TRANSIENT PHOTORESPONSES OF A PHOTOTACTIC MICROORGANISM, *HAEMATOCOCCUS PLUVIALIS*, REVEALED BY LIGHT SCATTERING

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ABSTRACT A new method, using incoherent light scattering, has been developed to measure the flagellar beating frequency of swimming microorganisms. By means of this method, transient changes of flagellar beating frequency in response to white light flashes have been revealed in samples of a phototactic microorganism, *Haematococcus pluvialis*. An increase of flagellar beating frequency occurs when the flash dose (flash intensity × flash duration) is sufficient. Reciprocity between light intensity and flash duration holds for durations not exceeding 60–80 ms. For lower doses a bimodal distribution of flagellar beating frequency is revealed. No effect is observed for very low flashes or for red stimuli, whereas green light is effective. A detailed analysis of experimental results has allowed us to determine the characteristic time of the effect and follow its evolution. The correlation of this effect with visually observed behavior is discussed and a possible underlying mechanism is suggested.

INTRODUCTION

Phototactic microorganisms are able to detect the light direction and swim towards the light source. Phototaxis is an integrated response; what is required is a model for the single steps that lead to the microorganism orientation. A common hypothesis (Diehn, 1973; Lenci and Colombetti, 1978; Diehn, 1979; Feinleib, 1980) is that these microorganisms detect the direction of the light by a time comparison between the light intensities successively absorbed by the photoreceptor; owing to the rototranslatory motion of the microorganisms, a shading (Diehn, 1973; Lenci and Colombetti, 1978; Diehn, 1979; Feinleib, 1980), or, in an alternative model, a light-focusing (Foster and Smyth, 1980) mechanism is suggested. In some preparations (i.e., *Euglena gracilis*) both photoreceptor and shading organelle are rather clearly identified (Benedetti et al., 1976; Benedetti and Lenci, 1977; Ghetti et al., 1985). In both cases (shading or light-focusing organelle) phototaxis should be the result of successive motor responses to intensity variations of the light absorbed by the photoreceptor. So it appears that responses to sudden changes in light intensity may yield information about the mechanism of phototaxis.

Behavioral responses to flashes or step stimuli are usually observed under the microscope (Boscov and Feinleib, 1979), but a better insight into the phenomenon requires quantitative measurements of motion parameters, such as flagellar beating frequency or amplitude, which are directly related to the motor apparatus. In *Chlamydomonas reinhardii* quantitative measurements of flagellar beating frequency during light stimulation were obtained by high-speed cinematography on single cells (Schimdt and Eckert, 1976). This seems the best way to obtain an image of the process but it is unsuitable for a statistical study because of the long time required to follow a single cell. In previous experiments (Ascoli et al., 1978; Ascoli et al., 1980; Ascoli and Frediani, 1980) the flagellar beating frequency of a population of swimming algae was measured by a laser light scattering technique. As will be argued below, similar measurements, which exploit the anisotropy of the scattering from large inhomogeneous particles, can also be made by using an incoherent analyzing light. This new method detects the light scattered from a population of microorganisms and performs the spectral analysis of the photocurrent.

In this paper we report measurements of the flagellar beating frequency of a phototactic alga, *Haematococcus pluvialis*, stimulated by brief flashes of white light. Smyth and Berg (1982), by a method based on a nonuniform illumination of the microscope field, measured changes in flagellar beating frequency in response to flashes on single mutants of a biflagellated alga, *Chlamydomonas reinhardii*, which had lost one flagellum. Their method of analysis is unsuitable for studying populations of swimming microorganisms.

DESCRIPTION OF THE METHOD

Laser light scattering experiments (Ascoli et al., 1978; Chen and Hallett, 1982) on swimming microorganisms that are larger than the laser wavelength show that the

light scattered is (*a*) frequency shifted by the Doppler effect; and (*b*) intensity modulated, because the intensity of the light scattered from a cell depends on its orientation with respect to the incident beam and to the detector, and the cell rotates periodically during the motion and wiggles.

