Two components of photoreceptor potential in phototaxis of the flagellated green alga *Haematococcus pluvialis*

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ABSTRACT The kinetics of the photoreceptor potential of phototaxis in biflagellated green alga *Haematococcus pluvialis* in response to a 10-ns laser pulse of three wavelengths (465, 550, and 590 nm) were measured in single cells with 30 μs time resolution. The rise and the decay of photoinduced potential are both at least biphasic. The first component of the rise is very stable and has no measurable (<30 μs) time delay. The second component is triggered after a 120–400-μs lag period, depending on flash intensity. Its appearance is sensitive to the physiological state of the cell and the amplitude can be increased by phototactically ineffective red background illumination. The electrical generators for both components are localized in the same region of the cell membrane (on the stigma-bearing side) and these components have the same depolarizing sign. The results indicate that the photoreceptor potential in phototaxis comprises two components, which could be interpreted as light-induced charge movement within the photoreceptor molecules and changes in ion permeability of the cell membrane.

INTRODUCTION

Green flagellated algae possess a highly developed photoreception mechanism which enables them to sense spatial distribution of light intensity and the direction to the light source (Foster and Smyth, 1980; Lenci et al., 1984; Nultsch and Hader, 1988; Sineshchekov and Litvin, 1988). Photoreceptor molecules for light-induced motile responses are believed to be localized in a special part of plasma membrane overlying the stigma, a pigmented shading device involved in phototaxis (Nultsch and Hader, 1988; Walne and Arnott, 1967). The photoreceptor molecules are probably retinal proteins similar to visual rhodopsins (Foster et al., 1984; Hegemann et al., 1988).

It was previously found that phototactically active short-wavelength light induces in a cell of *Haematococcus pluvialis* a complex of electrical responses (Litvin et al., 1978; Sineshchekov, 1978; Sineshchekov et al., 1978). Primary photoinduced potential in the millisecond range, or photoreceptor potential, depends gradually on the intensity of continuous light or energy of the flash up to the values of 10^3 W · m^{-2} and 10 J · m^{-2}, respectively. When the intensity of stimulus light exceeds a threshold level, it becomes superimposed by an all-or-none regenerative response, which is Ca^{2+}-dependent and follows the light stimulus with a delay of several tens of milliseconds. And finally, permanent late-photoinduced potential is observed under the continuous illumination, which has low light intensity saturation.

The detailed action spectra of photoelectric responses both for the flash and continuous light excitation are coincident with action spectra of photomotile behavior in *Haematococcus*, even in their complex fine structure (Litvin et al., 1978; Sineshchekov and Litvin, 1988). The electrical generator for the photoreceptor potential is localized in the stigma part of cell membrane (Litvin et al., 1978; Ristori et al., 1981; Sineshchekov et al., 1978). These facts, in line with the evidence based on the action of inhibitors, blockers, and ion composition of medium, indicate that the measured electrical responses play the key role in light signal transduction in phototaxis. According to the direct microscopic observation and the registration of flagella movement (as the cell is fixed on the pipette), the regenerative response mediates photophobic behavior and late-photoinduced potential is involved in photoorientation of the cell (true phototaxis) (Sineshchekov, 1988; Sineshchekov and Litvin, 1988).

Both the regenerative response and low saturating late-photoinduced potential are obviously due to changes in ion permeability of the membrane. The question arises whether the photoreceptor potential reflects only the fast appearance and decay of ion currents or whether it originates from charge movement in photoreceptor molecules.

One approach to this problem is to measure electrical response with a good time resolution and with particular attention to the existence and length of the delay between light pulse and the beginning of potential generation. In our previous experiments we were limited to 1 ms time resolution (both by light pulse and registering electron-

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ics). In the present study we describe fast kinetics of photoreceptor potential in phototaxis in response to nanosecond light pulses with time resolution up to 30 μs. The results indicate that both light-induced charge movement in receptor molecules and changes in ion permeability of the cell membrane give rise to the generation of the photoreceptor potential in phototaxis.

