by point, from the curve of the unpolarized ReP. A special computer program was made for this curve subtraction.

An example for the resulting membrane resistance change as compared to the course of the ReP of a *Limulus* photoreceptor cell is shown in Fig. 9.

Our experiments were intended to show whether the membrane resistance change and the time course of this change were different in dependence of the intensity of the stimulating light in the visual cell of *Limulus*.

The stimulus program was similar to that described before for the measurement of the intensity dependence (maximal light intensity ca 3 000 lx, decreased in logarithmical units, stimulus duration 2 000 msec, stimulus interval 2 min).
The number of experiments was 7 (HSL 048, 050, 054, 055, 058, 060, 062). In each experiment a stimulus sequence of falling and rising light intensities was applied. At each intensity at least four RePs were recorded; in two cases currents were injected through the microelectrode, alternatively in both current directions. (Sequence of the four RePs: unpolarized, hyperpolarized, unpolarized, depolarized). Between these sequences at different light intensities reference potentials at maximal light intensity were recorded. Polarization currents of up to $\pm$ 1.2 nA were used.

The difference between the pre-stimulus membrane potential pMP caused by the polarizing current in the dark, as opposed to the normal value of the pMP was taken as a reference measure for the resistance before stimulation by light, R_{dark} (sum resistance of electrode, cell and membrane).

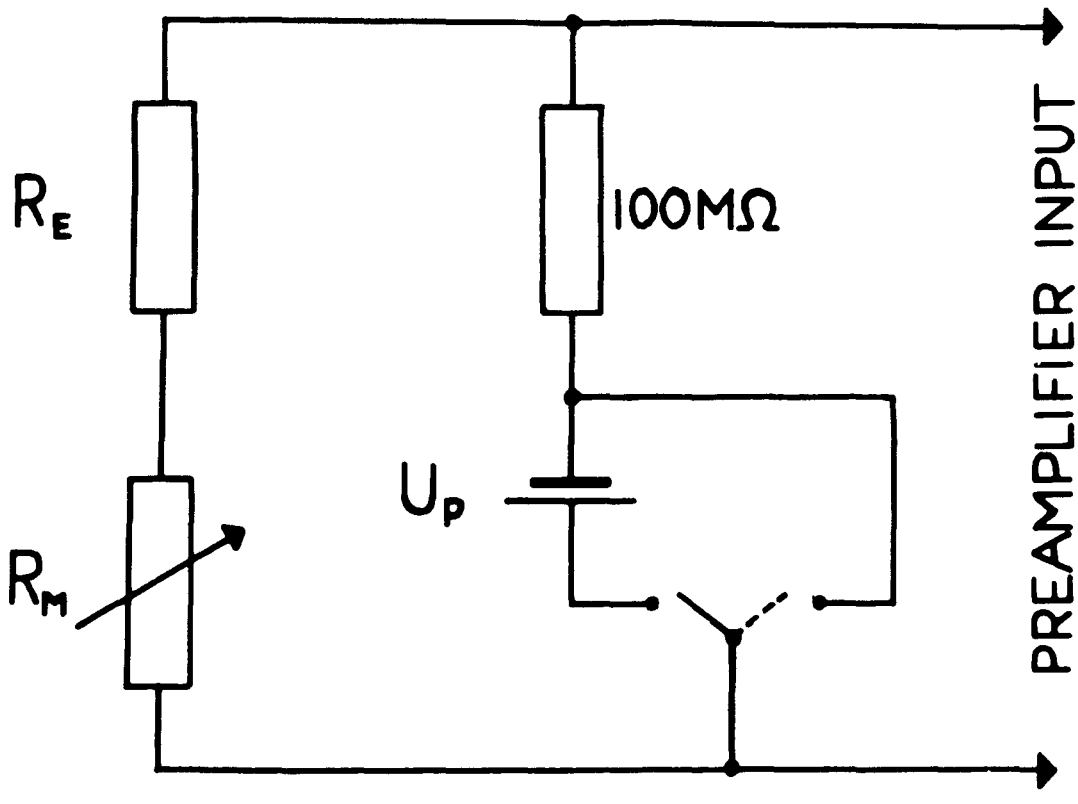

Fig. 8

Schematic drawing of the circuit used to measure the membrane resistance change of the visual cell. R_E: sum of electrode and cell resistance; R_M: membrane resistance; Up: source of polarizing current (Stieve 1965).

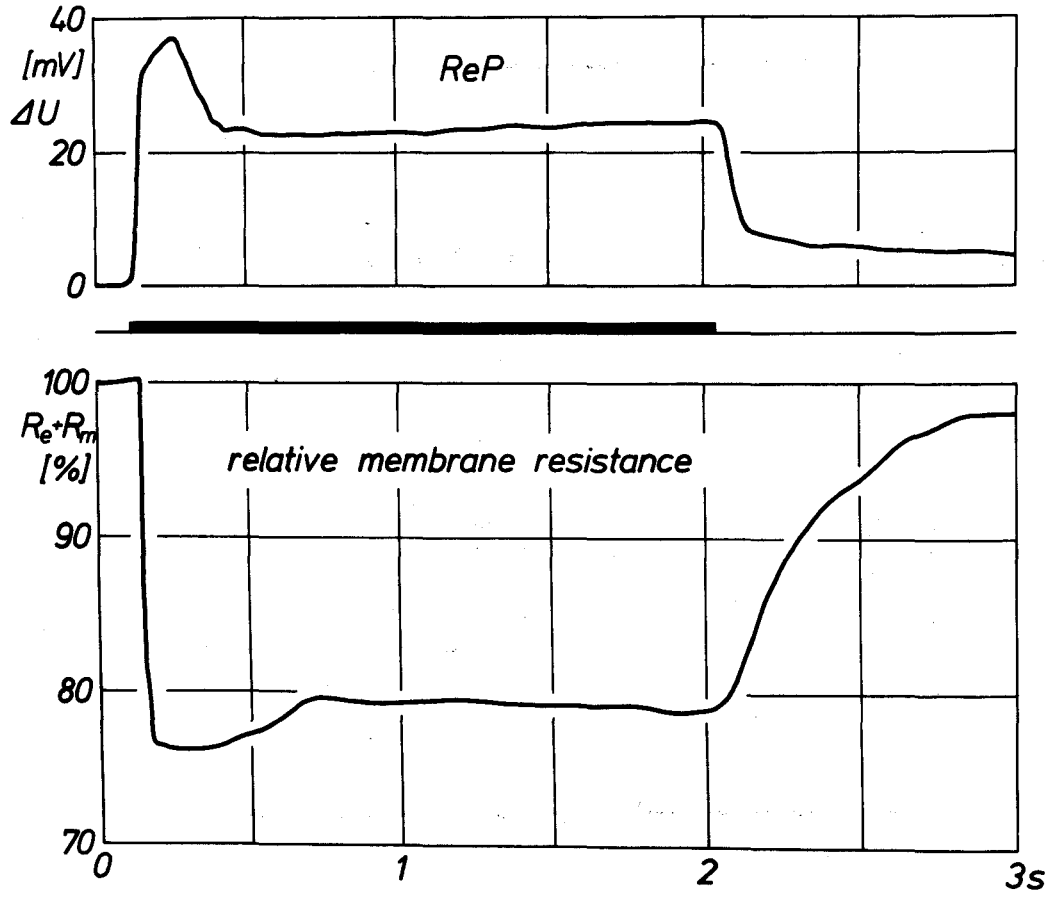

Fig. 9

Receptor potential (upper curve) and membrane resistance change (lower curve) during stimulation by light (black bar). Polarization current + 1.2 nA. (*Limulus*, retinular cell) (Stieve 1965).

The changes of the membrane resistance under stimulation by different light intensities were normalised with respect to the value of R_{dark}.

In order to avoid a point-by-point subtraction of the ReP recorded under polarization from the normal ReP, only the difference of the three most important parameters of the ReP was measured: the peak amplitude of the transient, h_{max}, the plateau-value h_e, and the after-potential h_a, determined by the computer program (with noise reduction) mentioned above.

This method is not quite correct concerning the measurement of the differences of the amplitudes h_{max} and h_a, because these parameters are measured at different times in the polarized ReP as compared to the unpolarized one (referring to all experiments the absolute time interval of the occurrence of h_{max}, polarized and unpolarized, was ca 40 msec and of h_a ca 50 msec).
Therefore it would have been desirable to determine the membrane resistance at such times of the polarized ReP corresponding exactly with t_{max}, t_e, and t_a of the respective unpolarized ReP. This was the case only with the time t_e which was clearly defined in all experiments by the end of the stimulus. For this reason the determination of the membrane resistance change ΔR_e is the only measurement which is free from this inaccuracy. To keep the error introduction by shifting of the time of measurement as low as possible only such pairs of RePs were evaluated where the time difference between polarized and unpolarized ReP was below 20 msec concerning t_{max} and below 25 msec concerning t_a.
In each single experiment which was chosen for evaluation the sum resistance before stimulation, R_{dark} (measured as difference between the pre-stimulus membrane potential pMP under polarized conditions and the pMP in the state of polarization) was not allowed to differ by more than 25 per cent from an estimated central value obtained from all experiments.

4.2.2 Description of the findings

The values of the membrane resistance change, normalised with respect to R_{dark}, were averaged and recorded as difference to R_{dark} for each intensity of stimulating light. These values ΔR_{max}, ΔR_e, ΔR_a and the quotient $\Delta R_{max}/\Delta R_e$ are listed in Table 1.

The averaged values of the three measurements of the relative membrane resistance change were compared statistically by t-test at each intensity of stimulating light (i.e. ΔR_{max} was compared with ΔR_e and ΔR_e with ΔR_a) and p was determined (Table 2).
The course of the change of the membrane resistance during the ReP apparently depends on the light intensity. At high light intensities (log I = 0, -1) the membrane resistance between t_{max} and t_e seems to be relatively constant (insignificant difference between ΔR_{max} and ΔR_e). The decrease between ΔR_e and ΔR_a, however, is significant ($p = 0.05$). At the lower light intensities (log I = -2, -3, -4) the difference of the membrane resistance between t_{max} and t_e is significant ($0.01 < P < 0.1$ between ΔR_{max} and ΔR_e) whereas the difference between ΔR_e and ΔR_a is significant only at the stimulus intensity log I = -3 ($p = 0.05$). For the lowest intensity (log I = -5) p was not determined because the standard error of the mean was too great, probably due to the occurrence of bumps.

Table 1

Values of (1) the membrane resistance change ΔR_{max}, ΔR_e, ΔR_a (normalised with respect to the 100 per cent value of R_{dark} = sum resistance of electrode, cell and cell membrane before stimulation) and the quotient $\Delta R_{max}/\Delta R_e$ and (2) the ReP amplitudes h_{max}, h_e, h_a, (in mV) and the quotient h_{max}/h_e at six intensities of stimulating light. Maximal intensity ca 3 000 lx (log I = 0), stimulus duration ca 2 000 msec.
(HSL 048, 050, 054, 055, 058, 060, 062, *Limulus*)

log I	ΔR_{max} [%]	ΔR_e [%]	ΔR_a [%]	$\frac{\Delta R_{max}}{\Delta R_e}$	h_{max} [mV]	h_e [mV]	h_a [mV]	h_{max}/h_e
0	14 ± 2.5	13.5 ± 2.1	8 ± 2.1	1.04	41.5 ± 0.45	23.6 ± 0.43	17.1 ± 0.50	1.74
-1	14 ± 2.3	12 ± 2.3	7 ± 1.7	1.17	36.4 ± 0.47	17.5 ± 0.74	9.7 ± 0.37	2.08
-2	19 ± 1.5	10 ± 1.9	9 ± 2.3	1.90	28.9 ± 1.18	13.1 ± 1.01	6.1 ± 0.53	2.21
-3	15 ± 3.6	9 ± 1.9	3 ± 2.7	1.67	20.3 ± 1.92	9.4 ± 0.99	2.7 ± 0.56	2.16
-4	18 ± 10.3	3 ± 3.3	2 ± 1.5	6.00	19.5 ± 1.92	10.55 ± 0.83	2.5 ± 0.65	1.85
-5	-11 ± 22.3	5 ± 4.1	-10 ± 3.7	-2.20	10.8 ± 1.93	6.2 ± 0.85	0.95 ± 0.39	1.74

Table 2

Statistical comparison by t-test and determination of p for the values of table 1 of changes of the membrane resistance ΔR_{max} and ΔR_e, and ΔR_e and ΔR_a, resp., at each intensity of stimulating light. For an explanation see text.
(HSL 048, 050, 054, 055, 058, 060, 062, *Limulus*)

	p	
log I	$\Delta R_{max} - \Delta R_e$	$\Delta R_e - \Delta R_a$
0	> 0.5	0.05
-1	0.6	0.05
-2	0.01	> 0.5
-3	0.1	0.05
-4	0.1	0.6

Table 3

Membrane resistance change ΔR_{max}, normalised with respect to R_{dark} = sum resistance of electrode, cell and membrane before stimulation (100 per cent = -9.3 $\pm$ 0.7 MegΩ), and maximal amplitude h_{max} of the ReP, normalised with respect to an average value of reference potentials recorded before and between the measurements (100 per cent = 46.3 $\pm$ 0.8 mV) at four intensities of stimulating light. Maximal intensity ca 1 000 lx (log I = 0). Stimulus duration ca 50 msec.
(JB 47-50, *Limulus*)

log I	ΔR_{max} [%]	h_{max} [%]
0	101 $\pm$ 3.2	99 $\pm$ 0.9
-1	90 $\pm$ 6.2	82 $\pm$ 6.7
-2	81 $\pm$ 9.2	57 $\pm$ 7.8
-3	56 $\pm$ 9.4	25 $\pm$ 3.5

To allow a comparison between the course of the ReP and the changes of the membrane resistance at the different light intensities, the values of the peak amplitude of the transient h_{max}, the plateau-value h_e, the after-potential h_a and the shape-quotient h_{max}/h_e were determined from the non-polarized RePs recorded during the same experiments. The values of these parameters, averaged for each intensity of stimulating light, are also plotted in Table 1. A comparison of this dependence (which is basically similar to the dependence of the ReP of the intensity of the stimulating light described before) with the change of the membrane resistance in the time-course of the ReP at different light intensities shows:

(1) The measured membrane resistance change ΔR_{max} fluctuates between relatively wide limits. This is probably due to the inevitable error concerning the time of measurement. Therefore ΔR_{max} seems not to depend on the intensity of the stimulating light, whereas the peak amplitudes of the RePs recorded in the same experiments show the typical S-shaped dependence of the light intensity.
These results seem not to represent the true behaviour of ΔR_{max}, which is shown by a comparison with the course of ΔR_e and ΔR_a.