Fig. 1 schematizes the light scattered from an ellipsoidal microorganism moving perpendicularly to the incident beam, onto a plane orthogonal to the beam itself; the scattering pattern appears highly asymmetric. This scheme reproduces pictures taken with *Euglena gracilis* by using a laser beam (Ascoli et al., 1978); in fact, the spatial coherence of laser light makes it easier to observe the scattering from a single microorganism. During the *Euglena* motion, the sharp bright zone in Fig. 1 appears to oscillate as *Euglena* rotates on itself. Spectral analysis of the photocurrent confirms this visual observation, showing a peak at ~2 Hz; it also shows a higher frequency peak (~30 Hz), (Ascoli et al., 1978). These two peaks are clearly light-intensity modulations, since they are independent of scattering angle, while the Doppler distribution depends on it. The 2-Hz peak is the body rotation frequency and the 30-Hz peak, which depends on temperature just as the swimming speed does (Ascoli and Frediani, 1980; Chen and Hallett, 1982), has been considered the flagellar beating frequency distribution. Measurements by high-speed cinematography of flagellar beating in *Chlamydomonas reinhardii* (Schmidt and Eckert, 1976) give a slightly different value of ~50 Hz. As the scattering cross section of the cell body is greater than that of the thin flagellum, this last intensity modulation is really due to the cell body vibrations induced by the flagellar beating.

Light intensity modulations are not interference effects and they are simply due to the anisotropy of the scattering, so they can be detected by using a common incoherent light source, the only constraints being that the incident beam should be well collimated and that it should not fall on the detector.

This new light scattering set up is schematized in Fig. 2. The infrared component of the microscope lamp passes through a dark-field condenser and falls on the sample; the incidence angle is restricted by a slit placed adjacent to the annular diaphragm of the condenser. The infrared light scattered from the sample, after being collected by the microscope lens, is detected by a photocell placed at the output pupil of the eye-piece.

Fig. 3 shows the photocurrent obtained when a single microorganism (*Euglena gracilis*) is swimming within the microscope field; the signal is amplitude modulated at two different frequencies (1.2 Hz and 40 Hz) corresponding to cell body rotation frequency and flagellar beating frequency, respectively.

This can be written

$$i(t) = \beta I^{\dagger}(1 + M_f \cos 2\pi\nu_f t + M_r \cos 2\pi\nu_r t),$$

where $I^{\dagger}$ is the mean value of the intensity of the light scattered from the microorganism into the microscope objective, β is the quantum efficiency of the photodetector, and M_f and M_r depend on both the microorganism motion and the anisotropy of the scattering pattern.

With a population of microorganisms, the photocurrent spectrum contains the distributions of body-rotation frequencies and of flagellar-beating frequencies (Fig. 4 *a* and *b*). A zero-centered band also appears in the spectrum; it is

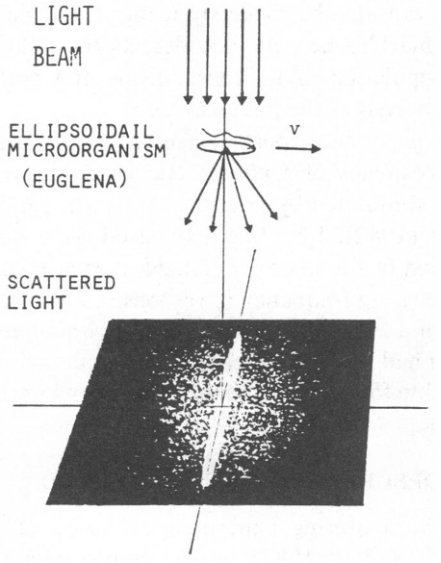

FIGURE 1 Schematic drawing of light scattering from *Euglena* onto a plane perpendicular to the incident beam.

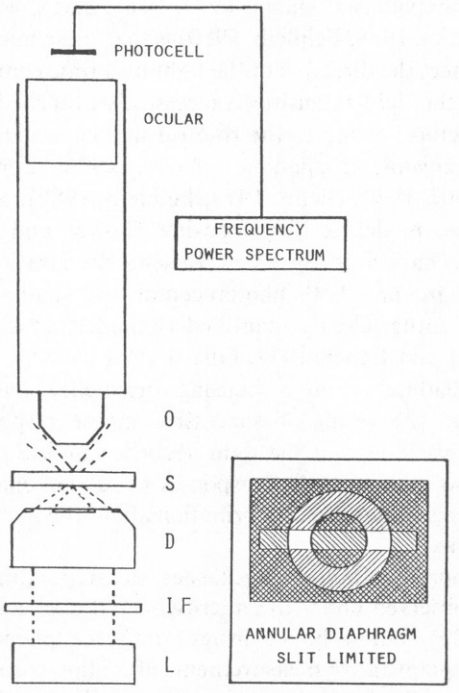

FIGURE 2 Scheme of the optical apparatus; the inset shows the annular diaphragm of the dark field condenser with the split laying on it. *O*, objective; *S*, sample; *D*, dark field condenser; *IF*, infrared filter; *L*, light source.