MATERIALS AND METHODS

The biflagellated green alga *Haematococcus pluvialis* is a close relative of *Chlamydomonas*, a classical object in phototaxis research (Foster and Smyth, 1980; Nultsch and Hader, 1988). It has been shown previously that *H. pluvialis* demonstrates all the types of light-induced behavior known for *Chlamydomonas*, with similar characteristics, but is more convenient for electrical measurements because of its relatively large size and its elastic cell wall (Sineshchekov, 1978). The cells were grown at room temperature under constant illumination with fluorescent lamps in a medium containing 1 mM KNO_3, 0.4 mM K_2HPO_4, 0.3 mM $MgSO_4$, 0.3 mM $Ca(No_3)_2$, and standard solution of trace elements. 3–5-d-old cultures were used.

The setup for photoelectric response measurements in a single cell (see Fig. 1) was basically the same as described previously (Litvin et al., 1978; Sineshchekov, 1978). The cell was sucked into the tip of a glass micropipette, causing two parts of the cell surface to become electrically isolated by the glass of the pipette. If an electrical response is local and takes place in only one part of the cell membrane, a potential difference appears between the inside and outside of the pipette. The sign of potential difference would then depend on the position of the part of the cell membrane with the electrical generators (in the pipette or in the chamber), the absolute amplitude of the signal being linearly proportional to the shunt resistance under the given conditions (Sineshchekov, 1978).

This signal was picked up with silver electrodes immersed into the solution of the chamber and the pipette and connected to a preamplifier with capacity compensation (UB-2; Engineering Group of the Institute of Biophysics, Biological Research Center, Szeged, Hungary). The preamplified signals were further amplified with an electrometer (model 604; Keithley Instruments, Inc., Cleveland, OH) and stored in a transient recorder (NEO200-B; product of Central Research Institute of Physics, Budapest, Hungary). Up to 100 signals were averaged, in some cases, to improve signal-to-noise ratio.

To improve the time resolution over that obtained in previous studies, we used a "double pulling" method (Corey and Stevens, 1983) for preparing the micropipette. These pipettes have a resistance of <30 MΩ when filled with the growth medium in which the experiments were carried out. The pipettes were chosen by their diameter so that the shunt resistance between the two parts of the cell membrane (inside and outside the pipette) was not >25 MΩ. The time resolution of the system was checked by passing a current pulse through the system and was as low as 30 μs in the best experiments.

The 10-ns stimulus light pulse was provided by a nitrogen laser pumped dye laser with coumarin (465 nm), fluorescein (550 nm), and rhodamine G (590 nm) dyes. The laser pulse was transmitted to the cell via the microscope objective. The intensity of laser pulses passed through the set-up optics were measured with light meter DC1010 (Karl Lambrecht Corp., Chicago, IL), and the energy density of the flash on the position of the cell was calculated taking into account the dimensions of the light beam. The calibrated neutral density filters were used to attenuate the stimulus intensity.

An electrical artifact from the laser was minimized by placing it far from the measuring system (~15 m) and by avoiding any electrical connection between the laser and the measuring system. The remaining part of the laser artifact was eliminated by digital subtraction in the multichannel analyzer of the artifact measured with the light beam blocked.