(2) The changes of the membrane resistance ΔR_e and ΔR_a are influenced by the intensity of the stimulating light in the same direction as the amplitudes h_e and h_a. With decreasing stimulus intensity ΔR_e and ΔR_a (except for ΔR_a at log I = -2) as well as the plateau-value h_e and the after-potential h_a of the ReP become smaller. The decrease of the membrane resistance changes is less marked than that of the amplitudes. The values of ΔR_e are at each intensity always smaller than those of ΔR_{max}.

(3) Both the shape-quotient h_{max}/h_e and the quotient $\Delta R_{max}/\Delta R_e$ have a maximum value at log I = -2. In the case of $\Delta R_{max}/\Delta R_e$ only the values from log I = 0 to log I = -3 were considered. The extreme values at log I = -4 and log I = -5

are probably due to unreliable measurements caused by the occurrence of bumps at the lower light intensities.

The findings (2) and (3), i.e. that ΔR_e is at each intensity smaller than ΔR_{max} and that the change of the quotient $\Delta R_{max}/\Delta R_e$ corresponds with the change of the shape-quotient, suggest that a similar dependence of the light intensity as observed for ΔR_e could be expected for ΔR_{max} provided that the exact time of measurement could be found.
Our results indicate a correlation between the amplitude of the ReP and the membrane resistance. The fact that the values of ΔR_{max} at the different light intensities are widely scattered suggests that the time of measurement of ΔR_{max} differed too much from the respective time of measurement of h_{max}.
In this connection the results of other experiments at the Limulus photoreceptor may be interesting (Stieve, unpublished). In these experiments the preparation was stimulated by short light flashes (50 msec duration) of different intensity (maximal intensity ca 1 000 lx, decreased in three logarithmical steps) and polarized by means of current pulses. By this procedure a better agreement of the times of measurement of h_{max} and ΔR_{max} can be obtained. The results of these experiments are shown in Table 3:
Both the amplitude h_{max} of the ReP and the membrane resistance change ΔR_{max} decrease significantly with reduced intensity of the stimulating light. Again the decrease over the range of light intensities used is much steeper for h_{max} (to ca 25 per cent of the reference value) than for ΔR_{max} (to ca 50 per cent of the reference value).
This correlation of the membrane resistance change ΔR_{max} and the maximal amplitude of the transient of the ReP at decreasing light intensities supplements our present results where similar correlations were found for the plateau-value and the after-potential of the ReP and the corresponding membrane resistance change ΔR_e and ΔR_a. Therefore it can be safely assumed that the wide scattering of the measurements of ΔR_{max} in our experiments is due to the inexact determination of the time of measurement.

Our statistically examined measurements of the change of the membrane resistance in the course of the ReP are in good agreement with the experiments cited above (see Fig. 9, Table 3).

Obviously an interrelation exists between (1) the course of the ReP and the course of the membrane resistance change during the ReP and (2) the changes of the amplitudes of the ReP at flashes of different light intensities and the corresponding changes of the membrane resistance. This interrelation seems to be of a complex nature because the changes of the membrane resistance observed at different intensities of stimulating light are not proportional to the changes of the ReP. though tending toward the same direction as those (i.e. reduction of the ReP amplitudes with falling stimulus intensity corresponds with decreased values of ΔR_M).

4.3 The effect of light and dark adaptation on the receptor potential

4.3.1 Test program and evaluation

The term adaptation is used here to describe the light-induced sensitivity changes of the visual cell, measured as change of the size and shape of the RePs evoked by constant test flashes, during exposition of the preparation to a period of background illumination (light adaptation, LA) and, during an immediately adjacent period of darkness (dark adaptation, DA).

Before the actual measurements the two light beams used as background illumination and stimulating light were adjusted, as exactly as possible, to the same spot of the ommatidium concerned, and calibrated at the maximal intensity available to cause RePs of roughly the same size (difference not exceeding 20 per cent referring to h_{max}).
To test the light adaptation the background illumination (beam I) was applied for 10 min. During that period the preparation was stimulated (beam II) at geometrically increasing time-intervals after 0.5, 1, 2, 4, and 8 min. After switching off the light test measurements were made at geometrically increasing time-intervals starting from 0.5 to 16 min, and afterwards at regular intervals of 8 min until 64 min dark adaptation.
A typical sequence of RePs recorded during light and dark adaptation is shown in Fig. 10 (HSL 042).
The total number of experiments in which the adaptation to light and darkness was tested was 15. In 9 of these (which were finally averaged) the

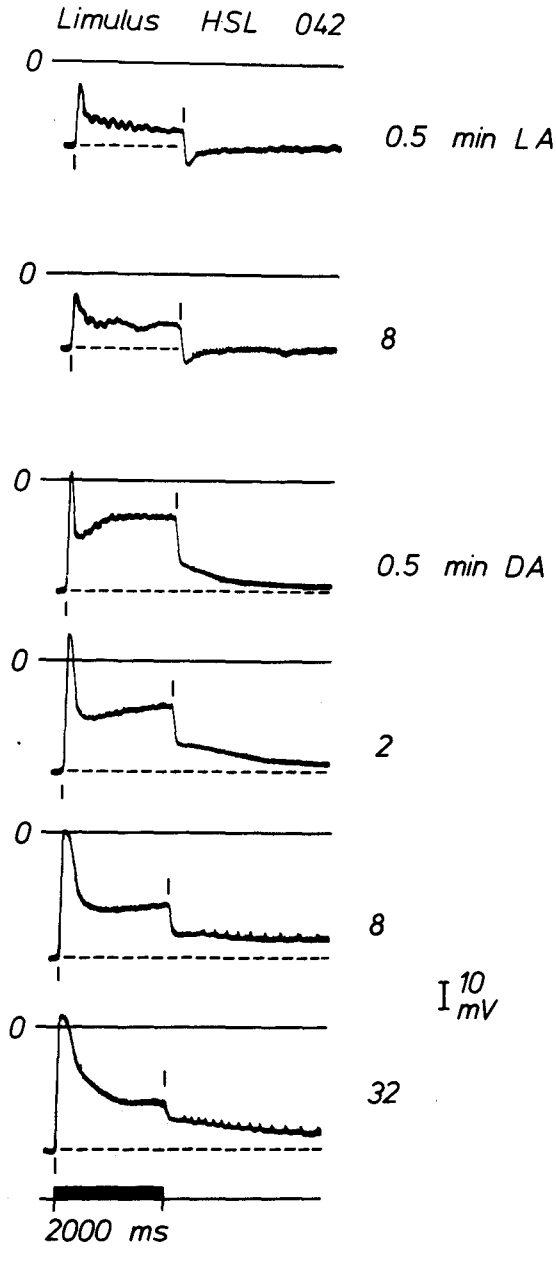

Fig. 10

Receptor potentials recorded at room temperature during 10 min light adaptation (LA) and subsequent dark adaptation (DA). Thin line: zero reference level. Intensity of stimulating light and background illumination during LA ca 3 000 lx, stimulus duration ca 2 000 msec.
(HSL 042, *Limulus* retinular cell)

visual cells responded until at least 32 min dark adaptation. In four experiments 64 min dark adaptation were reached. Therefore the curve sections beyond 32 min (dotted lines) are represented by less measurements.

The evaluation of the experiments corresponds on principle with that described for the intensity dependence. The results were averaged and normalised with respect to the parameters of the RePs recorded at 8 min DA. (This value was chosen as it was reliably reached in all experiments).

The normalised curves were than recalculated on the basis of the absolute value (in mV and ms) of the mean value at 8 min DA (which had been set equal to 100 per cent) to allow a comparison of the relative dimensions of the different parameters.
In six experiments both beams had maximal intensity, while filters were used in three cases. As in these experiments the intensity of the background illumination and the stimulus light was attenuated to the same degree, this difference was neglected.

4.3.2 Curve description

A typical pen recording of the beginning of the light adaptation and the subsequent transition from light to darkness is shown in Fig. 11 (HSL 042). Switching on the background illumination causes a ReP with a transient and steady state value which represents the pMP for the following RePs evoked by the test flashes superimposed on the background light. When the background light is turned off the membrane potential immediately increases again. This change of the membrane potential at beginning and end of the background illumination is shown in Fig. 12 a und b (HSL 042).

The averaged curves of the parameters of the RePs recorded during light and dark adaptation are shown in Figs. 13 to 16 (Experiments HSL 014, 018, 021, 025, 033, 034, 036, 042, 043).

Fig. 13 shows the changes of the pre-stimulus membrane potential pMP. During the measured period of light adaptation the pMP is depolarized to about -25 mV. Switching off the light causes a rapid increase to a maximum of nearly -50 mV. The pMP remains on this value for about 2 min DA and then depolarizes steadily to about -35 mV at 64 min DA.

The peak amplitude of the transient, h_{max}, (Fig. 14, upper curve) decreases rapidly as immediate effect of the background light. The slope of this decrease is not resolved by our measurements; the maximal amplitude remains constant at ca 13 mV until 8 min LA. The dark adaptation is characterized by an increase of h_{max} reaching a maximal value of nearly 40 mV at 2 min, followed by a slow decrease to an approximately constant level (ca 35 mV). The restoration of the sensitivity of the photoreceptor (measured as h_{max}) takes place more slowly