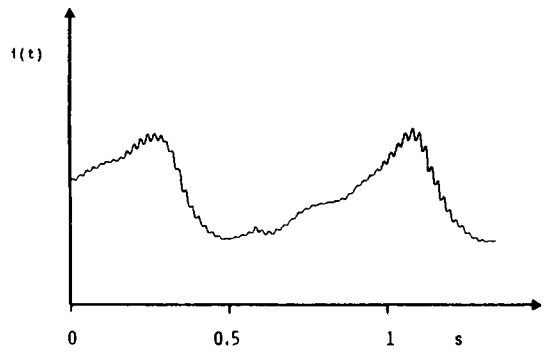

FIGURE 3 Time course of photocurrent due to light scattered from a single swimming *Euglena*.

caused by the fluctuations in the number of microorganisms moving in the illuminated field (Schaefer and Berne, 1975). With N swimming microorganisms in the microscope field, given the statistical independence of the scatterers, the amplitudes of the photocurrent modulations are $A_f = \beta I^{\dagger} M_f \sqrt{N}$ and $A_r = \beta I^{\dagger} M_r \sqrt{N}$, respectively. The signal is affected by the photon shot noise and by the current amplifier noise; the first is proportional to $\sqrt{(N + n)I^{\dagger}}$ where n is the number of nonmotile scatterers in the field, while the last does not depend on either N or the intensity of incident light. With increasing the intensity of the analyzing light, the signal-to-noise ratio increases with respect to both the photon shot noise and the current amplifier noise. We worked with many (10 to 100) microorganisms in the microscope field to rapidly achieve statistically meaningful results; many spectrum averages (~20) are required for reliable distributions.

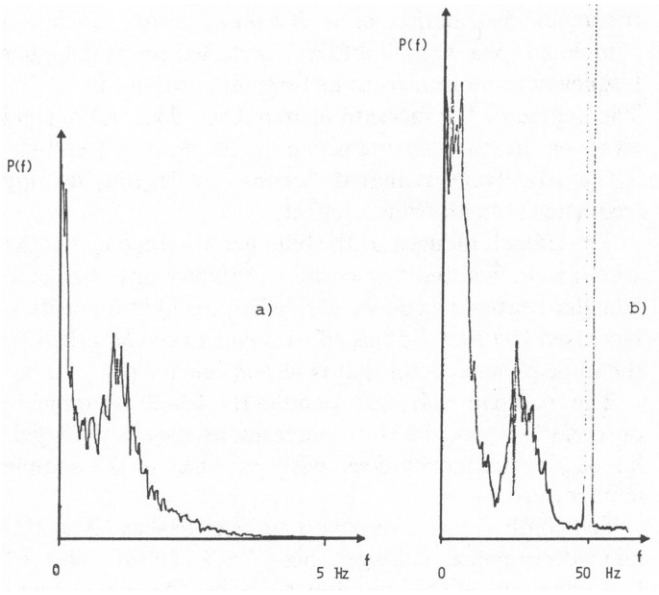

FIGURE 4 Power spectrum for a population of *Euglena gracilis*, obtained by the real time spectrum analyzer Federal Ubiquitous. (*a*) Body rotation frequency distribution (high pass filter 0.3 Hz, 32 averages); (*b*) flagellar beating frequency distribution (high pass filter 7 Hz, 64 averages). In both cases there is a zero-centered band, due to fluctuations in the number of microorganisms in the illuminated field.

APPLICATION TO THE STUDY OF *HAEMATOCOCCUS PLUVIALIS* PHOTORESPONSES

Haematococcus pluvialis is a quasi-spherical biflagellated alga, whose diameter is ~20 μm (Fig. 5). It is not optically homogenous, so scatters the light anisotropically and it is possible to measure its flagellar beating frequency by the method illustrated above. Fig. 6 shows the spectrum for a population of *Haematococcus pluvialis*; the flagellar beating frequency is clearly defined.