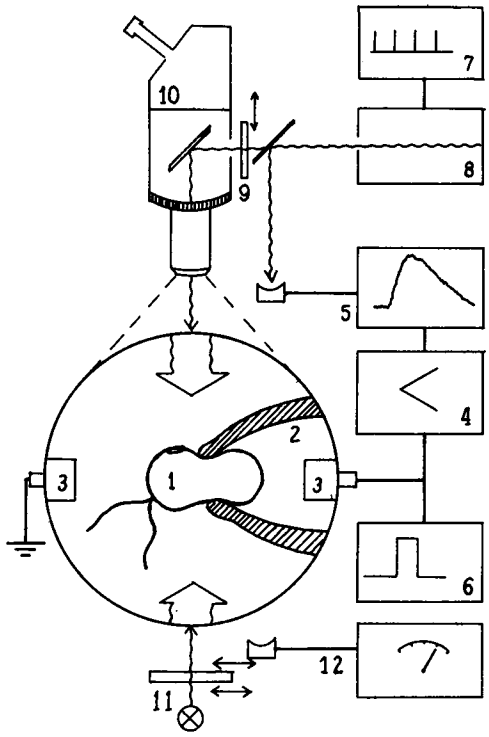

FIGURE 1 Scheme of the setup. (*1*) Cell with photoreceptor and flagella; (*2*) micropipette; (*3*) silver electrodes; (*4*) amplifier; (*5*) transient recorder triggered by photodiode; (*6*) constant current source for measurement of the resistance and time resolution of the system; (*7*) time controller; (*8*) dye laser; (*9*) neutral filter; (*10*) microscope; (*11*) light source for observation and background illumination; (*12*) laser pulse intensity meter.

RESULTS

Photoelectric response to nanosecond laser flashes includes the two previously described steps of light signal transduction: photoreceptor potential and regenerative response (Fig. 2). The regenerative response occurs when there is a relatively long (3–10 s) dark interval between the flashes, and as a rule, it disappears during the course of an extended experiment on a particular cell. Although this type of response is not dealt with in this paper, it should be mentioned that there is a direct relationship between the amplitude of laser-induced primary potential difference and the reciprocal of the lag period of regenerative response (which mediates the photophobic behavior), even in the range of light intensities where the

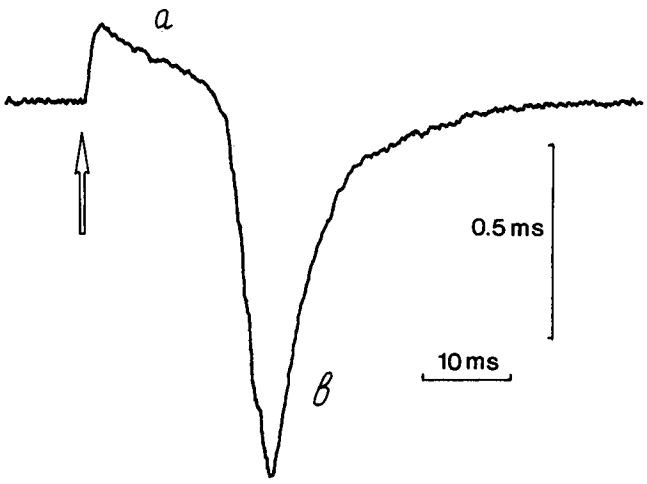

FIGURE 2 Two stages of photoelectric response in *Haematococcus pluvialis* to 10-ns, 590-nm, 25 J · m^{-2} laser pulse. (*a*) Primary photoreceptor potential; (*b*) regenerative response. The curve is an average of ten signals with a 5-s interval between flashes.

