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The Correlation of the Receptor Potential with the Light Induced Transient Increase in Intracellular Calcium-Concentration Measured by Absorption Change of Arsenazo III Injected into *Limulus* Ventral Nerve Photoreceptor Cell

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Abstract. The light-induced membrane voltage response (receptor potential, ReP) and the absorption change of the intracellularly injected calcium indicator arsenazo III (arsenazo response) were recorded simultaneously in *Limulus* ventral nerve photoreceptor cells. A double pulse technique was applied for stimulation. After pressure injection of the indicator into the cell absorption changes were measured at 646 nm to obtain a measure of the changes of the intracellular calcium ion concentration.

1. The size of the arsenazo response increases with increasing intensity of the light stimulus. The intensity dependence of the size of the arsenazo response ΔA_{max} shows almost no correlate with the peak amplitude of the ReP, but correlates rather well with the time integral of the ReP.

2. Decreasing light adaptation (caused by prolongation of the repetition time of the pulse pairs) leads to an increase in size of the arsenazo response. Also here ΔA_{max} correlates better with the time integral of the ReP than with its peak amplitude.

3. Lowering the calcium concentration in the superfusate (from 10 mmol/l to ca. 40 μmol/l) causes after ca. 10 min a drastical diminution of the arsenazo response to values below the noise level, and a less marked reduction in size of the ReP. The falling phase of the ReP is slower. After return to normal calcium concentration the arsenazo response recovers partly in ca. 50 min, while the ReP recovers faster.

The results show two opposite correlations between ReP and arsenazo response:

Increase in size and duration of the ReP causes a greater transient increase of the intracellular calcium ion concentration. This in turn tends to reduce and shorten the ReP. Which effect dominates obviously depends on the conditions of the experiment: when the calcium concentration in the superfusate is reduced to ca. 40 μmol/l a slow decrease of the ReP is accompanied by a small increase of the intracellular calcium ion concentration.

Key words: Receptor potential – Intracellular and extracellular calcium concentration – Intensity dependence – Adaptation – Sensitivity control.

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Introduction

As shown by Brown and Blinks (1974) the light response of *Limulus* and *Balanus* photoreceptors is accompanied by a transient increase in intracellular calcium ion concentration, measured by intracellulary injected aequorin.

Brown et al. (1977) have shown that also the intracellularly injected indicator arsenazo III can be used for measuring transient changes of the intracellular calcium ion concentration in the *Limulus* ventral nerve photoreceptor. As this transient increase in $[Ca^{2+}]_i$ plays an important role in regulating the state of light adaptation according to Lisman and Brown (1972, 1975a, b) and Fein and Charlton (1977), the time course and the size of the light induced change in intracellular calcium are of great interest.

The time course of the aequorin response and its dependence on membrane voltage, stimulus intensity and of state of adaptation, and the influence of extracellular calcium and magnesium was investigated by Brown and Blinks (1974). As the measurements were made with intracellularly injected aequorin, a quantitative interpretation is difficult (Brown et al. 1977). Moreover, aequorin is a very large, not freely diffusible molecule, and the kinetics of the luminescent reactions are slow. Therefore Brown et al. (1977) used intracellularly injected arsenazo III, to confirm the light-induced increase in $[Ca^{2+}]_i$, and measured how the arsenazo response as well as the receptor potential depend on the concentration of the intracellular dye.

Their results showed a transient increase of the free $[Ca^{2+}]_i$ followed by a decrease even during sustained illumination. In the *Limulus* ventral nerve photoreceptor a major part of the light-induced calcium increase did not disappear in a calcium-free saline with 5 mmol/l EDTA (Brown and Blinks 1974).

The half time of the calcium-arsenazo complexation reaction is reported to be under 3 ms (Scarpa et al. 1978; Brown et al. 1975).

In our experiments we use a double flash technique to compare the light-induced arsenazo absorption change as a measure of the change of the $[Ca^{2+}]_i$, in correlation to the simultaneously measured light induced change in membrane voltage, the receptor potential (ReP). We measured the effect of changing the intensity of the light flash, or the state of light adaptation, or lowering the calcium ion concentration of the bathing solution with which the ventral nerve was superfused.

The results show a strong correlation between the shape of the ReP and the size of the arsenazo response, or $[Ca^{2+}]_i$ response, when light intensity or state of light adaptation were changed. The reduction of the external Ca^{2+}-concentration was followed by a reversible disappearance of the arsenazo response, and a marked slowing down of the falling phase of the ReP.

Material and Methods

Limulus ventral nerves were excised, desheathed, and treated with 0.5 pronase for 45 s (according to Millecchia and Mauro 1969). The nerve was fixed on a piece of Sylgard and impaled with a single electrode filled with 0.5 mol/l KCl containing 2.5 mmol/l arsenazo III. The tip of the electrodes was smaller than 1 μm. The electrode resistance was 2–3 MΩ. Arsenazo III was prepared and purified according to Kendrick (1976). By pressure moderate amounts of this solution could be injected into the cell.

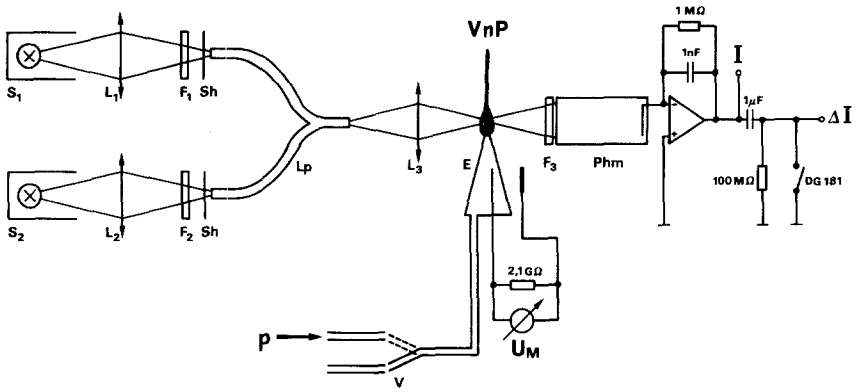

Fig. 1. Schematic drawing of the experimental set-up. Two light sources, S_1 and S_2, a quartz-iodine lamp, and a mercury high pressure lamp were used. The lenses L_1 and L_2 focus the light in UV-light pipes (L_p) of 1 mm diameter. The filters F_1, F_2 and the electro-mechanical shutters Sh define wavelength, intensity and length of the light pulses. L_3, a microscope objective, focusses the light in a spot of less than 50 µm diameter on the ventral nerve photoreceptor, VnP. Filter F_3 separates stimulus and measuring light; the measuring light is measured by the photomultiplier Phm. The micropipette E is used for the intracellular measuring of the membrane voltage U_M and for injection of arsenazo III. Injection by pressure is possible by switching the valve v

The piece of Sylgard with the nerve was mounted in an experimental chamber which was constantly superfused with physiological saline (PS) of a temperature close to 21° C. The constitution of the saline was 488.3 mmol/l Na^+, 10 mmol/l K^+, 55 mmol/l Mg^{2+}, 10 mmol/l Ca^{2+}, 566 mmol/l Cl^-, 30 mmol/l SO_4^{2-}, 2.3 mmol/l HCO_3^-, 0.1 mmol/l PO_4^{3-}, and 10 mmol/l glucose. In the third group of experiments described in this paper the perfusion of the chamber was switched, after an initial period in PS, to a low calcium saline which contained only ca. 40 µmol/l calcium, but the normal amount of magnesium ions. The osmotic pressure was kept constant by the addition of a corresponding amount of NaCl for the omitted $CaCl_2$. The actual calcium concentration of this low calcium saline was $\leq$ 40 µmol/l.