We have used this detection method to study transient photoresponses of *Haematococcus pluvialis* stimulated by brief light flashes. *Haematococcus pluvialis* is a phototactic microorganism whose photoreceptor is unknown; the action spectra both of phototaxis and of electrophysiological responses (Litvin et al., 1978) show that the response is obtained for stimuli within the range 410–560 nm. The stigma, an organelle that maximally absorbs blue–green light, is believed to be the shading organelle because of the similarity between *Haematococcus pluvialis* and other algal systems, such as *Euglena* or *Chlamydomonas*.

When responding to unilateral light stimulation, *Haematococcus pluvialis* shows both positive (orientation toward the light source) and negative (orientation away from the light source) phototaxis depending on both the light intensity and the growth conditions. We have used unilateral intense flashes to cause changes of flagellar beating frequency; for continuous light of the same intensity the cells displayed negative phototaxis. The flashes fell on the microslide containing the sample directly, not through the microscope optics and the sample was uniformly illuminated, so any effect depending on spatial nonuniformity of the stimulus was absent.

Fig. 7 shows the analysis apparatus schematically: the experiment was controlled by a computer: the computer samples the photocurrent for a given time τ_0 (usually τ_0 = 400 ms), triggers the flash occurrence and again samples the signal for a time $n\ \tau_0$ (usually we used $n = 7$). By performing an FFT (Fast Fourier Transform) of the sampled signal, we can obtain the spectrum for a population in an unperturbed state, and n successive independent spectra corresponding to different delays from the flash

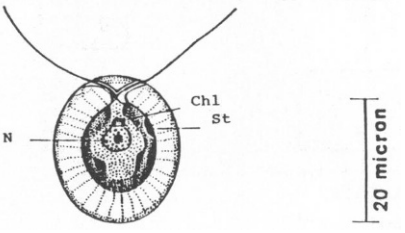

FIGURE 5 Schematic drawing of *Haematococcus pluvialis*; N, nucleus; Chl, chloroplast; St, stigma (inside the chloroplast membrane).

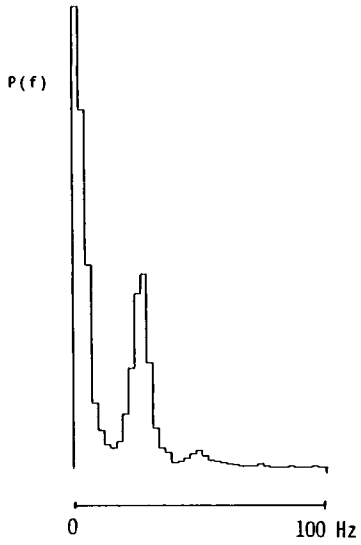

FIGURE 6 Averaged power spectrum (20 averages) for a population of *Haematococcus pluvialis*. Frequency resolution Δf = 2.5 Hz; sampling frequency f = 320 Hz; high pass filter frequency 3.5 Hz.

occurrence. The highest frequency that can be detected is equal to half of the sampling frequency (usually f = 160 Hz), while the frequency resolution Δf in the spectrum depends on the duration of the sampled signal τ_0 ($\Delta f = 1/\tau_0$). After a time interval (~20 s) long enough to avoid light adaptation the measure was repeated and spectra at the same distance from the flash occurrence were averaged to obtain stable distributions and to enhance the signal-to-noise ratio. In this way we were able to compare perturbed and unperturbed spectra and follow the time evolution of flagellar beating frequency distribution.

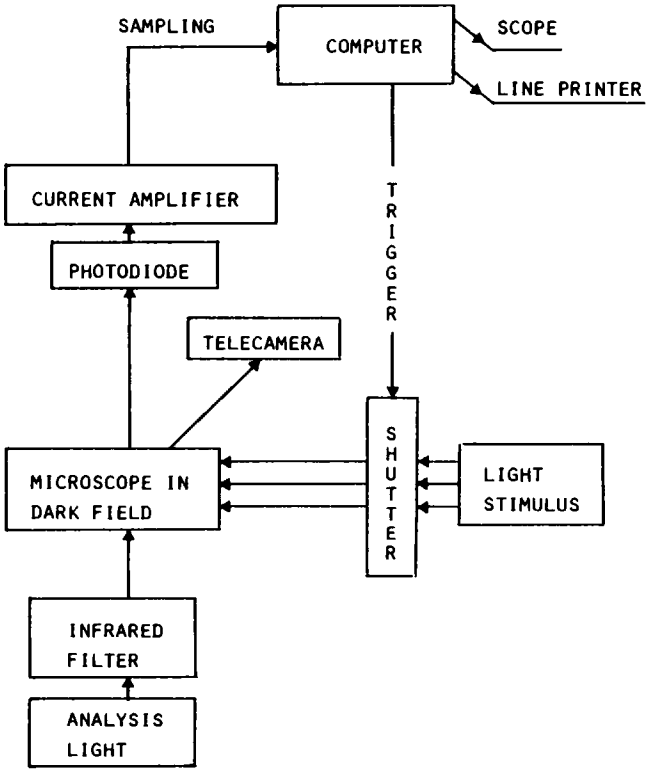

FIGURE 7 Block scheme of the experimental set up.