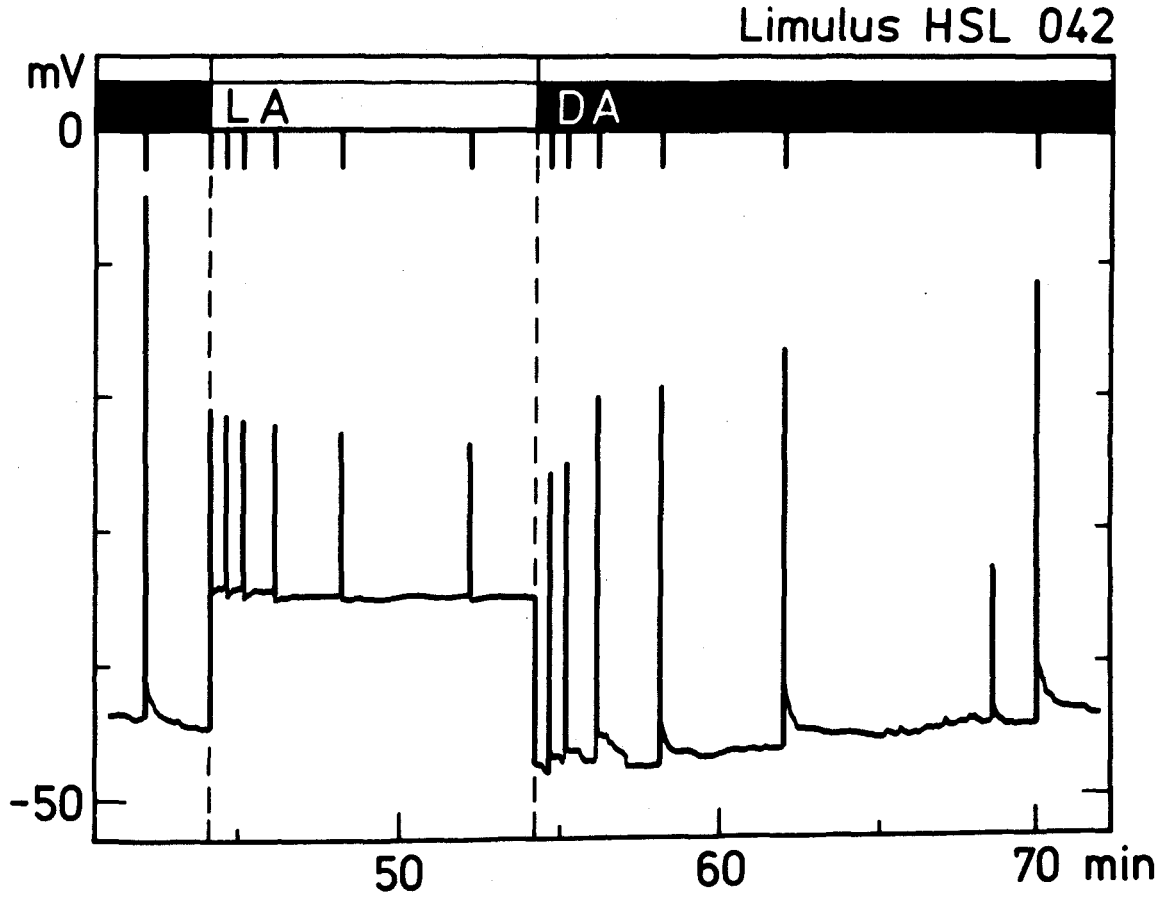

Fig. 11

Pen recording of membrane potential and RePs (lower trace) during adaptation to light (LA) and darkness (DA). Upper trace: zero reference line, vertical bars indicate time of light flashes. Intensity of light flashes and background illumination during LA ca 3 000 lx, flash duration ca 2 000 msec. Amplitude height of RePs is not reliably shown due to slugishness of pen deflection.
(HSL 042, *Limulus* retinular cell)

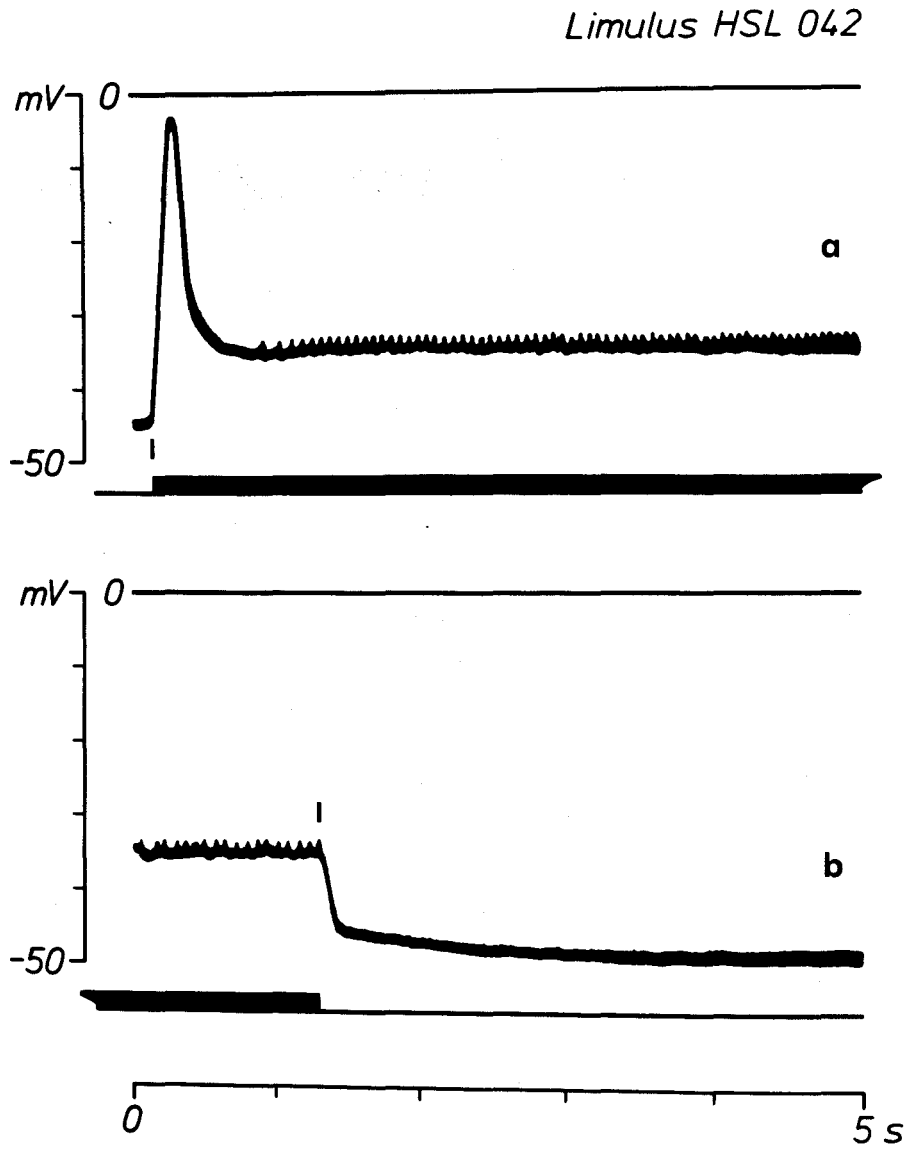

Fig. 12

Changes of the membrane potential at turning on (a) and off (b) the background illumination. Beginning and end of the background light (ca 3 000 lx) is indicated by the vertical black bars. Thin lines: zero reference level.
(HSL 042, *Limulus* retinular cell)

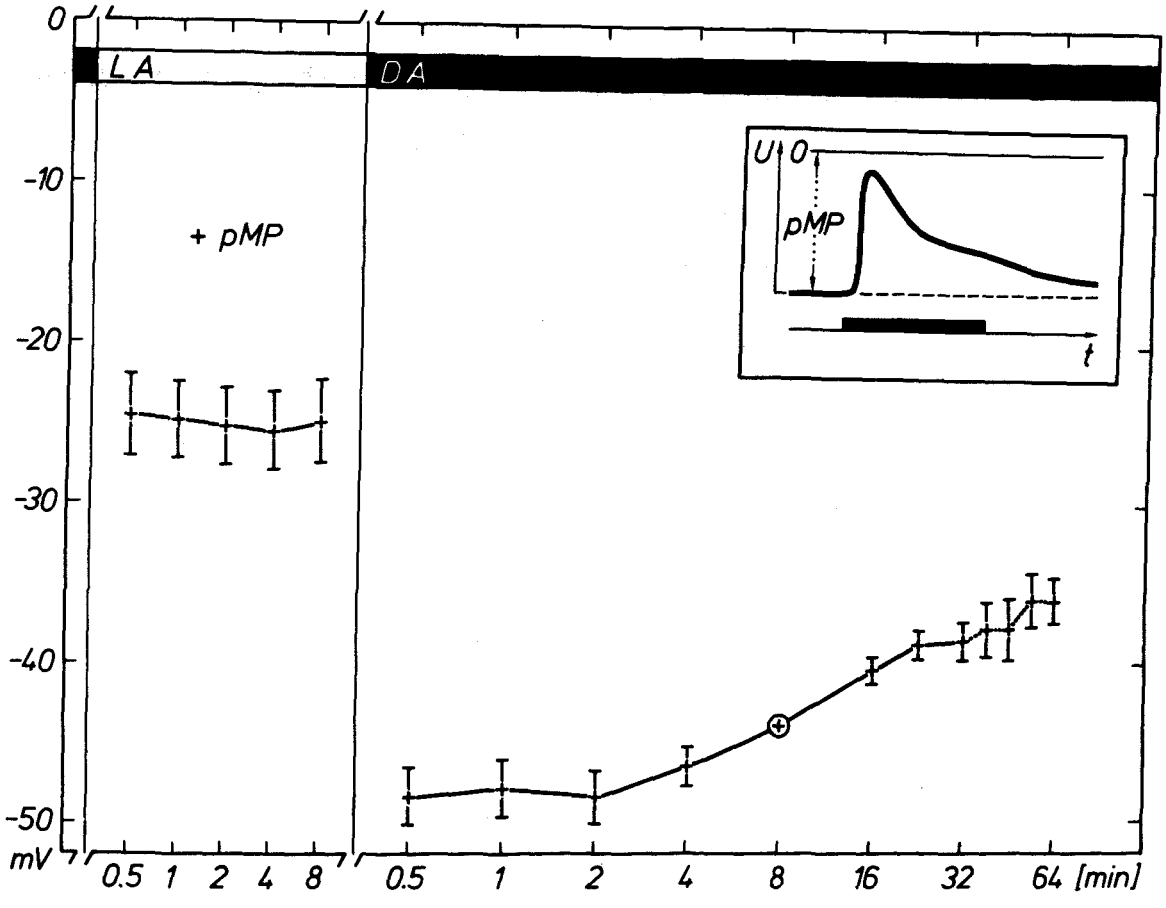

Fig. 13

Averaged values of the pre-stimulus membrane potential pMP (recorded in mV as difference to the zero reference level) at room temperature during 10 min light adaptation (LA) and subsequent 64 min dark adaptation (DA), versus time (in min). The horizontal bars indicate the standard error of the mean. Intensity of stimulating light and background illumination during LA ca 3 000 lx, flash duration ca 2 000 msec.
The curve is normalised with respect to the circled value at 8 min DA (average reference value: -43.9 mV). The values from 32 to 64 min DA (dotted line) are represented by fewer measurements.
(HSL 014, 018, 021, 025, 033, 034, 036, 042, 043; *Limulus* retinular cells)

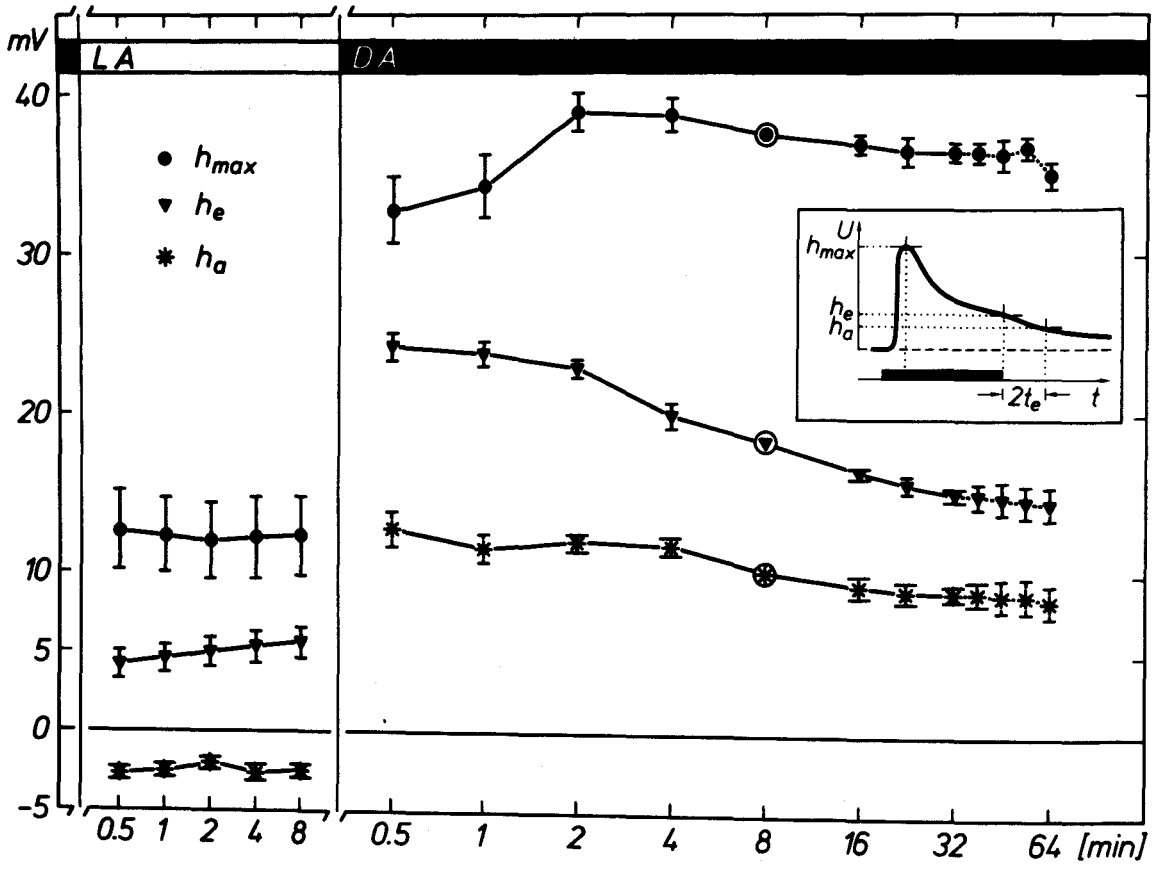

Fig. 14

Averaged values (recorded in mV as difference to the pre-stimulus membrane potential pMP) of maximal amplitude h_{max}, plateau-value h_e, and after--potential h_a (average reference values: h_{max} 37.57 mV, h_e 18.19 mV, h_a 10.24 mV) during LA and DA, versus time in min.
Further details as described in Fig. 13.
(HSL 014, 018, 021, 025, 033, 034, 036, 042, 043; *Limulus* retinular cells)