The experimental set-up is shown in Fig. 1. The membrane potential (U_M) and its light induced changes were recorded in the usual way. Only the cell tested was illuminated, the rest of the ventral nerve was kept dark. The measuring light came from a quartz-iodine lamp of 15 V, 150 W (Osram) with an Oltronix power supply B32–10R, filtered with an interference filter of λ_{max} 646 nm and a half width of 10 nm (Schott). The stimulus light came from a mercury high pressure lamp HBO 100/2 (Osram) and was filtered with a BG 12 and a DT-blue filter (Schott and Balzers). The wave-lengths were sometimes restricted by a cut-off filter OG 550 or by an interference filter of 361 nm (Schott). Sometimes the light of the second lamp was used as background light while the other lamp was used for the pulsed stimulus light. In front of the photomultiplier tube a RG 610 cut-off filter (Schott) and an interference filter of λ_{max} 645 nm and a half width of 5,3 nm (Anders) separated the measuring light from the stimulus light. The photomultiplier tube (EMI) allowed to measure the intensity of the measuring light, **I**, and the variation of this intensity, ΔI, (between 0.002 Hz and 30 Hz).

During a preperiod the cell was tested with 50 ms white light pulses, repeated every 5 s, later in the main part of the experiment the measuring light beam of 646 nm was constantly applied. The measuring light intensity was ca. $3 \cdot 10^{16}$ photons $cm^{-2}s^{-1}$ of 540 nm (maximal sensitivity of *Limulus*), which excited the photoreceptor cell to a certain steady degree, i.e., the potential increase from the dark potential of -40 ± 11 mV to a potential of -36 ± 10 mV. According to Brown et al. (1977) the absorption changes of arsenazo III at this wavelength only reflect changes in the intracellular calcium concentration. Recently signal analysis studies have been made regarding the possible effects of Mg^{2+} and of interaction between Ca^{2+} and protons (Ahmed and Connor 1979). In preliminary control experiments (Maaz 1978) we found no changes in light scattering during light responses in arsenazo-free photoreceptor cells of *Limulus*.

After pressure injection of the dye the photoreceptor cell was stimulated by pairs of identical light flashes of 10 ms duration. The stimulus light was blue light with a maximum of 420 nm and a maximal intensity I_0 of $7.5 \cdot 10^{17}$ photons cm^{-2}/s^{-1} of 540 nm. The two flashes of the pair were 5 s apart. The pairs of flashes were repeated with a variable interval t_{rep} between 10 s and 45 s throughout the experiment. Normally the ReP and the arsenazo response were averaged by a signal averaging device (Datalab DL 4000) to improve the signal to noise ratio. Figure 2 shows the time schedule of stimulus and recording program.

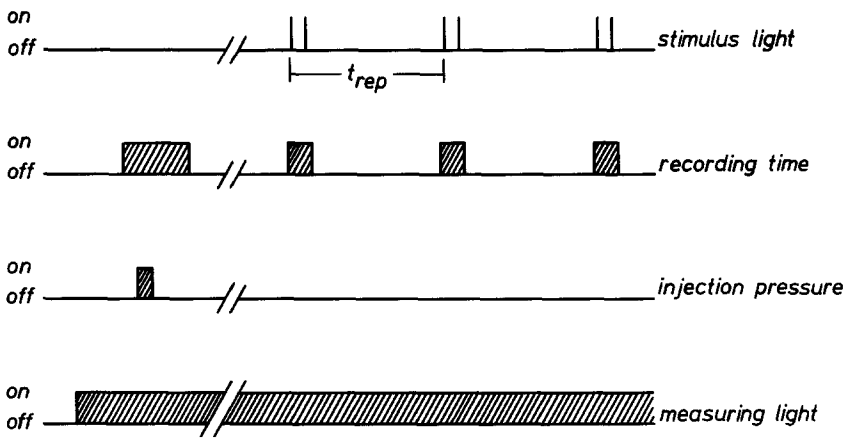

Fig. 2. Time schedule of the stimulus and recording program

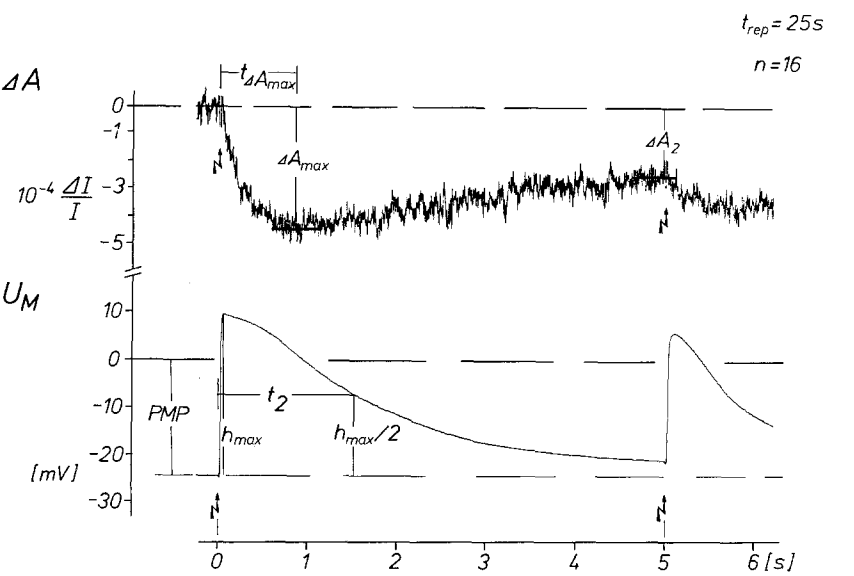

Fig. 3. Illustration of the measured parameters. The averaged measurements (ΔA) of the transmitted light of 646 nm, top trace, and the simultaneously measured membrane voltage (U_M), or receptor potential, lower trace, are recorded. The photoreceptor was stimulated by 50 ms pulse pairs, indicated by the bars and flashes, with a repetition time $t_{rep} = 25$ s; n = 16 measurements were averaged. In the recording of the transmitted light the dashed line indicates the light level existing prior to the first stimulus of a pulse pair. In the recording of the membrane voltage, U_M, the zero level and the PMP are indicated by the dashed lines

The apparatus was able to measure at least an intensity change of $I/I_0 = 2.5 \cdot 10^{-4}$ with a signal to noise ratio of S/N = 1 after 64 averages. The concentration of arsenazo III in the cells was estimated to be lower than 1 mmol/l.