MATERIALS AND TECHNIQUES

The cells of *Haematococcus pluvialis* were grown at 20°C in a medium containing 1 mM KNO_3, 0.03 mM $MgSO_4$, 0.3 mM $Ca(NO_3)_2$, 0.1 mM K_2HPO_4 and a standard solution of trace elements; illumination was provided by a fluorescent day-light lamp; the light intensity was ~1 mW/cm^2. Cultures of 3–7 d were in the exponential phase of growth. Cells grown in this condition do not respond to constant dim light; at higher light intensities a negative phototaxis occurs. Light flashes (the highest intensity was 10 mW/cm^2, which corresponds to 100 a.u. quoted in the following figures) were obtained from a halogen lamp (100 W Osram, West Germany) in line with an electronically controlled shutter (Uniblitz, Vincent Associates, Rochester, NY), triggered by the computer; the shortest flash duration was 6 ms. The infrared component of the halogen lamp was cut off by a filter (Kodak, Rochester NY); interference filters (Balzers, Liechtenstein) were used to get monochromatic flashes and neutral filters (Balzers, Liechtenstein) to attenuate them.

The microscope (Leitz, West Germany) was supplied with a dark-field condenser. To prevent the photocell (Hamamatsu, Japan) from collecting the stimulus, a filter (Kodak, Rochester, NY) that cut off UV and visible light was put in front of the photocell. The behavior of *Haematococcus* populations was monitored by a video system (Nical, Italy) during the experiments. The spectral analysis was performed by a real time FFT routine implemented on Nova 4 64 KBytes minicomputer (Data General, Westboro, MA), supplied with analog-to-digital and digital-to-analog converters, digital input-output interface, a 10 MBytes mass memory disk for storing the sampled points and performing further off-line spectral analysis, a Video (Hantarex, Italy) and a graphic printer (Centronics, Hudson, NY) for data presentation.

RESULTS

Fig. 8 shows a typical result: the flagellar beating frequency distribution of a *Haematococcus* population stimulated by a white light flash is shifted towards higher frequencies and returns to its original position after ~2 s. The degree of the increase in frequency does not depend either on the stimulus intensity or on its duration (see Figs. 9 and 10); it means that the change in flagellar beating frequency is an all-or-none effect.

The effect is induced by the blue-green component of the stimulus; in fact, red flashes do not produce any change in flagellar beating frequency even when the light intensity is increased 10 times. So this effect seems to be controlled by the same photoreceptor that is responsible for phototaxis.

The frequency shift was found in the 10–20 Hz range in different samples; the shift increases, as the steady flagellar beating frequency does, with increases in the sample temperature.

The comparison of responses to brief flashes (T > 100 ms) with responses to longer ones (T > 1 s) clearly showed that switching off the light does not affect the response and that the light that falls on the sample after the shift occurs is ineffective.

Fig. 9 shows the trend of the response with flash intensity. The arrows show the position of the maximum reached by the steady flagellar beating distribution. At the

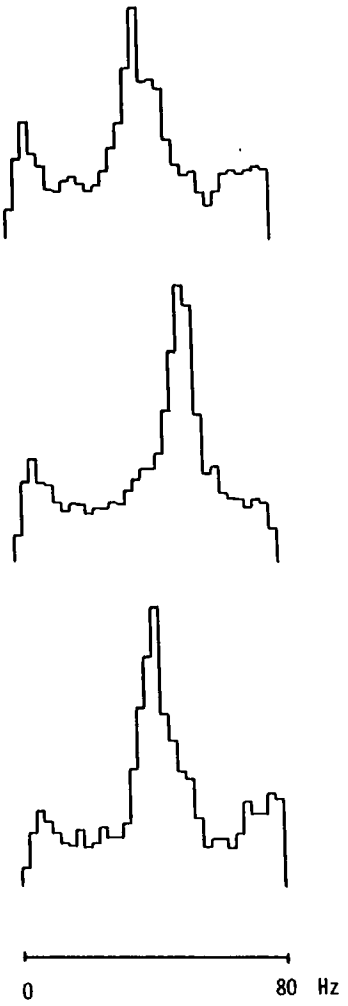

FIGURE 8 Flagellar beating frequency distribution: (*a*) before the flash occurrence; (*b*) 0.4 s after the flash; (*c*) 1.4 s after the flash. The flash intensity was 10 mW/cm^2 and the flash duration was 6 ms.