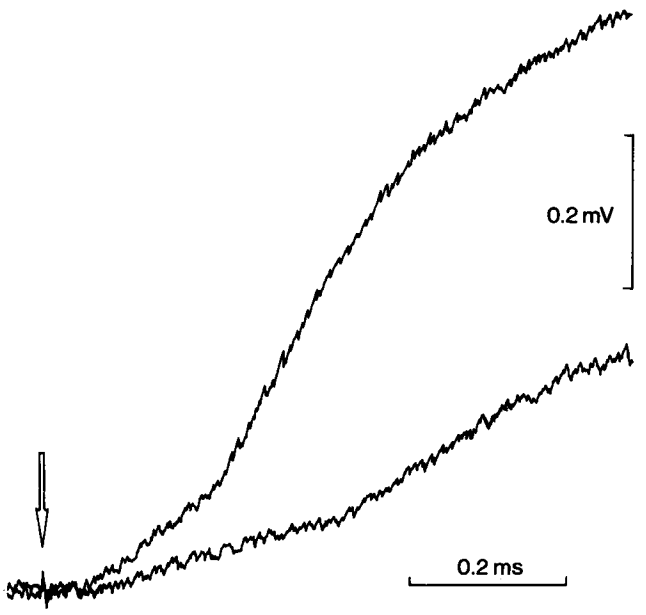

FIGURE 3 Fast kinetics of photoreceptor potential at two intensities of 465-nm laser flashes. Pulse energy in the plane of the cell was 0.1 J · m^{-2} for upper curve and 18% of this value for lower curve. Curves are averages of 20 signals with 1-s interval.

saturation begins. Attenuation of the light stimulus to 18.5% leads to the decrease of both parameters to equal values of 30%. This fact indicates that the laser-induced electric signal is related to phototaxis.

The photoreceptor potential is very stable and can be measured even after many hundreds of flashes. In some experiments photoinduced electric signals were registered from a single cell for over 10 h.

The photoreceptor potential was not saturated under the most effective available laser pulses (465 nm, up to 0.1 J · m^{-2} in the plane of the cell; Fig. 3), and in our experimental condition its absolute amplitude was usually in the range of 0.5–1 mV, depending on the shunt resistance.

According to the spectral sensitivity of phototaxis, three dyes (among those available) were chosen, which provided laser flashes with 465 and 550 nm for the short and long wavelength parts of the phototaxis spectral sensitivity band, and 590 nm for its longest wavelength end. All tested wavelengths were effective in eliciting electric responses. In good agreement with previously obtained detailed action spectra for the electrical and photomotive responses (Litvin et al., 1978; Sineshchekov, 1978; Sineshchekov and Litvin, 1988), the efficiency of the 465-nm laser pulse was several times higher than that of 550 nm, whereas 590 nm light was at least two orders of magnitude less effective (compare the intensities and amplitudes in Figs. 2, 3, 5, 6, and 7).

Maximum amplitude of the photoreceptor potential is found in the response to the first flash after long dark or red background light adaptation. The amplitude of the response to the second flash is smaller than that to the first and all subsequent flashes, thus showing complex adaptation of the sensory transduction system to a light-stimulating condition (Fig. 4). Similar adaptation phenomena is observed in photomotile behavior: the light sensitivity of the dark-adapted microorganisms decreased after the first flash, then partially recovered under repetitive stimulation.

A small delay between the laser flash and the start of potential rise could be observed on the curve (Figs. 3 and

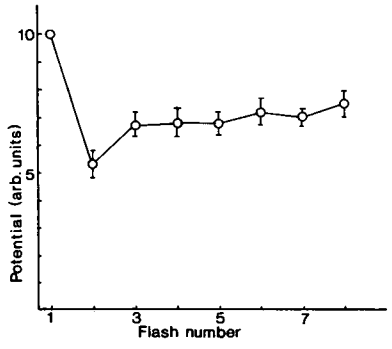

FIGURE 4 The dependence of the photoreceptor potential amplitude on the flash number with 3-s interval between 550-nm, 0.7 J · m^{-2} flashes. Amplitudes were normalized to the first signal in each series; mean values of four different experiments are presented.

5–7). The duration of this delay corresponds roughly to the time resolution of the experimental system and changes parallel to it from one experiment to another. (The variation in time resolution is due to difference in pipette shape, relative size of the cell as compared with that of the pipette, etc.) Under the best conditions, the delay does not exceed 30 μs (Fig. 6). For a given cell the delay does not depend on the intensity of the flash, temperature, or red background illumination (Figs. 3, 5, and 7).