From the recorded RePs (Fig. 3) we measured: the prestimulus membrane potential (PMP); the amplitude of the transient (h_{max}); the decrease time (t_2) which was needed for the decrease from the maximal to half the maximal height; and the time integral of the membrane voltage displacement (U_M) during the ReP, measured from the PMP [$\int(U_M\text{-PMP})dt$]. From the arsenazo response (Fig. 3) we measured: the maximal amplitude of the absorption changes of arsenazo (ΔA_{max}); the time from the beginning of the stimulus until the maximum of the arsenazo signal is reached ($t_{\Delta Amax}$); the time until half the maximum of the arsenazo signal is reached ($t_{\Delta Amax/2}$); and the difference between the level before the second stimulus and the level before the first stimulus (ΔA_2).

The indices 1 and 2 denote the response to the first or the second pulse of the pairs. More details are described in Maaz (1978).

Results

The moderate amounts of the arsenazo III containing KCl solution which were injected did not harm the preparation in so far as the response size and shape did not change much following the injection; the response was slightly attenuated and the decrease was prolonged.

In most cases pressure injection of moderate amounts of arsenazo was sufficient to induce absorption changes in the light path of the measuring light (Fig. 5). If more arsenazo was injected, the electrical response to light was changed in a similar way as when the external [Ca^{2+}] was lowered or the internal [Ca^{2+}] was buffered by calcium buffers like EGTA, i.e., the decrease of the ReP became slower (Fig. 4).

After injection of arsenazo the light absorption changes, while the membrane potential remains almost constant (Fig. 5, upper record). In typical averaged records of

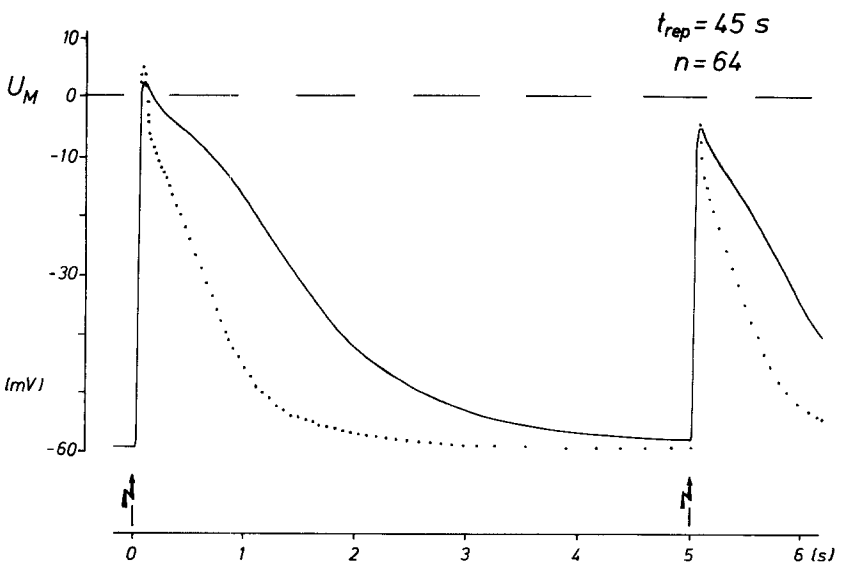

Fig. 4. Averaged RePs from 64 measurements to pulse pairs with t_{rep} = 45 s prior to a second pressure injection (dotted line) and after the injection under the same stimulus conditions (continuous line). The 10 ms light pulses are indicated by bars and flashes

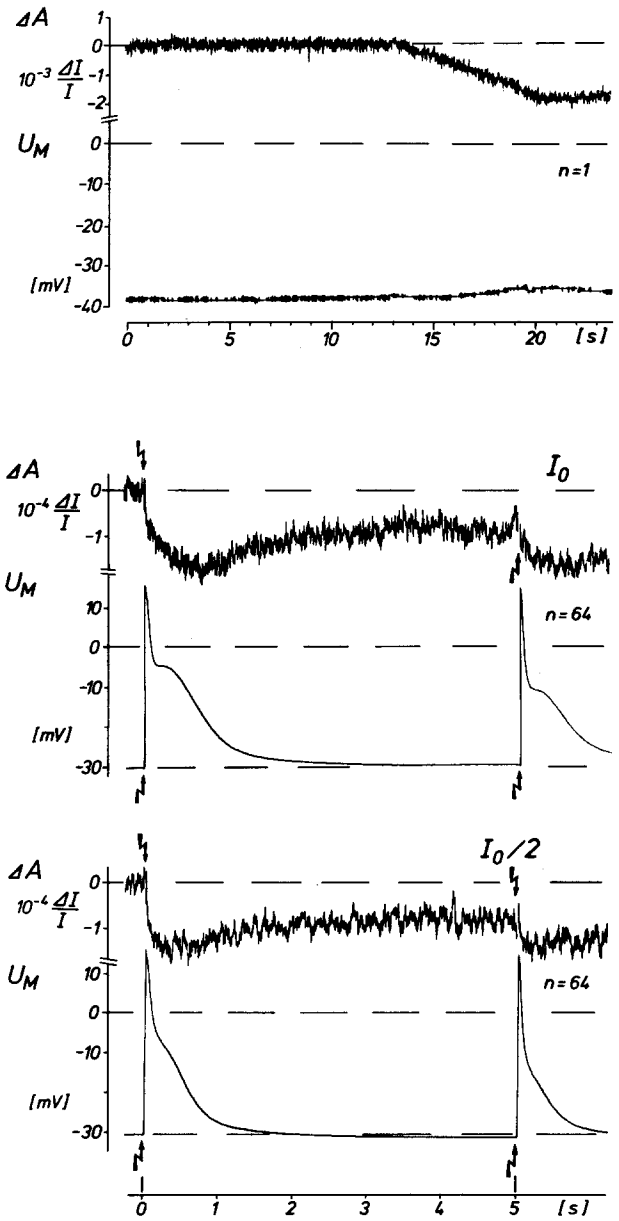

Fig. 5. During the injection of arsenazo III the intensity of the transmitted light of 646 nm (ΔA) and the simultaneously measured intracellular ReP (U_M) were recorded in the first plot. During injection the intensity of the transmitted light decreased. Afterwards in the second and third plot the cell was stimulated with pulse pairs. The plots of transmitted light intensity and membrane voltage were averaged from 64 measurements. The light intensity of the pulses (10 ms) was the maximal intensity I_0 (second plot) and $I_0/2$ (third plot). The repetition time was 45 s. (The small upward deflection before the second arrow in the light trace of the middle record is probably a flash artefact)