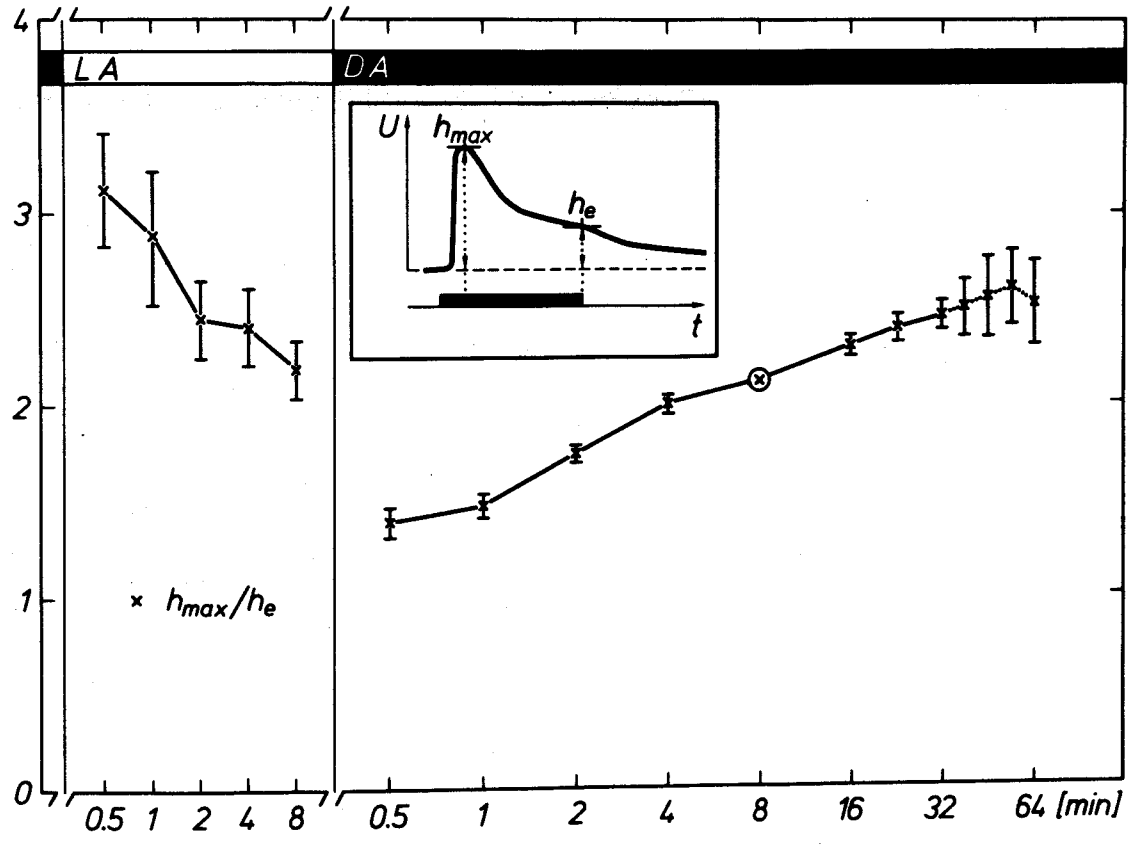

Fig. 15

Averaged values of the shape-quotient h_{max}/h_e (average reference value: 2.12) during LA and DA versus time in min.
Further details as described in Fig. 13.
(HSL 014, 018, 021, 025, 033, 034, 036, 042, 043; *Limulus* retinular cells)

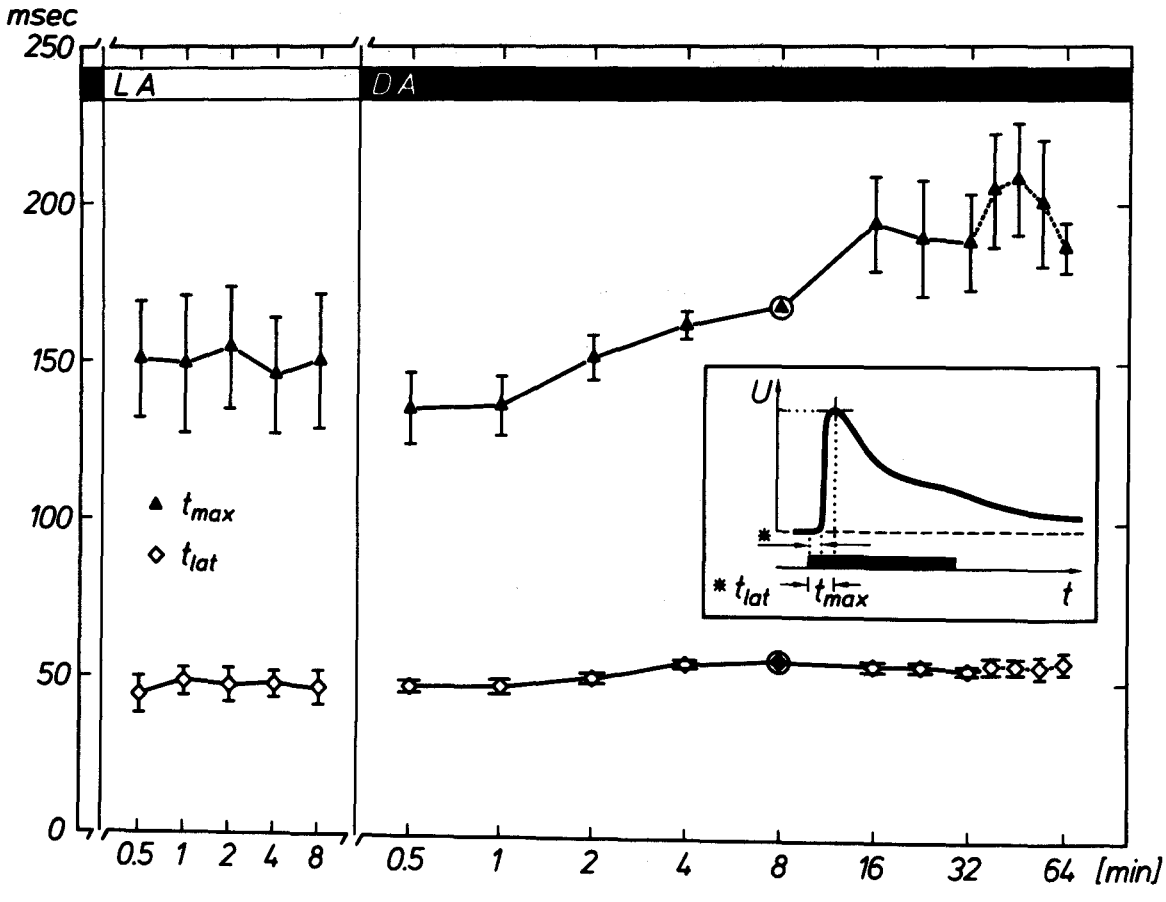

Fig. 16

Averaged values in msec of the latent period t_{lat} (average reference value 56.5 msec) and the peak-amplitude-time t_{max} (average reference value 167.8 msec) during LA and DA, versus time in min.
Further details as described in Fig. 11.
(HSL 014, 018, 021, 025, 033, 034, 036, 042, 043; *Limulus* retinular cells).

in the dark as compared to the membrane potential which is even hyperpolarized beyond its stationary level 30 seconds after switching off the light.

The plateau-value h_e (Fig. 14, medium curve) is reduced to ca 4 mV 30 sec after switching on the light but increases slightly and steadily until the end of the light adaptation. After switching off the background light it increases rapidly to ca 25 mV at 0.5 min DA (by about the same amount as h_{max}) and then decreases steadily until the end of the dark adaptation, with a saturation tendency at about 16 mV.

According to the course of the two curves described before the shape-quotient h_{max}/h_e (Fig. 15) has its maximum at the first measured value of the light adaptation (ca 3.2), which means that the background illumination initially causes a relatively greater reduction of the plateau-value as compared to the maximal amplitude. In the course of the light adaptation the shape-quotient decreases steadily to ca 2.3. The immediate effect of switching off the background light consists in a further decrease of the shape-quotient to ca 1.4 before it increases again to a steady value of ca 2.5 (slightly below the initial maximum).

During the measured period of the light adaptation negative values (-2 to -3 mV) were recorded for the after-potential h_a (Fig. 14, lower curve). This means that a strong background illumination apparently causes the last part of the ReP to drop below the level of the pMP. During the dark adaptation the after-potential, following a rapid initial increase (at 0.5 min DA) to ca 11 mV as immediate effect of the end of the background illumination, decreases slowly and constantly to ca 8 mV at 64 min DA.

The latent period t_{lat} (Fig. 16, lower curve) does not change much under the adaptation conditions applied. It remains constant between 40 and 60 msec. The small increase observed in the course of the dark adaptation is not significant.

The peak-amplitude-time, t_{max} (Fig. 16, upper curve) remains on a level of about 150 msec (below the reference value) in the measured light adaptation period. After a small decrease to about 130 msec at 0.5 min DA as immediate effect of the dark adaptation it increases relatively steadily toward saturation (ca 180 msec at 17 min DA). As already stated before the values recorded after 32 min are not representative because they were averaged from fewer experiments than the other curve points.

4.3.3 Adaptation experiment with short test flashes

An additional single experiment was carried out (at a temperature of 15°C) to test the adaptation to light and darkness of the lateral eye of Limulus under basically the same conditions as in the experiments just described, except for the fact that short light flashes (50 msec instead of 2 000 msec) were applied (JB 78). The results correspond fairly well with those obtained in the experiments with long stimuli.

The pre-stimulus membrane potential pMP (Fig. 17) changes in rather the same way but less marked in the experiment with short flashes as compared to the experiments with long stimuli: depolarization by light, repolarization during the dark adaptation. The only major difference of the curves is that in the experiments with long stimuli the initial hyperpolarization step in the dark adaptation is followed by a slow depolarization toward a constant value, whereas in the experiment with short flashes the final repolarization level is almost reached at the first measured value of the dark adaptation.

The value of the peak amplitude of the transient, h_{max}, (Fig. 18) is still decreasing, after a rapid initial decrease to ca 25 mV at 0.5 min LA, throughout the light adaptation (ca 20 mV at 8 min LA), whereas it remains constant during the same measured period of light adaptation in the experiments with long stimuli.
In the dark adaptation a sudden fast increase of h_{max} within the first 30 seconds to a maximal value followed by a slight decrease toward a constant value is observed in both types of

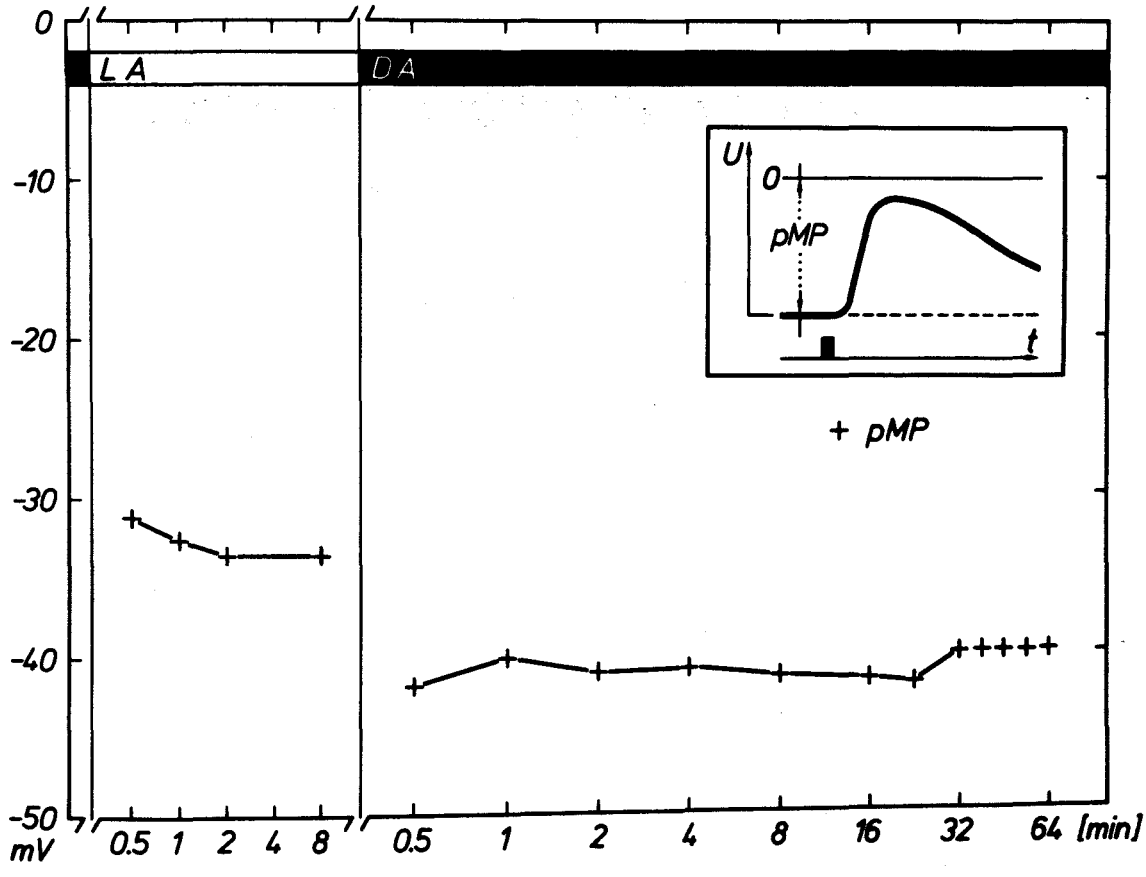

Fig. 17

Values of the pre-stimulus membrane potential pMP (recorded in mV as difference to the zero reference level) at 15°C during 10 min light adaptation (LA) and subsequent 64 min dark adaptation (DA) versus time (in min). Intensity of stimulating light and background illumination during LA ca 500 lx, flash duration ca 50 msec.
(JB 78, *Limulus* retinular cell)