highest intensity the shift of flagellar beating frequency is complete, since the whole distribution has shifted; the corresponding observation of the population behavior shows a sudden stop followed by a slow backward motion and then a change of swimming direction. At lower intensities the distribution of flagellar beating frequency becomes bimodal, with a peak in the unperturbed position and the shifted peak in the same position it had at the highest intensity; in this case only some of the cell population is observed to stop. The area of the unperturbed peak increases at lower intensities, while the area of the shifted peak decreases and eventually disappears at a very low intensity.

Fig. 10 shows the trend of the response with the flash duration T for high light intensity; brief flashes give bimodal distributions of flagellar beating frequency, the area of the shifted peak increases with every increase in duration and at last the whole distribution is shifted. This trend is identical with that in Fig. 9, where the variable is the light intensity. This reciprocity between light intensity

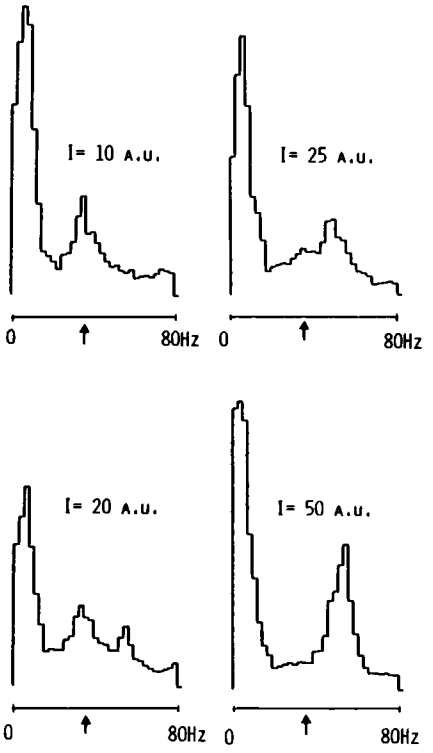

FIGURE 9 Trend of the responses with the flash intensity I; the arrows show the mean value of the unperturbed distribution. The flash duration was 20 ms and I = 100 a.u. corresponds here and in the following figure to 10 mW/cm^2.

I and flash duration T suggests that the meaningful stimulus parameter is really the light dose $I \times T$.

Fig. 11 supports this suggestion. We report the flash duration in abscissae and the light intensity in ordinates. The squares correspond to a complete shift of flagellar beating frequency distribution, the dots to a partial shift of the cell population (bimodal distribution), and triangles

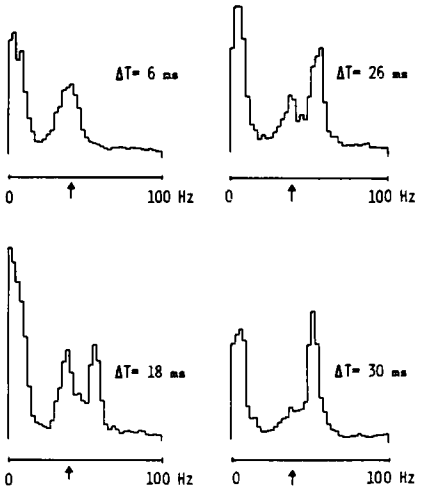

FIGURE 10 Trend of the response with the flash duration; the arrows show the position of the mean value of the unperturbed distribution; I = 50 a.u.