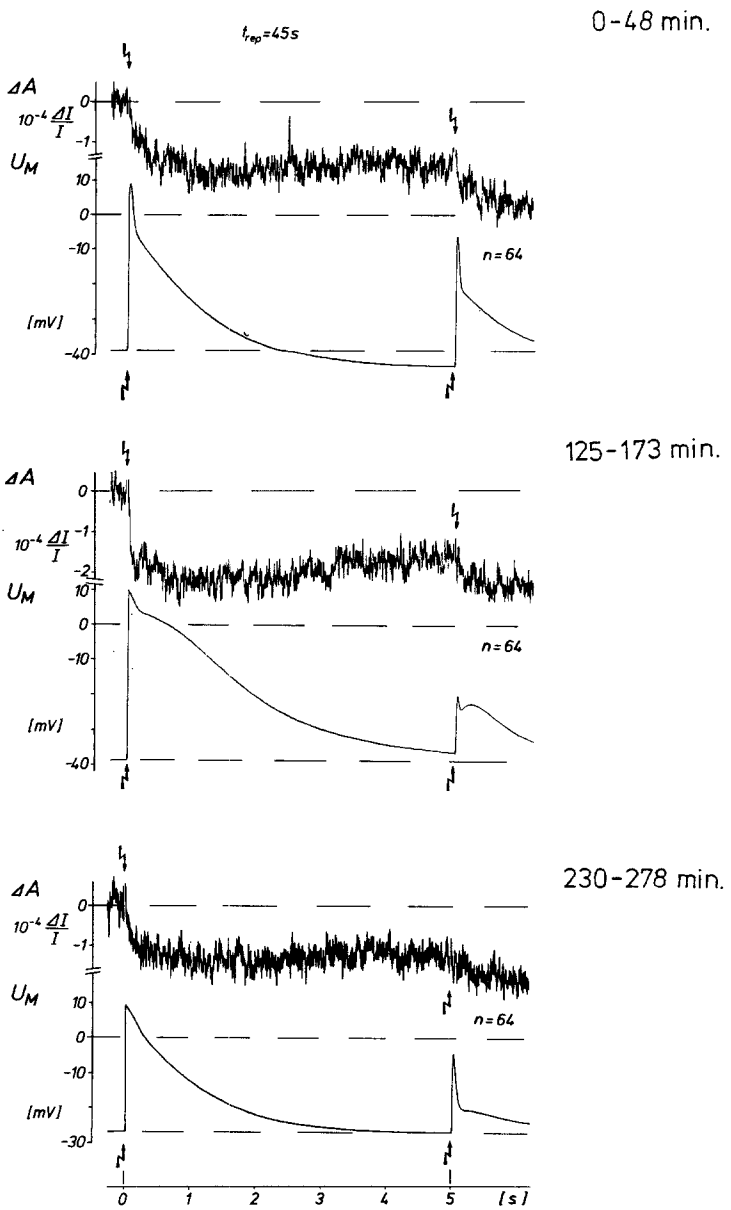

Fig. 6. Comparison of arsenazo response (ΔA) and ReP (U_M) at different times after injection of arsenazo III. The top figure was recorded immediately after the injection; the middle figure was recorded 125 min later and the bottom figure still 105 min later. The stimulus conditions were identical. The pairs of light pulses of maximal intensity were repeated every 45 s. The plots are averaged from 64 measurements. 10 ms light pulses

arsenazo responses and RePs to 10 ms light pulses (Fig. 5, for details see legend) the ReP to the first pulse of the pair shows a relative fast rise, a time-to-peak of nearly 50 ms, and a second shoulder or maximum almost 300 ms after the light stimulus. The ReP to the second pulse of the pair shows practically no reduction in the transient height but a faster decrease or a lower shoulder.

The Arsenazo signal induced by the first pulse of the pair has sometimes a fast component ($t_{1/2}$ ca. 20 ms) in the beginning but always a slow component with a time-to-peak of nearly 800 ms. The Arsenazo signal declines slowly and does not reach the original level within the five seconds after which the second pulse is delivered. There remains a difference ΔA_2 between the levels before the first pulse and the second pulse of the pair; the intracellular calcium concentration has not yet decreased to the original level as before the first flash. The second flash of the pair causes a much smaller arsenazo signal. The ReP however is not much changed in the height of the transient, but in the falling phase.

In the course of a longer experiment, when after the arsenazo injection the ventral nerve photoreceptor was stimulated continuously for a long period of time, the size and time course of both ReP and arsenazo signal may change (Fig. 6). After injection of large amounts of Arsenazo and after many stimulations both signals decline and may even disappear finally.

Receptor Potential and Arsenazo Response Depending Upon Different Intensities of the Stimulating Light

In six experiments the arsenazo response and the ReP were measured at different intensities of the light of the pulse pairs. The repetition time t_{rep} was kept constant (25 s).

Both, ReP and arsenazo response, depend on the intensity of the light stimuli (Fig. 7). With decreasing light intensity (compare the two top records in the left row) the arsenazo response vanishes in the noise, while small RePs are still evoked. With increasing light intensity the arsenazo response continues to increase gradually while the RePs have already reached nearly saturation height with a stimulus light intensity of $I_0/10$.

The amplitude of the arsenazo response, ΔA_{max}, to the first pulse of a pair (Fig. 8), increases with increasing stimulus intensity. The arsenazo response to the second pulse of the pair goes through a maximum. Its relative height in comparison with the amplitude of the response to the first stimulus becomes smaller. Also the time to reach the maximum of the arsenazo response $t_{\Delta Amax}$ generally increases with increasing stimulus intensity whereas the value of $t_{\Delta Amax/2}$ is not much changed. This means the initial steepness of the increase of the arsenazo signal remains unchanged. The ReP to the first pulse of a pair increases in height and in length with increasing light intensity and saturates at high intensities (Figs. 7, 8). The ReP of the second pulse of a pair goes through a maximum but decreases again with increasing light intensity (Figs. 7, 8) due to the light adaptation, caused by the first pulse.

With increasing light intensity the amplitude of the arsenazo response, ΔA_{max}, does not parallel the amplitude of the transient of the ReP, h_{max}, which saturates at much lower light intensities than the arsenazo response (Fig. 8). Also the relative values of the arsenazo response, $\Delta A_{max2}/\Delta A_{max1}$, change much more than the relative values of the