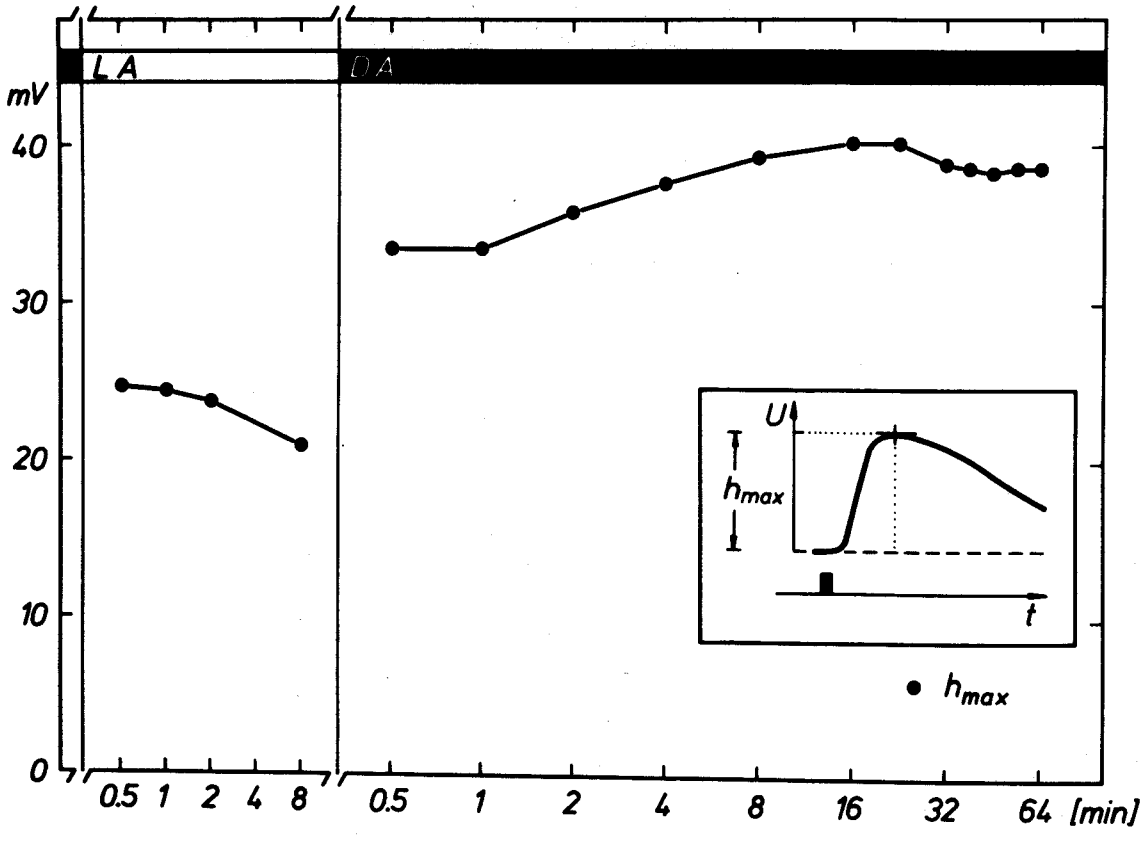

Fig. 18

Values of the maximal amplitude h_{max} (recorded in mV as difference to the pMP) during LA and DA, versus time in min.
Further details as described in Fig. 15.
(JB 78, *Limulus* retinular cell)

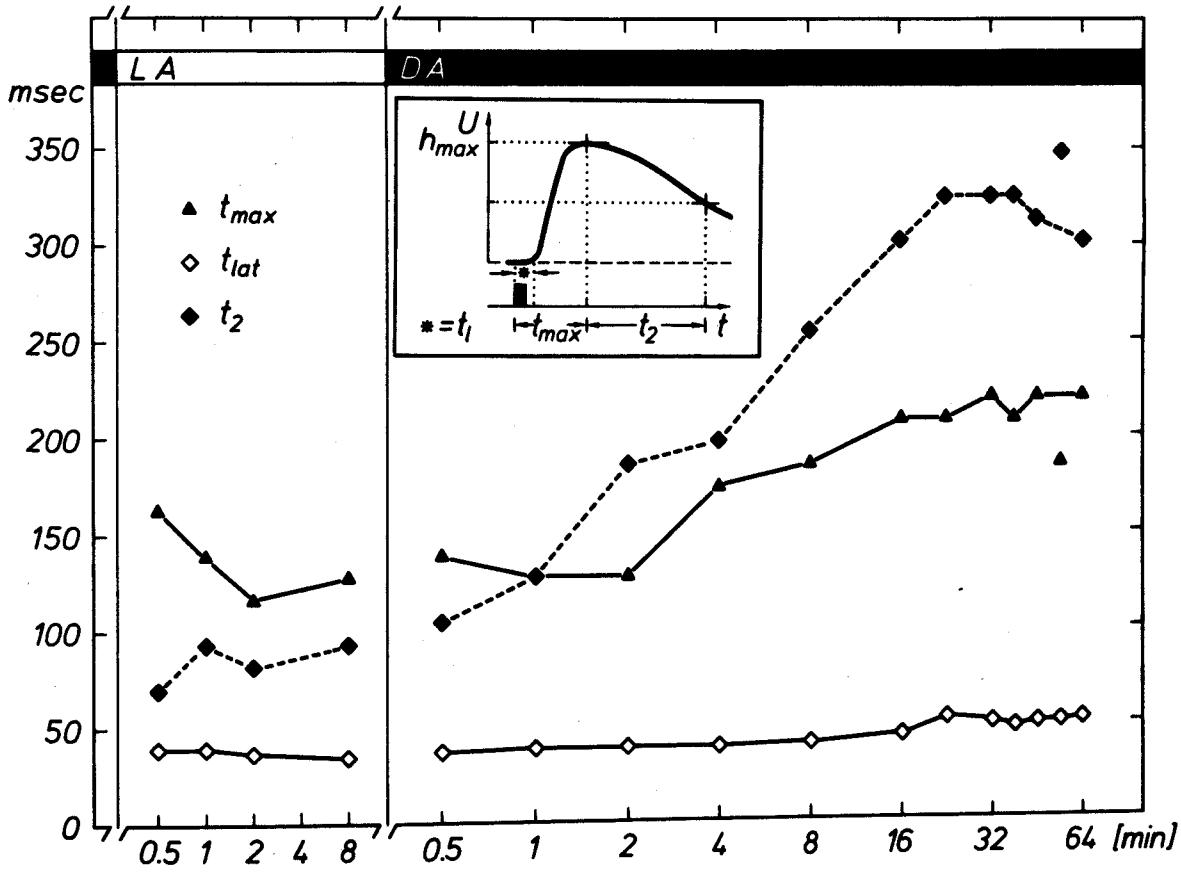

Fig. 19

Values of the latent period t_{lat}, the peak-amplitude-time t_{max}, and the decrease-time t_2 (in msec) during LA and DA, versus time in min. Further details as described in Fig. 15.
(JB 78, *Limulus* retinular cell)

experiments, but the maximal value is reached later (at 24 min) when short flashes are used than in the case of long stimuli (at 2 min). The comparison between plateau-values and after--potentials recorded in the experiments with long and short flashes must be omitted as these amplitudes do not exist in the RePs caused by short flashes.

The latent-period t_{lat} (Fig. 19) hardly changes during light and dark adaptation in the experiment with short stimuli, similar as for long stimuli.

The peak-amplitude-time t_{max} (Fig. 19) decreases in the experiment with short flashes during the measured period of light adaptation (from ca 170 msec at 0.5 min LA to ca 120 msec at 2 min LA, final value at 8 min LA ca 185 msec), as opposed to the constant level observed in the experiments with long stimuli. During the dark adaptation t_{max} increases, after a slight initial decrease from ca 130 msec at 0.5 min DA to ca 125 msec at 2 min DA in the same way as in the averaged curve and saturates at about 200 msec after 16 min DA.

The decrease-time t_2 (Fig. 19), the time until the amplitude $h_{max}/2$ is reached - a suitable parameter to describe the slope of the decline of a ReP caused by a short flash - increases during the measured period of light and dark adaptation from an initial value of ca 70 msec at 0.5 msec LA to a constant value of over 300 msec at 24 min DA. From 32 min DA to 64 min DA the decrease-time slightly decreases again.

As shown by the plots (Figs. 16 and 19) the time parameters of the RePs caused by short and long stimuli are hardly influenced by the transition from light to darkness. The changes, if any, occur mainly in the course of the long-term dark adaptation.

The adaptation effects observed in our experiments were fully reversible, i.e. the RePs recorded before the light adaptation period were not significantly different from those recorded after the end of the dark adaptation.
As compared to our results Benolken (1962) registered a somewhat slower increase of the sensitivity of the *Limulus* photoreceptor (to ca 50 per cent of the original value after 30 seconds) after the end of the light

adaptation. According to his findings the sensitivity of the photoreceptor cell changes markedly in the first two minutes. In his measurements he applied an arbitrary constant amplitude criterion and found that to cause RePs of the same magnitude the test flash had to be 10 000 times more intense at the time 3 sec after the end of the light adaptation period than two minutes later.

Our results suggest that the effect of the strong background illumination on the photoreceptor of *Limulus* is relatively weak as compared to adaptation experiments with *Eupagurus* (Stieve 1960, 1963) where the effects, though being basically similar, were more marked.
Particularly the time needed to regain the former sensitivity level after light adaptation is very short in the photoreceptor of *Limulus*. In our experiments 80 percent of the reference value of the maximal amplitude of the ReP were already reached after 30 seconds darkness.
The course of the dark adaptation is apparently slower in other arthropod species. This is shown by experiments with *Eupagurus* and *Carcinus* (Stieve et al. 1973) where the restoration of the sensitivity (measured as h_{max}) lasted considerably longer (ca 10 min) although the intensity of the preceding 10 min background illumination was much weaker (ca 150 lx) than in the present experiments.

4.4 The effect of the stimulus frequency on the pre-stimulus membrane potential

The way in which the stimulus frequency influences the level of the pre-stimulus membrane potential pMP was observed in those experiments which could be continued after the dark adaptation owing to a still satisfactory condition of the visual cell. The dark adaptation (stimulus interval 8 min from 16 min to 64 min DA) was followed by a dark period of 20 min with test stimuli every 2 min before the next series of measurements was started.