In many experiments there is a clear break on the curve of the photoreceptor potential, indicating the existence of two phases of the process (Figs. 3, 6, and 7). The second phase is most obvious in the beginning of an experiment with a given cell and with high-intensity 465- and 550-nm flashes. The clear break in the curve was never observed with less effective 590-nm light, although even in this case the signal was not a single exponential (Fig. 5). The existence of two phases on the averaged curve could not be due to the summation of the curves with different time-course, because in experiments with good signal-to-noise ratio the two phases are clearly seen without averaging.

The time interval between the flash and the beginning of the second phase depends on light intensity and decreases from 400 to 120 μs with rising light intensity (Fig. 3). The rise time of the whole photoreceptor potential depends only slightly on the intensity of the flash and lies between 0.6 and 1.5 μs for different experiments.

Phototactically ineffective red background illumination increases the amplitude of the photoreceptor potential up to two times. This increase is due to the activation of the second phase, the fast component and the lag period

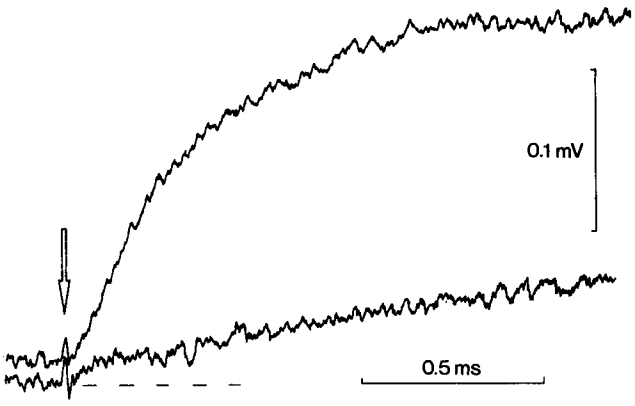

FIGURE 5 Temperature independence of time delay of the photopotential rise. (*Upper curve*) At room temperature; (*lower curve*) after cooling the cell by placing a piece of frozen culture medium into the chamber. Each curve is an average of 100 responses to 590-nm, 25 J · m^{-2} laser pulses with 1-s interval. The experiment was performed in the presence of 5 · 10^{-6} M of DCMU under the red background light for observation.

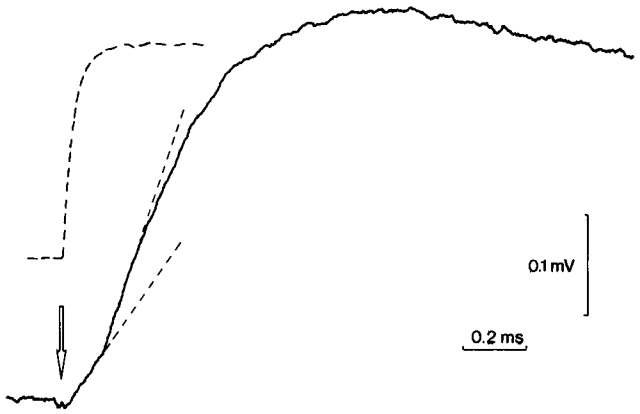

FIGURE 6 Rising part of photoreceptor potential in response to 550-nm laser flashes with 1-s interval. The curve is an average of 50 repetitions. Pulse energy was 0.7 J · m^{-2} in the plane of the cell. The dashed curve represents the time response of the system to a voltage jump of ~0.1 μs rise time.

of the second phase being unchanged (Fig. 7). This effect is in agreement with the strong and reversible influence of red background illumination on phototaxis in *Haematococcus* and its close relative *Chlamydomonas* (Sineshchekov et al., 1989).