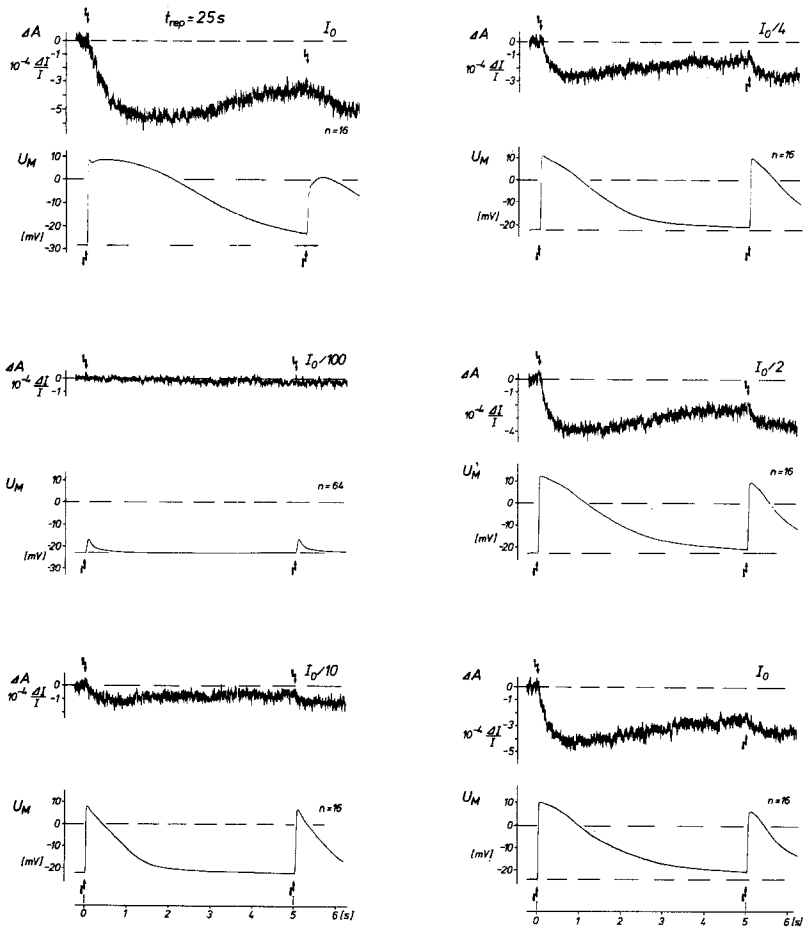

Fig. 7. Comparison of arsenazo response (ΔA) and ReP (U_M) in succeeding records when the light intensity was changed but the repetition time $t_{rep} = 25$ s was kept constant. Starting with the maximal intensity I_0 the intensity increased from $I_0/100$ to I_0 again. The plots were averaged from n measurements as indicated. 10 ms light pulses

ReP, h_{max2}/h_{max1}. But the amplitude of the arsenazo signals, ΔA_{max}, correlates much better with the decrease time t_2 of the RePs or the time integral of the ReP. A striking correlation is existing, too, between the time integral of the ReP and the value $\Delta A_{max} \cdot t_{\Delta Amax}$, an approximate measure for the time integral of the arsenazo signal. The increase of the value ΔA_2, caused by the increase of the stimulus light intensity, corresponds to a higher $[Ca^{2+}]_i$ level in the cell before the second stimulus in relation to the level before the first pulse of the pair. It is not correlated with h_{max}, or t_2 or the time integral of the second ReP.

The almost constant value of $\Delta A_2/\Delta A_{max1}$ when the light intensity is changed (also shown directly by the single curves of ΔA_2 and ΔA_{max1} in Fig. 8, which differ only by a scaling factor) means that the decrease of the $[Ca^{2+}]_i$ within a certain time is proportional to the height of the maximal increase of the $[Ca^{2+}]_i$. The decrease of the $[Ca^{2+}]_i$ could, e.g., be controlled by diffusion.

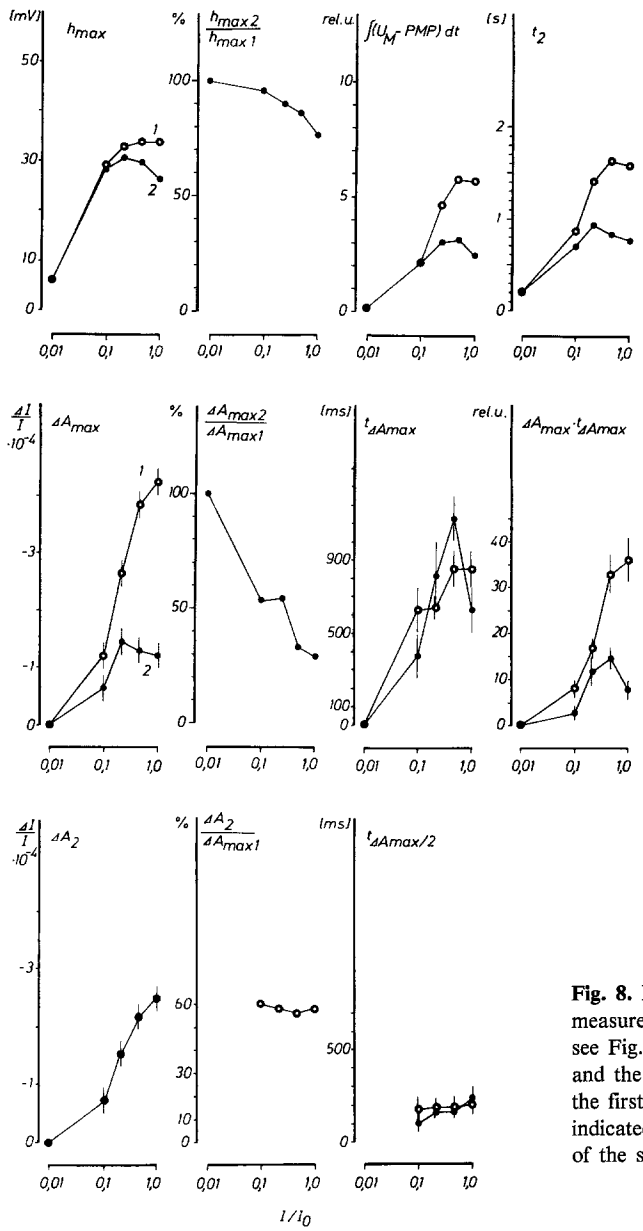

Fig. 8. Intensity dependence of the measured parameters (for explanation see Fig. 3) of the arsenazo responses and the RePs of Fig. 7. The values to the first pulse of the pulse pair are indicated by open circles, the values of the second pulse by solid circles

Receptor Potential and Arsenazo Response Depending Upon the State of Light Adaptation

We tested the effect of light adaptation in seven experiments by varying the repetition time, t_{rep}, of the pulse pairs between 10 s and 45 s, while the light intensity of the pulses was kept constant. The results of these experiments are shown in Figs. 9 and 10.

With decreasing light adaptation, i.e., increasing repetition time, the height of the arsenazo response to the first pulse of the pair increases much more than the height of

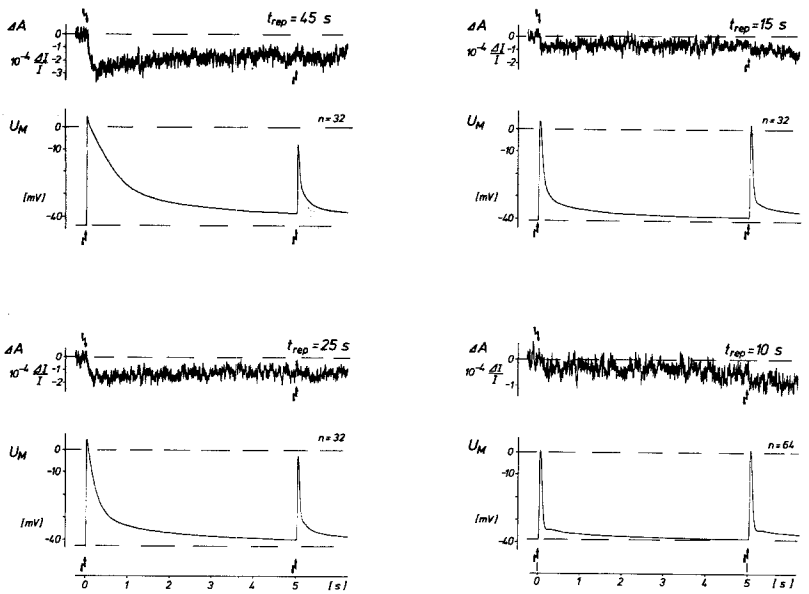

Fig. 9. Dependence of arsenazo response (ΔA) and ReP (U_M) on the state of adaptation. The state of adaptation was changed by variation of the repetition time t_{rep} of the pulse pairs. The stimulus light intensity was maximal. The number of measurements averaged for the records is indicated by n. 10 ms light pulses