The increase of the stimulus frequency in the way described is actually a form of weak increase in light adaptation. Normally the pMP was about -55 mV, with occasional minor fluctuations. At the transition from the stimulus interval of 8 min to the shorter interval of 2 min an increase to about -65 mV of the pMP was observed in some experiments. The pMP was raised in the repolarization phase at the end of each successive ReP. Fig. 20 shows a paper recording of such a hyperpolarization (HSL 018). In the same period during which the hyperpolarization by ca 10 mV of the pMP was observed as effect of the raised stimulus frequency the maximal amplitude h_{max} of the RePs and the plateau-value h_e increased by almost the same amount (ca 7 to

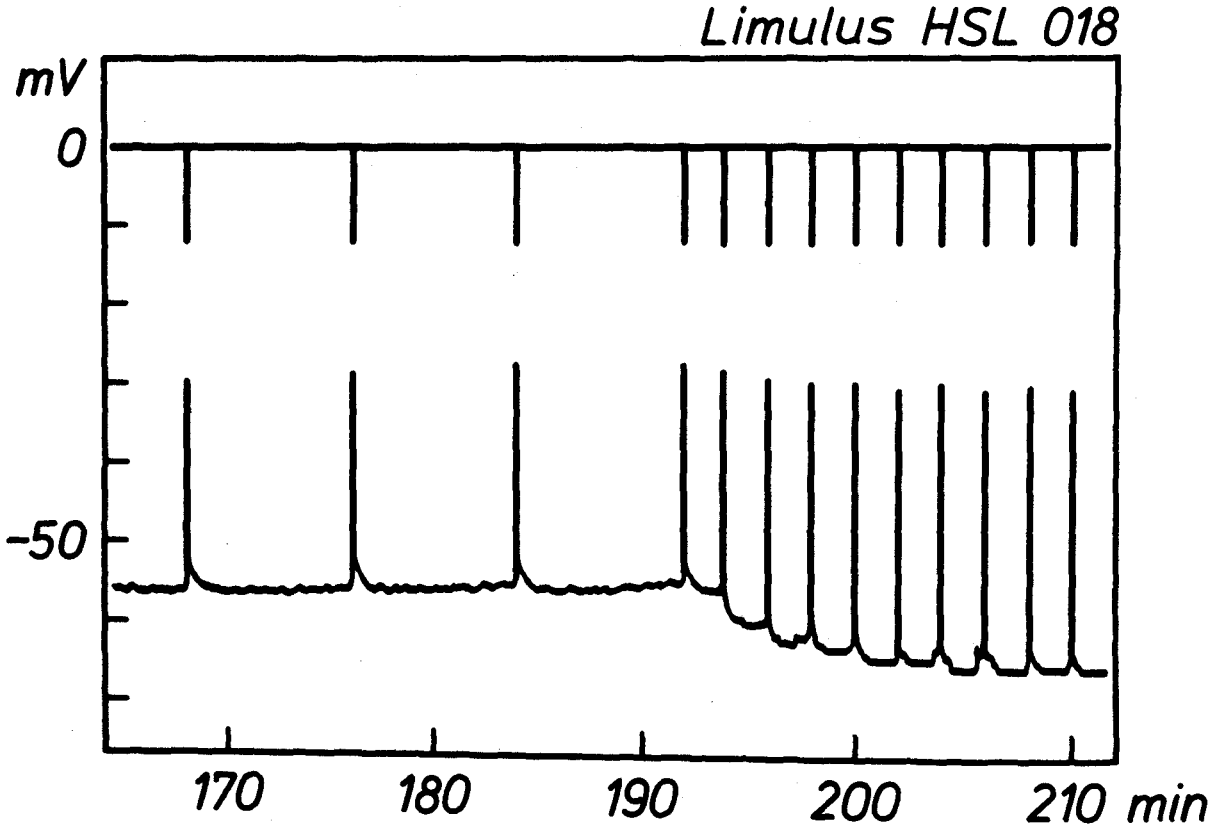

Fig. 20

Pen recording of the change of the pre-stimulus membrane potential pMP caused by decreasing the stimulus interval from 8 to 2 min.
Lower trace: recording of the membrane potential.
Upper trace: zero reference line, vertical bars indicate time of light flashes (intensity ca 3 000 lx, duration ca 2 000 msec).
Amplitude height of RePs is not reliably shown due to sluggishness of pen deflection.
(HSL 018, *Limulus* retinular cell)

10 mV). As a result of this increase of h_{max} the total maximal depolarization (measured as difference to the zero reference line) was only slightly reduced during the measured hyperpolarization of the pMP.

The hyperpolarization of the pre-stimulus membrane potential pMP apparently caused by a weak increase in light adaptation was not studied more closely. It was observed in three of ten experiments where the dependence of the RePs on increased stimulus frequency was measured. In four experiments the hyperpolarization was much less marked, while in the remaining three experiments the pMP was either depolarized or not changed at all.

Apparently the occurrence of a hyperpolarization of the pMP as a result of increased stimulus frequency varies from preparation to preparation. This is also suggested by adaptation experiments of Wulff et al. (in print) in the photoreceptor of *Limulus*.

The phenomenon that the membrane potential increases after each stimulus and the maximal amplitude is reached at a higher level in the eye of *Limulus* was also observed by Millecchia (1969). Actually this effect means that the sensitivity is greater in a state of moderate light adaptation than in the dark. This may also underlie the findings of Benolken (1962) who found that in the course of dark adaptation in *Limulus* (constant stimulus curve) an initial response maximum occurred which was followed by a decrease.

5. Discussion

The results of our quantitatively representative experiments on the electrophysiological response of the photoreceptor of the lateral eye of *Limulus* agree with those obtained in single experiments.
Basically the reactions in the *Limulus* eye are similar to the reactions observed in other arthropod species, but several details and differences will be discussed below.
Despite these particular problems our observations fit into a general concept of the electrophysiological conditions prevailing in the arthropod photoreceptor.
The main points of this concept will be shortly outlined in a very simplified way as a basis for the interpretation of our results.

The potential (interior of the cell negative against the surrounding medium) which exists across the visual cell membrane in the dark is assumed to be essentially caused by the existence of ionic concentration gradients across the membrane which are maintained by an active pump mechanism and different membrane permeabilities for various ion species.

According to voltage clamp experiments by Millecchia and Mauro (1969) the dark potential, which has a fairly consistent value of -40 to -60 mV for most invertebrates studied, is apparently produced by a dominant permeability of the visual cell membrane to potassium ions in the dark, as the value of the dark potential is close to the electrochemical equilibrium potential for potassium.
One of the reaction steps following the absorption of light quanta by the visual pigment triggers a change of the membrane permeability which leads to the generation of the ReP.
The depolarization of the membrane during stimulation by light (measured as maximal amplitude, h_{max}, of the ReP) is apparently mainly the result of a sudden transient influx of sodium ions into the visual cell caused by an increased membrane permeability to sodium (Stieve 1965, Millecchia and Mauro 1969, Mack

Brown et al. 1970). The following repolarization is mainly produced by a compensatory efflux of potassium ions (Stieve et al. 1972, Stieve 1974).
The transport mechanisms maintaining the ionic concentration gradients across the membrane against the passive ion fluxes can be electrogenic which means that during their activity they produce a potential difference. According to experience electrogenic transport effects a hyperpolarization in the eye of *Limulus*.

Erler (1972) assumed such an electrogenic pump activity to be responsible for a post-stimulus hyperpolarization of the membrane potential in the photoreceptor cell of *Balanus*.

Fig. 21 shows a simplified schematic representation of the ionic mechanism of the membrane potential in the dark and during excitation in an invertebrate visual cell.
One way to explain how the visual cell membrane is able to perform the functions involved in this concept consists in the assumption of ion-specific channels leading through the membrane, some of them controlled by light. Although this channel hypothesis, which seems to hold for the nerve membrane (Hille 1968), could not yet be proved for the visual cell membrane it seems to offer, in our opinion, the most simple and plausible explanation for the existence of the ion specific permeabilities and great ionic currents in the state of rest and during excitation.
In the simplest case there would be two types of channels:
(1) "Dark channels" which are permanently open and let preferably potassium ions pass and
(2) "Light channels" which are opened only during excitation and let preferably sodium ions pass.

The number of light channels being opened at a time increases with the number of the incident photons. This is consistent with the fact that at high light intensities a membrane potential can be reached (saturation response) which is near the electrochemical equilibrium potential for sodium.
Possibly there are different types of light channels cooperating to produce the ReP.

It is not yet known in which part of the retinular cell membrane the conductance changes during excitation take place. Fioravanti

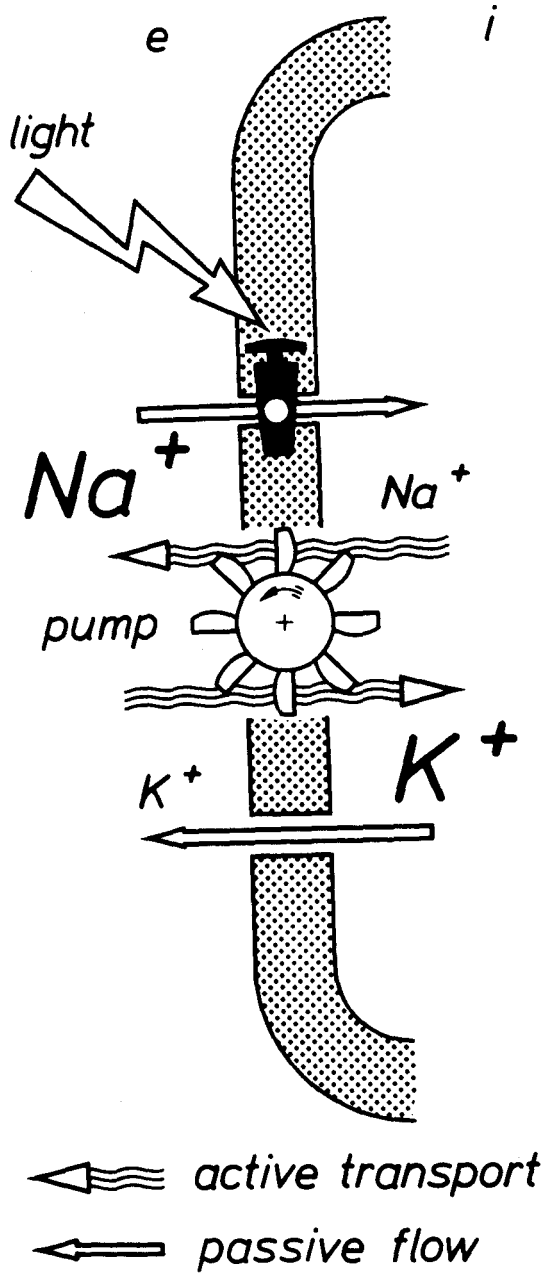

Fig. 21

Schematic drawing of the ionic mechanism of the membrane potential in the dark and during excitation in an arthropod visual cell (e - exterior, i - interior of the cell). The paddle-wheel symbolizes active transport mechanisms (Stieve 1971).

and Fuortes (1972) found that the response of the visual cell of the leech is caused by a conductance increase in the microvillar membrane. This finding is in agreement with earlier experiments of Walther (1965). This and other reasons make it probable that the membrane resistance change during excitation in the photoreceptor of *Limulus* occurs only in the rhabdomeric area, while the dark channels responsible for the membrane potential in the dark presumably are located all over the visual cell membrane.

In the following the results of the different series of experiments will be discussed separately with a view to their relevance to the basic concept described.

5.1 Intensity dependence

The characteristic curve of the visual cell of *Limulus* (maximal amplitude h_{max} versus log I, see Fig. 2) is, within the limits of our measuring accuracy, well fitted by the formula described by Pak et al. (1973). As the cell was kept in an equal state of slight light adaptation during the experiment by regular reference measurements at high light intensity the peak amplitude of the ReP could be expected to be a function of the light intensity alone.

In the experiments which were averaged for the plots the logarithmically linear increase of the peak amplitude before saturation extends over about 2 logarithmical intensity units. As in three experiments, which were not averaged, this log linear increase phase was considerably longer (exceeding 3 intensity units) the total light intensity range which can be registered by the *Limulus* photoreceptor apparently varies within certain limits. This phenomenon has not been investigated in earlier experiments.

The discrepancy between the calculated curve for the peak amplitude and the measured values at the low flash intensities is due to the frequent occurrence of discrete slow depolarizing potential fluctuations (bumps). These bumps, which can even be observed at zero light intensity, do not occur at low temperatures. Bumps are also responsible for the more or less straight beginning of the curve of the plateau-value h_e at the low light intensities. Without bumps this part of the plot could be

expected to be curved similarly (though less marked) than that of h_{max}, and approach zero.
The time-course of the response in the photoreceptor of *Limulus* is relatively slow as compared to other arthropod species (see results).
As the value of the latent period t_{lat} (Fig. 4) at the highest light intensity used was approached asymptotically this time of ca 50 msec seems to represent the minimal duration of the processes leading to the saturation response at room temperature.
The S-shaped curve of the peak-amplitude-time t_{max} extending from ca 200 msec for high light intensities to slightly over 500 msec for low intensities suggests that the processes responsible for the initial amplitude rise of the ReP become less influencial within this time than the processes leading to the decrease toward the plateau-value.

5.2 Membrane resistance change

The resistance change of the visual cell membrane was measured since it is known to be the main cause for the occurrence of the ReP.
The results of our averaged experiments indicate that the amplitude of the ReP, while certainly being a function of the membrane resistance change, is not directly proportional to this change at different light intensities.
It must be considered that a close and complex relation exists between the resistance of the visual cell membrane and the degree of depolarization during the ReP. Millecchia and Mauro (1969) showed that in the photoreceptor of *Limulus* the membrane resistance and the light induced conductance changes depend on the membrane potential. The different phases of the ReP in turn have an influence on the course of the membrane resistance change. Regarding this chain of actions with the membrane resistance occurring as link twice in different positions our measured values of the overall membrane resistance as compared to the amplitudes of the ReP can merely provide clues.