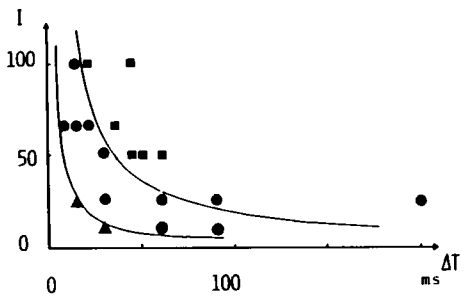

FIGURE 11 Meaning of symbols: ▲, no effect; •, partial effect; ■, complete effect. Equation of the upper curve $I \times T = 1{,}900$ (a.u. × ms); equation of the lower curve $I \times T = 380$ (a.u. × ms).

indicate that the stimulus is completely ineffective. If the frequency shift depends on the dose, two hyperboles should separate the no-effect zone, the partial effect zone and the complete effect zone in the XY plane. This is, in fact, true, but only for flash durations that are not too long (see Fig. 12 also).

In Figs. 9 and 10 the degree of the frequency shift does not depend on the stimulus strength, whereas the area of the shifted peak increases with every increase in stimulus strength. The ratio $R = A_1/(A_1 + A_0)$, where A_1 and A_0 are the areas of the shifted and unshifted distributions, respectively, gives the percentage of the responding cells and then it measures the intensity of the response.

The trend of R against flash durations is shown in Fig. 12, where different curves correspond to different flash intensities. R increases linearly with duration, for short durations. Then, the increase of the duration becomes less effective until it becomes ineffective for durations longer than 60–80 ms, depending on the sample.

The time evolution of the response can be followed by shifting the starting point of spectral analysis. To analyze spectrally a signal with a frequency resolution Δf one must sample the signal for a time duration $\tau_0 = 1/\Delta f$ (we used $\Delta f = 2.5$ Hz and then $\tau_0 = 400$ ms and the sampling frequency $f = 160$ Hz). By shifting the starting point of the analysis, we obtained many successive nonindependent spectra; the time resolution of this analysis is limited only by the sampling frequency f. Fig. 13 shows schematically how gradually an instantaneous change in signal frequency affects the spectrum. By computing the areas A_0 (old peak) and A_1 (new peak) for each successive spectrum, the time

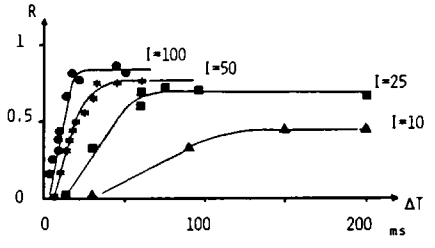

FIGURE 12 Trend of R vs. flash durations for several light intensity I (I in arbitrary units).