the corresponding ReP. The amplitude h_{max} of the ReP is only slightly enlarged, the decay or the decrease-time t_2 of the ReP is considerably prolonged (Fig. 10). Again the correlation of the height of the ReP, h_{max}, with the height of the arsenazo response, ΔA_{max}, is poor. With increasing repetition time h_{max} saturates while ΔA_{max} keeps rising. Also again the correlation of the amplitude of the arsenazo response ΔA_{max} with the decrease-time t_2 of the ReP and with the time integral of the membrane voltage displacement during the ReP is relatively good. There is also a very good agreement between the time integral or t_2 and the product $\Delta A_{max} \cdot t_{\Delta Amax}$ (Fig. 10).

The prolongation of the repetition time, t_{rep}, also influences the arsenazo response and the ReP to the second pulse of the pair. The arsenazo response to the second pulse as compared with the response to the first pulse is in all cases much more diminished than the ReP to the second pulse. This is clearly shown by a comparison of the curves of h_{max2}/h_{max1} and $\Delta A_{max2}/\Delta A_{max1}$ (Fig. 10). They show again that ΔA_{max2} as well as the relative values $\Delta A_{max2}/\Delta A_{max1}$ do not parallel h_{max2}/h_{max1}. There is also no correlation of $\Delta A_{max2}/\Delta A_{max1}$ with the decrease-time t_2 or the time integral of the second ReP. But the values A_{max} or $\Delta A_{max} \cdot t_{\Delta Amax}$ are correlated with the decrease-time t_2 or the time-integral of the RePs corresponding both to the first and the second pulse.

Since the arsenazo response has not declined to zero in the 5 s time interval between the two pulses of the pair the level of the $[Ca^{2+}]_i$ before the second pulse should influence the height of the second ReP. Figures 9 and 10 show that as ΔA_2 is becoming larger h_{max2} is becoming smaller and the difference between the heights of the two RePs of the pair is becoming greater too. However decrease-time t_2 and the time integral of the ReP to the second pulse are almost constant and do not correlate with ΔA_2.

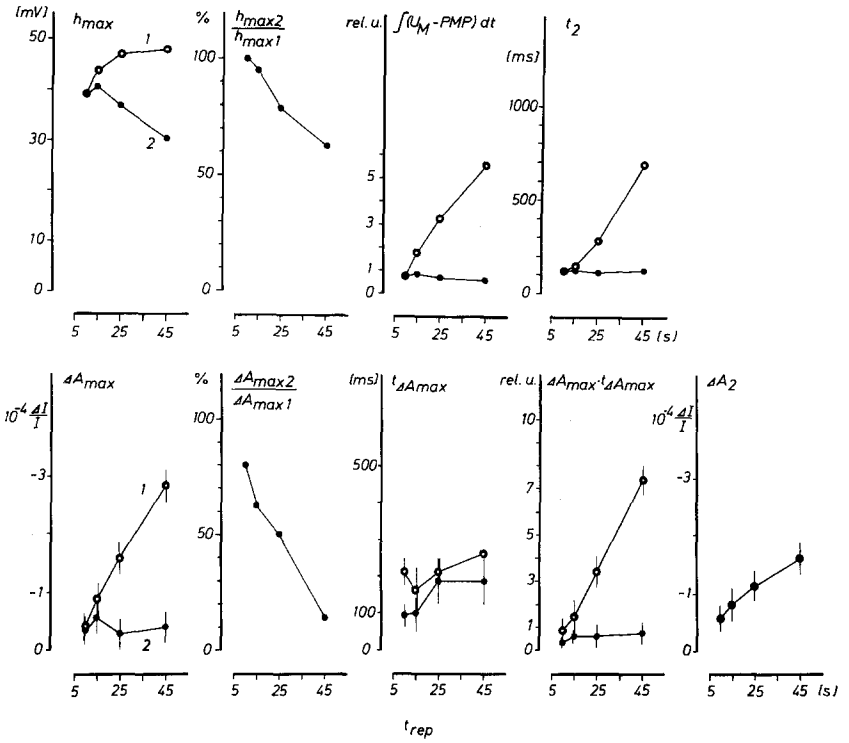

Fig. 10. Dependence of the measured parameters (see Fig. 3) of the arsenazo response (ΔA) and the ReP (U_M) on the state of adaptation. (Same experiment as Fig. 9). The values to the first pulse of the pulse pair are the open circles, the values to the second pulse are the solid circles

Receptor Potential and Arsenazo Response at Different External Calcium Concentrations

In six experiments the physiological saline (10 mmol/l Ca^{2+}) superfusing the ventral nerve was replaced by a saline with no added calcium but a normal concentration of magnesium ions. The calcium ion concentration at the photoreceptor became smaller than 40 μmol/l. The results are shown in Figs. 11 and 12. The values are averages recorded during periods of 6 to 40 min as indicated.

In the low calcium saline (Fig. 11, left column) the arsenazo response becomes smaller and slower. In the last record of this column it is diminished to a level where it is no longer recognizable (after 18 to 24 min stay in the low calcium saline). At the same time the ReP shows the usual change in low calcium saline; prolongation of t_2, diminution of h_{max} and PMP (Figs. 11 and 12). In the first 6 min after the switch to low calcium the ReP is slightly prolonged and its shoulder is higher. After 18–24 min after the switch to the low calcium saline the ReP to the first pulse of the pair is reduced to ca 50% (as compared to the value in normal calcium), the fast initial peak is lost, the decrease is very much slowed down. The ReP to the second flash of the pair is still more depressed, and instead of the fast initial peak a small hyperpolarisation (probably the early receptor potential, ERP) is observed.