A correlation between the membrane resistance and the ReP is shown in our experiments by the fact that the relative membrane

resistance change ΔR increases with increasing amplitudes of the ReP. As however the changes of the ReP are not proportional to those of the membrane resistance it is evident that the unspecific total membrane resistance determined by our measuring method cannot be directly proportional to the different phases of the ReP which depend on ion specific conductance changes, and active (electrogenic) pump mechanisms.

The reason why the total membrane resistance change cannot be directly parallel to the course of the ReP (see Fig. 9) is shown by a simplified electrical equivalent circuit for the visual cell membrane of *Limulus* (Benolken 1961). In this model (Fig. 22) the membrane contains a constant dark resistance R_d and a battery E_d producing the dark potential and, parallely, a battery E_L, producing the transient amplitude of the ReP, in series with the respective resistance R_L which decreases during excitation by light.

Assuming such a simplifying concept with a potassium battery representing E_d and a sodium battery representing E_L in the visual cell membrane the state of the membrane potential at any time of the excitation would be dominated by a weighted ratio between the specific sodium and potassium potentials.

Even this simple model makes clear that the change of the membrane resistance, though being the cause of the ReP, does not proportionally determine its magnitude.

Additionally there are other possible factors determining the ReP besides the membrane resistance change. The ionic gradients supplying the batteries can become exhausted during the excitation (this could hold especially for the sodium gradient). Furthermore the course of the ReP can be influenced by the activity of an electrogenic pump. For instance this additional electrogenic pump activity is presumably responsible for the fact that the potential decrease after the end of the flash is two-phasic which means that it is caused by two processes, the first of which coincides with the monophasic membrane resistance change.

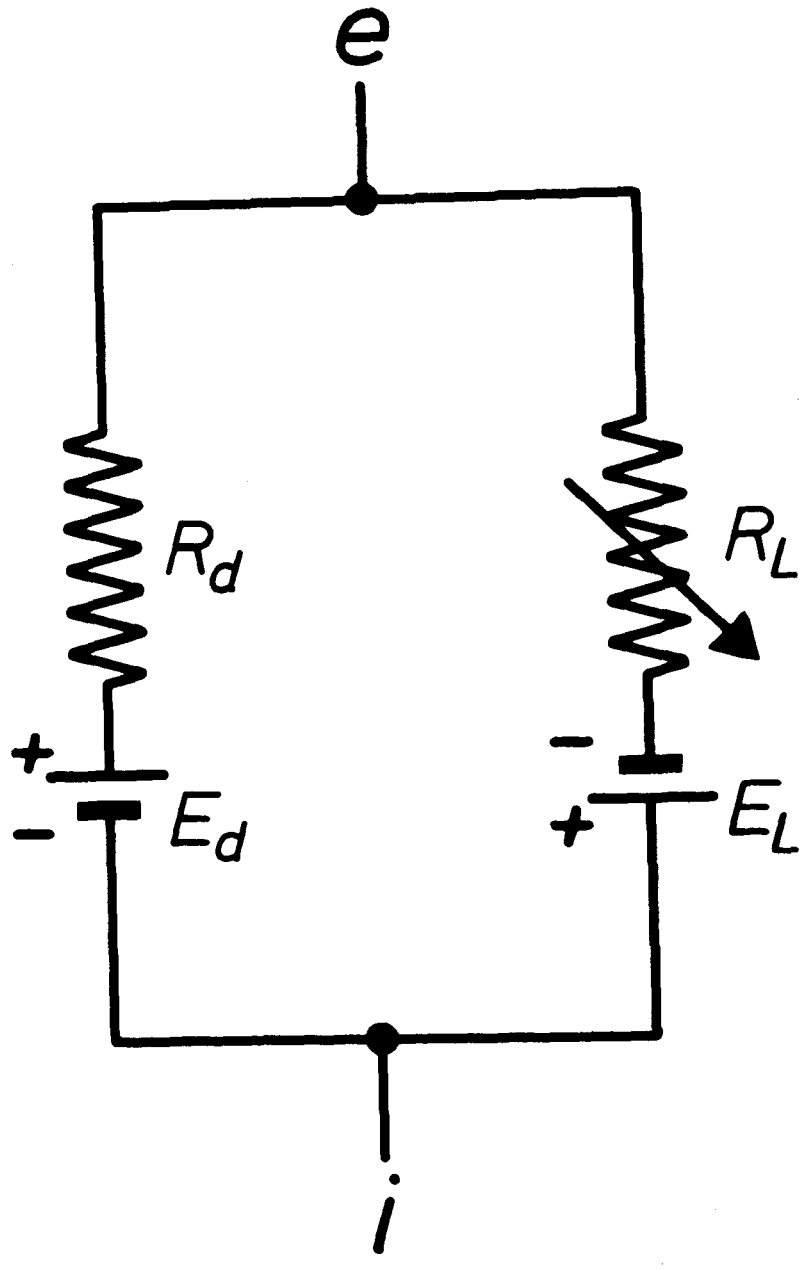

Fig. 22

Electrical equivalent circuit of the visual cell membrane of *Limulus* (Benolken 1961). E_d battery determining the membrane potential in the dark; R_d the respective resistance; E_L battery determining the transient of the ReP; R_L the respective resistance which is changed upon a light stimulus; e - exterior; i - interior of the cell.

5.3 Adaptation

The adaptation processes involved in our experiments are limited to the photoreceptor cell.
The short-time adaptation effects, i.e. the changes of the ReP during the first 30 seconds of background illumination resp. after switching off the background light were not measured in our experiments, but stress was laid on determining the long--term effects of the adaptation to light (10 min) and darkness (up to 64 min).
For this reason the observed effects must be ascribed to membrane adaptation because pigment adaptation is terminated within very short time (half period ca 100 msec) in the ventral photoreceptor of Limulus (Fein and Cone 1973, Fein and DeVoe 1973).

The term pigment adaptation refers to the dependence of the sensitivity of the visual cell of the quantity of bleached visual pigment. Contrary to the conditions in *Limulus* Rushton (1957) and Dowling and Ripps (1970) found a linear relation between the quantity of bleached visual pigment and the logarithm of the intensity needed for a response of constant magnitude in vertebrates. Apparently pigment adaptation is much slower and more prominent than membrane adaptation in vertebrates.
Also in invertebrates pigment adaptation can play a decisive role. Hamdorf and Rosner (1973) and Rosner (1974) found that under certain conditions the sensitivity of the photoreceptor of the fly is proportional to the bleached rhodopsin.
The way in which the visual pigment content actually controls the sensitivity of the visual cell is not completely understood.

The term membrane adaptation denotes those processes which influence the electrochemical properties of the visual cell membrane, and though these processes can be regarded separately from the visual pigment reactions the two types of adaptation are functionally interdependent.

The effect of the background light in our experiments consists in a reduction of the sensitivity of the photoreceptor (decrease of h_{max}). At the same time the response to the light flashes becomes faster (decrease of t_{max}) (Figs. 14-16).
During the time of background illumination the visual cell is strongly depolarized, which is shown by the reduction of the pre-stimulus membrane potential pMP to about half its value in the dark (see Fig. 11).
Correspondingly the maximal amplitude h_{max} of the ReP decreases during the light adaptation to a level of less than 50 per cent

of its reference value. Owing to these reversely directed changes of the pMP and h_{max} the resulting total depolarization at the peak of the ReP remains approximately equal during light adaptation and under normal conditions in the dark.
This is rather astonishing as the total light intensity (sum of background light and flash) of the stimuli during the light adaptation was almost double as high as compared to the test flashes in the dark because the intensity of the background light was presumably close to the saturation range.

The fast restoration of the sensitivity in the beginning of the dark adaptation despite the strong background light applied in the preceding 10 min suggests that the adaptative capacity in the *Limulus* photoreceptor seems to be greater than in certain other arthropods, e.g. crayfish and hermit crab (see results). The fact that the pre-stimulus membrane potential, after being depolarized to 50 per cent of its dark value already 30 seconds after turning on the background light, remains relatively constant at this value during the sustained background illumination indicates that the ionic gradients obviously remain on a constant (presumably reduced) level without decreasing further.
Adaptation effects due to changed ionic gradients influencing the membrane potential should increase during sustained illumination and would probably be slower than those we observed. This applies particularly for the change of the pMP at the transition from light to darkness. At the first measurement in the dark (after 30 seconds) the pMP has completely recovered to a value even higher than the reference value.
The constant course of the pre-stimulus membrane potential pMP and the maximal amplitude h_{max} of the response during the sustained background illumination can mean either that the long-term light adaptation effects in our experiments are not due to changes of the membrane potential, or that the membrane potential is changed only in specific areas (e.g. in the rhabdomeric region). In the latter case our measurement of the overall membrane potential would not show the relevant difference. A further possibility would be that only the sodium

gradient determining the ReP is noticeably changed during adaptation. Also in this case the pre-stimulus membrane potential in the dark which is mainly dominated by the unchanged potassium gradient would remain on a constant level.

In this respect our results tend into the same direction as those of Fein and DeVoe (1973) but while they assume that adaptation in the *Limulus* visual cell is wholly independent of the membrane potential our interpretation is restrained to the statement that the membrane potential is at least not the dominating cause of the long-term adaptation effects observed.

The fast repolarization of the pre-stimulus membrane potential in the dark may be partly due to increased activity of, perhaps electrogenic, ionic pump mechanisms which are stimulated by the intense background light and are sufficient to counteract the reduction of the ionic gradients mainly in the second phase (after 30 seconds) of the light adaptation period.
Electrogenic pump activity is also suggested by the phenomenon that an increase of the stimulus frequency (Fig. 20), which is actually a weak increase in light adaptation, resulted in a hyperpolarization of the membrane potential. This interpretation was also used by Millecchia (1969) to explain the results of similar experiments in *Limulus*.
Presumably ionic pump mechanisms are already very active during the light stimulus. After the end of the stimulus the pump becomes electrogenic for some time, or the electrogenicity becomes measurable because of increasing membrane resistance since the electrogenic pump has a relatively large internal resistance.
Obviously the activity of the electrogenic pump leading to the hyperpolarization of the membrane potential following increased stimulus frequency is different in different preparations.

The changes of the amplitude parameters of the RePs recorded during light and dark adaptation may yield some clues for the understanding of the long-term adaptation processes (see Fig. 14).

The only major change registered during the long-term light-adaptation is the continuous reduction of the shape-quotient (see Fig. 15). The reason for this is that the plateau-value h_e, following an initial reduction caused by switching on the light,

slightly increases again during the sustainment of the background illumination. This may be due to the restoration of ion reservoirs by the light-induced activity increase of ionic pump mechanisms. As shown by Stieve et al. (1972) the plateau-value is more influenced by active transport than the maximal amplitude h_{max}.
After switching off the background light the plateau value immediately rises to a value higher than its reference value but then decreases steadily during the dark adaptation period. This long-term decrease may be more easily understandable on the basis of certain assumptions concerning the processes leading to the formation of the plateau-value.

A light flash induces an influx of sodium into the cell which causes the maximal amplitude of the ReP. Presumable there are several factors responsible for the amplitude reduction toward the plateau-value.
Lisman and Brown (1972) found that in the visual cell of Limulus increased sodium concentration leads to a release of calcium at the inner surface of the visual cell membrane which results in a decrease of the conductance of the visual cell membrane and at the same time in a reduction of the ReP amplitude to the level of the plateau-value. Actually this means that the amplification factor for the effect of the incident light is altered while the light intensity remains unchanged.
According to a hypothetical assumption of Brown and Blinks (1974) this release of calcium plays a decisive role in light adaptation processes in the *Limulus* photoreceptor.
Another factor determining the plateau-value is ionic transport activated by high sodium concentration in the cell. Owing to the increased conductance of the membrane the electrogenic effect of the pump is very small during the sustained background illumination but the pump activity is still sufficient to prevent the ionic gradients to be much reduced. During the long-term light adaptation the activity of the ionic transport mechanisms possibly rises steadily. This could be the reason for the increase of the plateau-value in the course of the light adaptation, as the ionic gradient becomes less reduced from

flash to flash due to the increase of active (not electrogenic) pump activity.

While the increase of the plateau-value during the sustained background light does not correlate with the course of the pre-stimulus membrane potential pMP (Fig. 13) a comparison of the plot of the pMP with those of the plateau-value and after--potential (Fig. 14) during the dark adaptation suggests that these parameters are more or less determined by the same process, probably the restoration of the pre-stimulus membrane potential by active transport mechanisms.

The increase of the maximal amplitude during the first two minutes of the dark adaptation is not parallel to the course of the other amplitude parameters and the pre-stimulus membrane potential but seems to be caused by a change in amplification which will be discussed later.