In these experiments cells were usually sucked into the pipette by their posterior side, so that stigma and flagella were in the chamber. In this case, in agreement with our previous results, the sign of the photoreceptor potential was positive and both components of the resolved signal had the same polarity. Only when the small part of the stigma-bearing cell surface was inside the pipette was the sign of both components changed simultaneously to negative. According to this observation, the electrical genera-

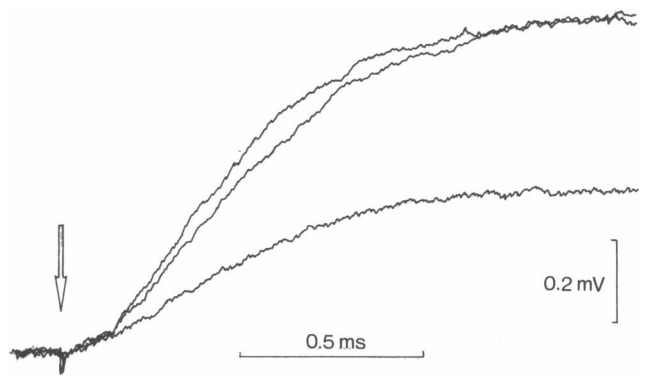

FIGURE 7 The effect of red background illumination (wavelength longer than 650 nm) on the photoreceptor potential. (*Upper curves*) Under red background before and after dark adaptation. Each curve is an average of 25 responses to 550-nm, 0.7 J · m^{-2} laser flashes with 1-s interval. 1 mM of KCN was added 5 min before start of experiment.

tors for both components should be located on the same (stigma-bearing) side of the cell, both components being depolarizing by their signs.

Under conditions where regenerative response disappears (long-term experiments and the short interval between flashes) the decay of the receptor potential can be decomposed into two exponential components with 2.5–6-ms and 14–32-ms time constants (Fig. 8). The ratio of the amplitudes of the fast and slow decaying components changes from 0.3 to 3.2 for different cells and different laser wavelengths, 590 nm light being less effective in generation of the fast decaying component.

Although the close relationship between the measured electrical responses and phototaxis has been proven in previous studies (see Introduction), control experiments were performed with our new high-resolution set-up to exclude the possibility that photosynthesis and energy metabolism may participate in the generation of any component of measured electrical signal. Photosynthetically active red light with wavelength longer than 650 nm and intensity up to >10 J $\cdot$ m^2 (from microsecond flash lamp) did not elicit any electrical signal. The described complex electrical response was observed in the presence of inhibitors of photosynthesis (DCMU, $5 \cdot 10^{-6}$ M) and respiration (KCN, 1 mM) (e.g., data for Figs. 5 and 7, respectively).

DISCUSSION

In our previous studies it has been proven that the electrical responses measured in *Haematococcus* by the developed suction micropipette technique are deeply involved in sensory transduction and photocontrol of movement in this organism (Litvin et al., 1978; Sineshchekov, 1978, 1988; Sineshchekov and Litvin, 1988; Sineshchekov et al., 1978). The lines of evidence reproduced in the present study show that the resolved laser-induced signal is due to phototaxis and is not related to photosynthesis or energy metabolism. They are: (*a*) the localization of the response on plasma membrane in relation to stigma; (*b*) the spectral sensitivity band, especially the position of its long-wavelength end; (*c*) the high level of light intensity saturation; (*d*) the character of inhibitors action; (*e*) the influence of red background illumination; (*f*) the quantitative relation with membrane excitation involved in photophobic response; and (*g*) the characteristic adaptation behavior.

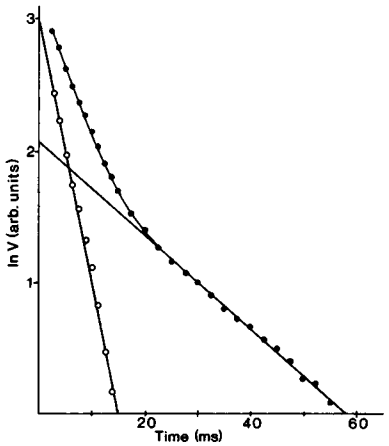

FIGURE 8 Semilogarithmic plot of photoreceptor potential decay and its decomposition into two exponentials with time constants of 5 and 28 ms. The data were obtained by averaging 10 responses to 550-nm, 0.7 J $\cdot$ m^{-2} flashes with 1-s interval.