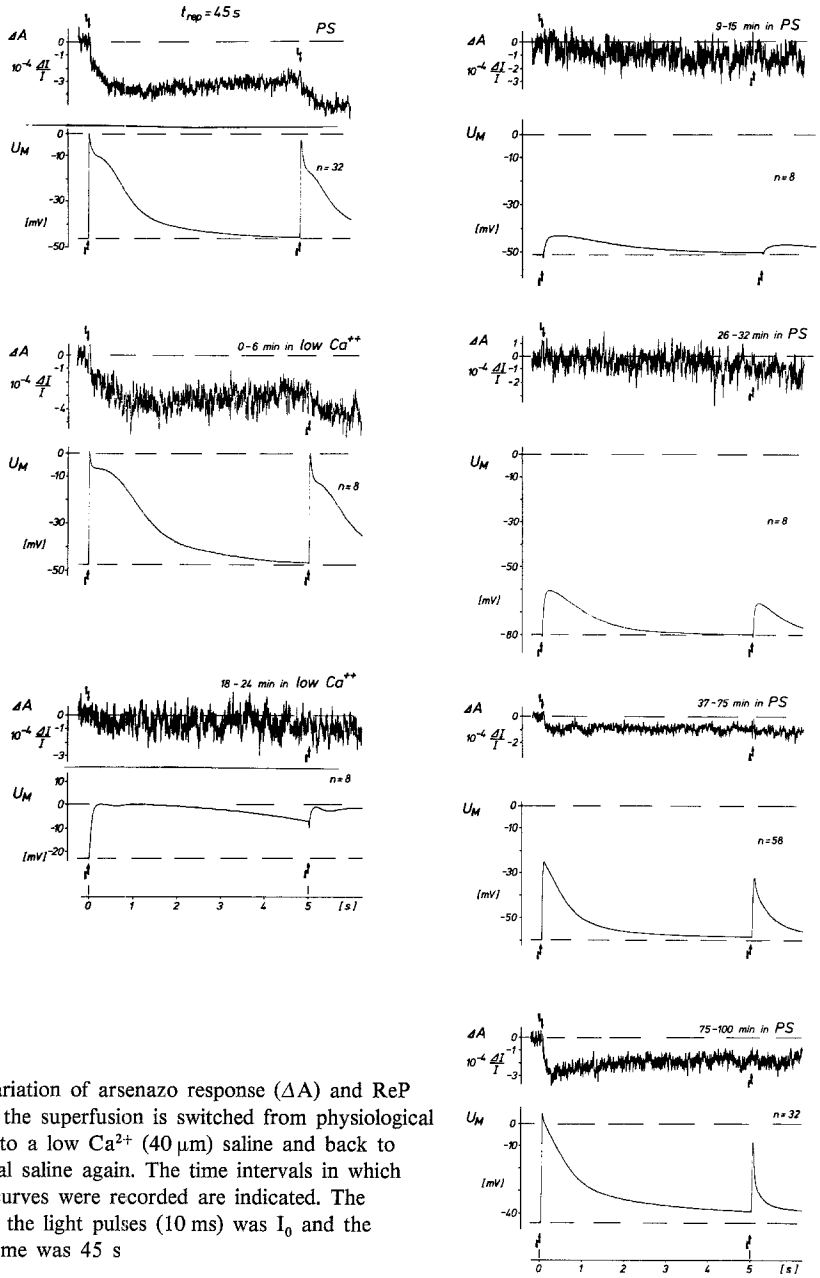

Fig. 11. Variation of arsenazo response (ΔA) and ReP (U_M) when the superfusion is switched from physiological saline, PS, to a low Ca^{2+} (40 μm) saline and back to physiological saline again. The time intervals in which the single curves were recorded are indicated. The intensity of the light pulses (10 ms) was I_0 and the repetition time was 45 s

After return into physiological saline (Fig. 11, right column) the arsenazo response remains undetectable for more than half an hour and recovers only in the time span of 37—75 min after the exchange of the salines. In the last record of Fig. 11 (75—100 min in Ps) the arsenazo response has recovered almost to its original value. In the time span of 9 to 15 min after return to physiological saline the pre-stimulus membrane potential,

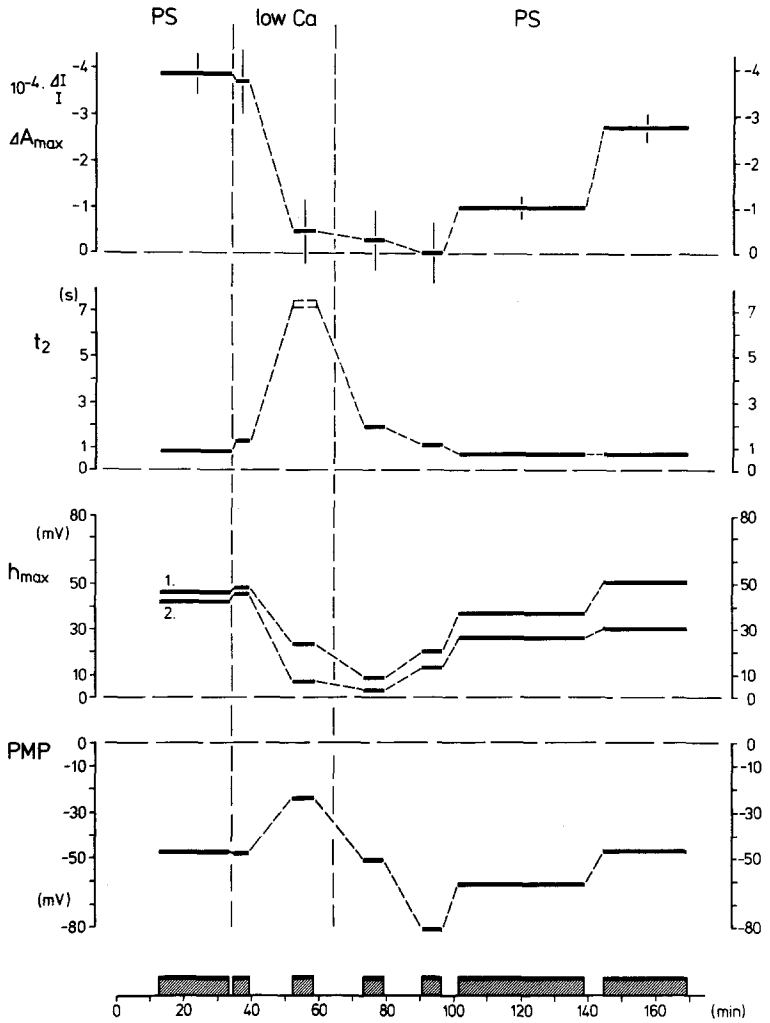

Fig. 12. Dependenc of the measured parameters of the arsenazo response and the membrane voltage on the extracellular calcium concentration. (Same experiment as Fig. 11). The values ΔA_{max}, and PMP of the first pulse of the pulse pair are plotted. The vertical bars in the ΔA_{max} plot indicate the noise amplitude. The highest value in the t_2 plot was measured in the ReP to the second pulse because t_2 of the first ReP was too long to be measured. The h_{max} values of the first ReP are indicated by 1, those of the second by 2. The bars parallel to the time axis indicate the time during which the records were averaged.

PMP, has recovered to a greater value than the original one but the height of the ReP is even more decreased; the falling phase of the ReP, however, has recovered already to a higher rate and so the decrease-time t_2 is short again (Fig. 12). In the time span of 26–32 min after return to normal calcium the PMP is even more hyperpolarized and the height of the ReP is more recovered. The RePs to both pulses of the pair still show the short hyperpolarisation (ERP) instead of the fast initial peak. In the following time the ReP recovers more and more, though its shoulder is still missing, whereas the PMP

depolarizes and stays at a somewhat greater value than the original level (last record of Fig. 11, time span 75–100 min).