A very intriguing result of the adaptation experiments is the fact that the after-potential h_a was negative during the light adaptation in all cases observed. This phenomenon is not easily understandable. Its biological significance could be an amplifying "light-off" information for the eye intensifying the contrast between the superimposed light flash and the strong background light. Kirschfeld (1961) described responses to dark stimuli - realised by lack of light stimulation - in the ommatidia of several beetle species.

The reaction mechanism of the whole visual cell should be taken into consideration in an attempt to explain the hyperpolarizing after-potential during the light adaptation.
The decrease of the ReP after the end of the stimulus normally has at least two phases with different time constants, a fast steep decrease (which coincides with a membrane resistance increase) and a slower approach toward the final level of the membrane potential. The negative after-potential which occurs approximately at that time after the end of the stimulus at which the first decrease phase would be normally finished might be caused by one of the following mechanisms.

(1) The permeability to potassium ions producing the decrease of the ReP after the stimulus could be relatively greater immediately after the flash tham some time later. This would result in a greater tendency toward the equilibrium potential for potassium immediately after the flash than during the steady state. Such an effect should be the more marked the more the membrane potential differs from the equilibrium potential for potassium. Consequently the negative after--potential should then have also occurred when the cell was depolarized by current injection. This was not the case. Furthermore there is not evidence for increased potassium permeability in the final phase of the ReP (Stieve and Malinowska 1973).

(2) Active electrogenic transport could be responsible for the hyperpolarizing after-potential. This possibility is improbable because the transient hyperpolarization occurs too fast after the stimulating flash and also does not last long enough to be the result of electrogenic pump activity (Erler 1972).

(3) As the negative after-potential was regularly only recorded when the eye was intensely light adapted (during the period of background illumination) the attempt seems justified to explain it by means of specific adaptation effects. The visual cell is light adapted (by the background illumination) already before the flash. During the superimposed flash the extent of light adaptation is even more increased. Immediately after the flash the sensitivity of the photoreceptor is still temporarily reduced below the state before the flash. A short time later the sensitivity increases again according to the level determined by the background light alone.
While this description certainly holds true for the changes in adaptation in a visual cell submitted to sustained background light with superimposed flashes there is no definite proof whether this effect is sufficient to explain the negative after-potential observed in our experiments.

In this context it may be interesting that Kramer (1975) in the description of a mathematical model of the ReP of *Limulus* suggests a negative after--potential which is explained by a change in the level of light adaptation.

Although part of the observed adaptation effects can be explained by active transport and other factors the processes controlling the main sensitivity changes in the *Limulus* photoreceptor cell during light and dark adaptation are not yet understood. Obviously the adaptative light effects an amplification change, i.e. a change of the conductance increase per photo-stereo-isomerization which determines the extent of the depolarization.

The existence of a mechanism which could explain the change in amplitude during the light stimulus from the maximal amplitude to the plateau-value was suggested by Lisman and Brown (1972), as mentioned before.

There are several hypothetical assumptions concerning the realisation of an amplification factor for the mechanisms causing the ReP,which is changed during adaptation.
One possibility would be that the conductance changes determining the size of the ReP are triggered by light in the way of a snowball effect, e.g. by secondary substances produced in the photochemical cycle. The extent of this snowball effect could be varied according to the state of adaptation.
Another possibility would lie in the existence of a cooperative effect between rhodopsin molecules. In this case a change of the amplification factor could mean that e.g. the number of rhodopsin molecules forming functional units is altered during adaptation.

In the already mentioned mathematical approximation to the size and time course of the ReP of *Limulus* which was developed by Kramer (1975) the initial amplitude increase of the ReP during the latent period can be satisfactorily described only by a snowball or cooperative effect.

Since there is no experimental evidence for cooperative interaction between rhodopsin molecules the explanation of an amplification mechanism by means of a snowball system seems to be more probable.

5.4 Final remarks

The results of our experiments in the photoreceptor of *Limulus* show several interesting aspects, particularly in the field of

adaptation. On the whole they are consistent with the basic concept of the electrophysiological processes in the invertebrate visual cell outlined in the discussion.
The question whether and how this concept is realised centralizes on structure and function of the visual cell membrane.
The correlation between the membrane resistance and the magnitude of the ReP which was shown in our measurements of the relative changes of the membrane resistance at different light intensities can be explained most plausibly on the basis of the channel hypothesis (assuming in the simplest case "light" (sodium) channels opened by light determining the ReP and permanently "dark" (potassium) channels responsible for the stationary dark potential) together with the respective ion batteries and ionic transport mechanisms.
Active ionic transport across the visual cell membrane serves to restore the ionic gradients necessary to keep the cell excitable.
According to our results light-induced ionic pump activity across the visual cell membrane and fast pigment regeneration contributes, additionally to the decisive amplification control and other factors, to a fast (as compared to other arthropod species) restoration of the sensitivity at the transition from background light to darkness.
Owing to the fast course of the pigment adaptation the effects of the background illumination are completely reversible.
While the main causes governing the long-term membrane adaptation in the *Limulus* photoreceptor cell can be understood only in terms of amplification control active transport phenomena apparently play a role in both light and dark adaptation. Some of our findings, e.g. the hyperpolarization of the membrane potential immediately after the end of the background illumination and the after-hyperpolarization of the ReP in the dark caused by the increase of moderate light adaptation, indicate electrogenic pump activity.

Judging from our results the processes governing the ReP occur approximately in the following way in the visual cell of *Limulus*.

Upon the absorption of a photon by a rhodopsin molecule one reaction step of the photochemical cycle induces a conductance increase in the visual cell membrane. The extent of this conductance change is controlled by an amplification which is probably realised by means of a snowball reaction or a cooperative effect. Presumably this amplification governing the magnitude of the depolarization by determining mainly the conductance change of the membrane is already reduced during the light stimulus, which is the main cause for the decrease toward the plateau-value.

The major part of the sensitivity decrease during light adaptation is due to the fact that sustained background illumination leads to a reduction of the amplification factor controlling the conductance increase causing the ReP.

Active transport is an important factor in the adaptation phenomena observed. Probably the ionic gradients for sodium and potassium, which are presumably reduced during the first 30 seconds after turning on the background light, remain relatively unchanged on this reduced level during the sustained background illumination owing to active transport. In the first phase of the dark adaptation the ionic gradients are probably still somewhat reduced as compared to the normal values in the dark. As soon as the background light is turned off the membrane resistance increases again toward its dark value. At the same time the amplification factor for the conductance to sodium (which in a simplifying assumption mainly determines the magnitude of the ReP) increases again owing to the changed state of adaptation. During this initial phase of dark adaptation the pump mechanism transiently becomes noticeably electrogenic (owing to increased membrane resistance), which leads to the hyperpolarization of the membrane potential after turning off the background light, until its activity is reduced again according to the normal conditions in the dark.

6. References

Benolken, R.M.
Reversal of photoreceptor polarity recorded during the graded receptor potential response to light in the eye of *Limulus*.
Biophys. J. 1, 551-564 (1961)

Benolken, R.M.
Effects of light- and dark-adaptation processes on the generator potential of the *Limulus* eye.
Vision Res. 2, 103-124 (1962)

Borsellino, A., M.G.F. Fuortes, and T.G. Smith
Visual responses in *Limulus*.
Cold Spring Harb. Symp. Quant. Biol. Vol. XXX, 429-443 (1965)

Brown, J.E. and J.R. Blinks
Changes in intracellular free calcium concentration during illumination of invertebrate photoreceptors. Detection with aequorin.
J. gen. Physiol. 64, 643-665 (1974)

Dowling, J.E. and H. Ripps
Visual adaptation in the retina of the skate.
J. gen. Physiol. 56, 491-520 (1970)

Erler, G.
Untersuchungen am lateralen Photorezeptor von *Balanus*: Wirkung von Pharmaka auf die Zellmembran. Lichtabhängige Reaktion.
Dissertation, T.H. Aachen 1972

Fein, A. and R.A. Cone
Limulus rhodopsin: Rapid return of transient intermediates to the thermally stable state.
Science 182, 495-496 (1973)

Fein, A. and R.D. DeVoe
Adaptation in the ventral eye of *Limulus* is functionally independent of the photochemical cycle, membrane potential, and membrane resistance.
J. gen. Physiol. 61, 273-289 (1973)

Fioravanti, R. and M.G.F. Fuortes
Analysis of responses in visual cells of the leech.
J. Physiol. (Lond.) 227, 173-194 (1972)

Frank, K. and M.G.F. Fuortes
Stimulation of spinal motoneurones with intracellular electrodes.
J. Physiol. (Lond.) 134, 451-470 (1956)

Fuortes, M.G.F.
Initiation of impulses in visual cells of *Limulus*.
J. Physiol. (Lond.) 148, 14-28 (1959)

Fuortes, M.G.F. and A.L. Hodgkin
Changes in times cale and sensitivity in the ommatidia of *Limulus*.
J. Physiol. (Lond.) 172, 239-263 (1964)

Hamdorf, K. and G. Rosner
Adaptation und Photoregeneration im Fliegenauge.
J. comp. Physiol. 86, 281-292 (1973)

Hartline, H.K., H.G. Wagner, and E.F. MacNichol
The peripheral origin of nervous activity in the visual system.
Cold Spring Harb. Symp. Quant. Biol. 17, 125-141 (1952)

Hille, B.
Pharmacological modofications of the sodium channels of frog nerve.
J. gen. Physiol. 51, 199-219 (1968)

Kirschfeld, K.
Quantitative Beziehungen zwischen Lichtreiz und monophasischem Elektroretinogramm bei Rüsselkäfern.
Z. vergl. Physiol. 44, 371-413 (1961)

Kramer, L.
Interpretation of invertebrate photoreceptor potentials in terms of a quantitative model.
Biophys. Struct. Mechanism 1 (1975, in press)

Lisman, J.E. and J.E. Brown
The effects of intracellular iontophoretic injection of calcium and sodium ions on the light response of *Limulus* ventral photoreceptors.
J. gen. Physiol. 59, 701-719 (1972)

Mack Brown, H., S. Hagiwara, H. Koike, and R.W. Meech
Membrane properties of a barnacle photoreceptor examined by the voltage clamp technique.
J. Physiol. (Lond.) 208, 385-413 (1970)

Millecchia, R.
The ventral photoreceptor cells of *Limulus*: An electrophysiological study.
Ph.D. Thesis, The Rockefeller University N.Y. (1969)

Millecchia, R. and A. Mauro
The ventral photoreceptor cells of *Limulus*. III. A voltage clamp study.
J. gen. Physiol. 54, 331-351 (1969)

Naka, K.J. and W.A. Rushton
S-potentials from color units in the retina of fish (*Cyprinidae*).
J. Physiol. (Lond.) 185, 536-555 (1966)

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

Rosner, G.
Korrelation zwischen Photoregeneration und Stoffwechselgröße beim Photorezeptor.
Dissertation, Ruhr-Universität Bochum (1973)

Rushton, W.A.H.
Physical measurement of cone pigment in the living human eye.
Nature 179 (4559), 571-573 (1957)

Stieve, H.
Elektrophysiologische Untersuchungen des Auges von *Eupagurus bernhardus* L.
Helgol. Wiss. Meeresunters. 7 (4), 149-188 (1960)

Stieve, H.
Veränderungen des Belichtungspotentials der Retina des Einsiedlerkrebses (*Eupagurus bernhardus* L.) bei Vergiftung des Stoffwechsels und bei Adaptation.
Z. vergl. Physiol. 47, 17-38 (1963)

Stieve, H. Interpretation of the generator potential in terms of ionic processes.
Cold Spring Harb. Symp. Quant. Biol. Vol. XXX, 451-456 (1965)

Stieve, H.
On the ionic mechanisms responsible for the generation of the electrical response of light sensitive cells.
In: Biochemistry of Sensory Functions (L. Jaenicke, ed.)
pp. 79-105. Berlin, Heidelberg, New York: Springer-Verlag (1974)

Stieve, H., H. Gaube, and T. Malinowska
The effect of some sodium substitutes on the receptor potential of the crayfish photoreceptor cell.
Z. Naturforsch. 27b, 1535-1546 (1972)

Stieve, H., H. Gaube, E. Hildebrand, and P. Thomsen
Long-term adaptation by different monochromatic light in the photoreceptors of three crustacean species.
Vision Res. 13, 1403-1414 (1973)

Stieve, H. and T. Malinowska
The effect of tetrodotoxin, veratridine, and tetraethylammonium chloride on the receptor potential of the crayfish photoreceptor cells.
Z. Naturforsch. 28c, 149-156 (1973)

Walther, J.B.
Untersuchungen über die Erregungsvorgänge einzelner Lichtsinneszellen in den Augen des Blutegels *Hirudo medicinalis*.
Habilitationsschrift, Göttingen 1965

Wulff, V.J., H. Stieve, J.L. Fahy
Dark adaptation and sodium activity in *Limulus* lateral eye retinular cell.
Vision Res. (in print)