The fact that the photoreceptor potential of maximum amplitude can be induced by a nanosecond flash after long dark adaptation shows that either a dark- or a light-adapted thermostable form of a pigment is effective in light signal transduction in phototaxis. Less probable is a two-quantum process with an intermediate which would appear in several nanoseconds.

The delay in the start of photoreceptor potential rise does not exceed 30 μs. In fact, even this short delay is probably due to the time resolution of the experimental system. It becomes progressively shorter as time resolution increases, and does not depend on light intensity, temperature, or background illumination. The delay is probably due to the time needed to charge the capacity of the pipette. Thus, in the limits of our method we could not find lag period of the photoreceptor potential. Its fast appearance, stability, and high intensity saturation indicate that at least the first component of the photoreceptor potential in phototaxis precedes biochemical steps of light signal transduction. Thus, this component should represent charge movement in photoreceptor molecules and is similar to the early receptor potential in vision (Cone and Pak, 1971). Both in our measurements and in extracellular registration of the early receptor potential in rod cells the photoinduced electric polarization of cells is due to asymmetric localization of pigment molecules in cell membranes. Both signals have the same order of magnitude and are in the same time scale. But, opposed to the early receptor potential in vertebrates, positive charges move into the cell in photoreceptor of *Haematococcus* under light excitation, as in the early receptor potential in blowfly (Stavenga, 1980).

The kinetics of the photoreceptor potential clearly show the existence of a second component. This component has more physiological characteristics. It has a clear lag period, which becomes shorter as the intensity of flashes increases. The second component is most obvious at the beginning of the experiment with the particular cell, and its contribution to the signal can differ in different

cultures. The amplitude of the delayed component could be increased by red photosynthetically effective background light, thus showing its dependence on the energetic state of the cell.

All of these features lead to the conclusion that the delayed component of the primary photoreceptor potential of phototaxis could be due to secondary changes in the state of cell membrane, namely its ion permeability. At this step of the transduction chain of phototaxis the previously stored energy of electrochemical gradients could be used for the amplification of the signal. As the second component of photoreceptor potential has the depolarizing sign, its activation by red background illumination can be directly due to the hyperpolarization of plasmalemma by photosynthesis found in this organism (Sineshchekov, 1978; Sineshchekov et al., 1976).

The conclusion that the primary photoreceptor potential comprises more than one component is also proven by its biphasic decay. Similar with the rising part of the signal, the relative amplitudes of the decay components strictly depend on the state of the culture and experimental condition and could change during the course of prolonged experiment on the particular cell.

The quantitative deconvolution of the rising part of the signal is difficult now because of the as yet low time resolution of the system (compared with the very fast component) and high overlap of the two components. The capacity of the cell membrane and the pipette should also be taken into account in deconvolution of the fast stages. Because of this, the fast and the delayed components of the rising part of the photoreceptor potential could not be correlated to each other or to the components of its biphasic decay. We do not exclude the possibilities that there is no simple causal relationship between those components and that they originate from different steps or intermediates of the photoreceptor cycle, or even from different photoreceptor pigments. Earlier, on the basis of the complex fine structure of the action spectra for both photoelectric and photomotile responses in *Haematococcus*, it was suggested that more than one pigment could participate in the reception of phototactically active light stimulus in this organism (Sineshchekov, 1978; Sineshchekov and Litvin, 1982, 1988). That two photoreceptor pigments are involved in the phototaxis of the halophilic bacteria *Halobacterium halobium* has been firmly established in recent years (Spudich and Bogomoini, 1988).

Independent of the interpretation, the results presented here reveal the existence of two processes that contribute to the primary photoreceptor potential in phototaxis. They can be interpreted as charge movement within the photoreceptor molecules and delayed changes in ion permeability of the cell membrane. These processes represent the two fastest up-to-date detectable steps in light signal transduction in phototaxis of flagellates and can be correlated with early and late receptor potentials in vision.

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