These results show two important things. (1) The transient increase in the $[Ca^{2+}]_i$ depends upon the external calcium concentration. (2) After return to normal external calcium concentration RePs of reasonable size and shape can be recorded without a measurable transient increase in $[Ca^{2+}]_i$ within the accuracy of our measurements. The arsenazo response recovers with the same course as the fast initial component of the ReP.

In low external calcium concentration and in the first hour after the return into normal calcium concentration the size of the arsenazo response no longer correlates with the time integral of the ReP or the decrease time t_2 of the ReP. On the contrary, if only the responses in the first hour of the experiments are compared, it appears that large arsenazo responses coincide with fast repolarization of the RePs (short t_2), as could be expected according to the hypothesis of Lisman and Brown (1972).

Discussion

If we assume that arsenazo has been distributed evenly all over the cell the $[Ca^{2+}]_i$ may vary within the cell, and the change in $[Ca^{2+}]_i$ probably has different magnitude and time-course in different intracellular regions, as was shown by Meech and Mack Brown (1976) for changes in pH and $[Ca^{2+}]_i$ in *Balanus* photoreceptor cells.

By measuring the net change of the $[Ca^{2+}]_i$ we therefore have only a limited measure for the changes in the immediate vicinity of the cell membrane. The discussion is nevertheless led in the hope that we measure a decisive part of the relevant calcium kinetics by the arsenazo response.

In correlation between arsenazo response and electrical response may be partially distorted when only the voltage response is measured due to voltage-dependence of membrane conductances. Current measurements under voltage clamp conditions should help to clarify this point.

If we assume the ReP to cause variations of the intracellular calcium concentration shown by the arsenazo response we should expect two effects which are opposite to each other:

(1) The greater in size the ReP is, the greater the arsenazo response should be.

(2) The greater the arsenazo response is, the shorter the decrease time of the ReP should be (according to the hypothesis of Lisman and Brown 1972, 1975a).

Which of the two effects is dominating may depend on the experimental conditions. The results of our measurements in physiological saline do not agree with (2): We observed a great arsenazo response in physiological saline, correlated with a long decrease-time of the ReP.

Size and duration of the arsenazo signal correlate poorly with the amplitude of the ReP, but well with the time integral of the ReP or the duration of the repolarization of the ReP when the intensity of the light stimulus or the state of adaptation were varied.

The decrease-time of the ReP is often a more sensitive measure for the state of adaptation than the peak amplitude. Therefore the lack of correlation between arsenazo

response and rate of decrease of the ReP when Ca^{2+} was present in the medium was unexpected. In the experiments with low calcium saline, however, the results agree with the assumption that a great arsenazo response coincides with an accelerated decrease of the ReP.

The existence of the difference of levels ΔA_2 before the beginning of the second stimulus makes it reasonable to assume that the absolute $[Ca^{2+}]_i$ level (not its transient change measured) is greater in the state of light adaptation than in the state of dark adaptation.

It seems conceivable that in physiological saline the decrease of the ReP is already greatly accelerated due to the light-induced increase in the $[Ca^{2+}]_i$. Under these conditions the effect (1) that a greater and longer ReP causes a greater arsenazo response predominates, whereas under low extracellular calcium conditions the lack of the increase in $[Ca^{2+}]_i$ (effect 2) is responsible for the very slow decrease of the ReP.

Several possible mechanisms might be responsible for the transient increase in intracellular free calcium concentration:

(a) Lisman and Brown (1972) suggested that the light-induced influx of sodium during the ReP could cause the release of calcium ions from internal sources in the photoreceptor cell where the calcium is sequestered. The calcium increase is transient since the calcium is sequestered again afterwards.

(b) Another possibility would be that calcium flows into the cell during the ReP, due to a change in membrane conductance. This would only be in accordance with the experimental results of Brown and Blinks (1974), if the exchange of calcium between the saline and the extracellular compartment adjacent to the photosensory membrane is slow, since they did not find a decrease of the light-induced aequorin response when the external calcium concentration was lowered. Our results indicate that this is the case (Stieve et al. unpublished). Meech (1978, personal communication), looking at our records, suggested the possibility of "late" calcium channels during the decrease of the light response. Fain and Lisman (personal communication) found a voltage sensitive calcium conductance in the *Limulus* ventral nerve photoreceptor which was activated by a depolarising voltage step.

(c) Our results could also be explained by a sodium/calcium antiport across the visual cell membrane: Calcium ions could be taken up into the cell in (perhaps electroneutral) exchange for the outward transport of intracellular sodium which had come into the cell during the light response. A sodium/calcium exchange mechanism was described by Mullins (1977) and Baker and Glitsch (1975) in the squid nerve, but in the opposite direction (sodium in, calcium out).

Our experiments with changed external calcium concentration indicate that a major part of the light-induced intracellular calcium increase observed originates from external sources. There are two possibilities: Either there is an increased calcium influx during each ReP which causes the arsenazo signal. Or there is a slow exchange of calcium between external and intracellular compartments in the dark. When in the latter case the intracellular store compartments are exhausted the light-induced release of calcium from these compartments is no longer possible. The long time delay between the change of the external calcium concentration and the change in the arsenazo response both after lowering and after increasing the external calcium concentration could be due either to the long time needed to exhaust the extracellular compartment im-

mediately adjacent to the photosensory membrane and/or to a slow exchange between the external and the intracellular compartments.

The comparison of the shape of the arsenazo response with the ReP in physiological saline shows that the light-induced increase in the $[Ca^{2+}]_i$ starts more slowly than the ReP and is finished earlier than the ReP. Therefore the measured calcium increase does not coincide with the fast initial sodium influx.

The ReP of the ventral nerve photoreceptor appears to be composed of different components (Maaz et al. in preparation). As there is such a strong correlation between the increase in the $[Ca^{2+}]_i$ and the shape of ReP in normal calcium saline, which is characterized by t_2 or the time integral, the calcium increase should be mainly caused by a component of the ReP which is connected with the slow part of the light response. The results of the experiments with varied calcium concentration did not show the same correlation as in physiological saline between the shape of the ReP and the size of the arsenazo response. In these experiments the decrease of the ReP was retarded in the low calcium saline whereas the arsenazo response disappeared completely. Immediately after the return to normal calcium saline the ReP decreased faster but there was still no arsenazo response. As in our experiments the existence of the arsenazo response seems to be coupled with the existence of the fast initial component of the ReP it may be possible that this fast component triggers or causes the intracellular calcium increase or that both effects need the same state of the membrane which depends on the calcium concentration.

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Note added in proof. The repolarizing phase of the receptor potential recovers much faster than the arsenazo response after return from low calcium saline to physiological calcium concentration (Figs. 11 and 12). This may indicate that the acceleration of the repolarizing phase of the receptor potential is not caused by a transient increase in intracellular calcium which is registered in our arsenazo measurements.