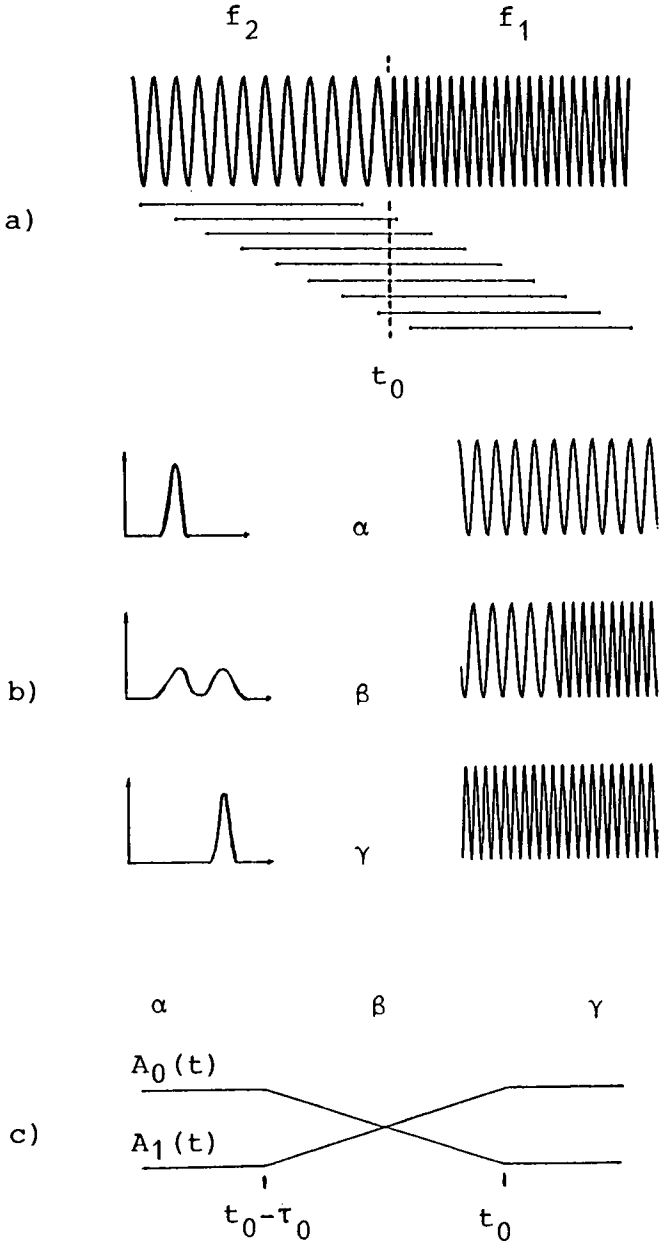

FIGURE 13 *a* shows a sinusoidal signal which changes its frequency at t_0; the segments below the signal with temporal length τ_0 indicate the sequences successively analyzed. In *b* we show schematically the spectrum of a temporal sequence of the signal placed before t_0, around t_0, and after t_0. In *c* the temporal trends of A_0 (area of the peak around f_1) and of A_1 (area of the peak around f_2) are reported. The instantaneous changes of the signal frequency affects the spectra gradually and the rise of the new peak at f_2 occurs in the time τ_0.

evolution of these areas can be followed (*bottom* of Fig. 13). Note that the rise time of A_1 is equal to τ_0, while there is a stepwise change in the frequency signal.

Fig. 14 shows A_1, A_0, and A^* (area of the zero centered peak) as functions of time; A_1 increases after the flash occurrence, while A_0 decreases, reaching zero if all the cells respond to light. The population recovers its steady flagellar beating frequency in ~2 s; note that A_0 increases more

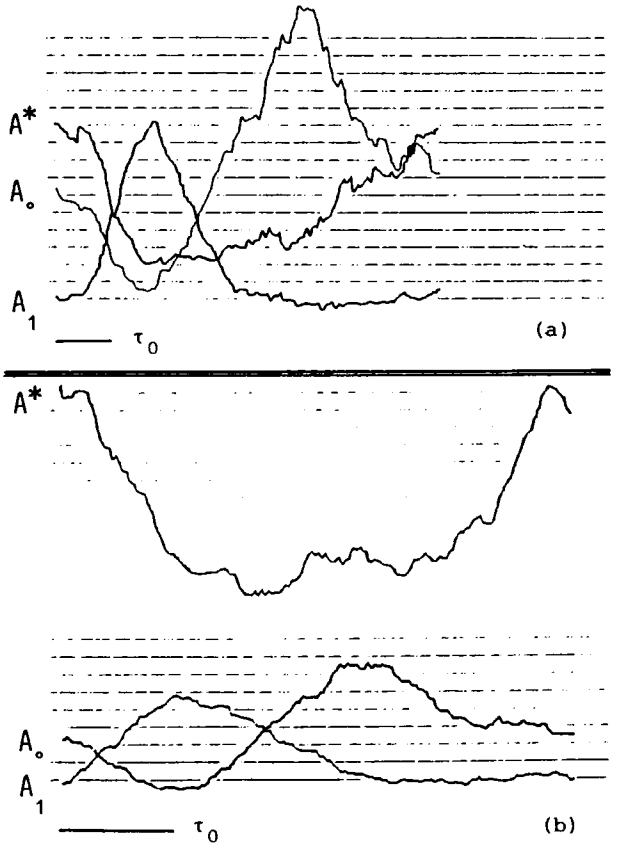

FIGURE 14 Time evolution of the response. Meaning of symbols: A_0, area of the old peak; A_1, area of the new peak, A^*, area of the zero centered band. τ_0 is always equal to 400 ms. In *a* a latency of the response of ~200 ms can be measured.

than its initial value and its increase continues even after A_1 has reached zero. This suggests that, during the recovery, there is an increase in flagellar beating amplitude, or at least in body vibration amplitude. A^* decreases after the flash occurrence and returns to its original value together with the flagellar beating distribution. As the zero-centered band is mainly due to photocurrent transients induced by the input and output of moving cells from the microscope field, the decrease in A^* corresponds to a lower motility of the sample.

In some samples a response latency can be measured (200 ms in Fig. 14 *a*), whereas in other samples no latency occurs (Fig. 14 *b*).

DISCUSSION

Two main topics will now be discussed; first, a comparison among the time evolution of spectra, population behavior, and previous results concerning flagellar beating after flash stimuli; and, second, a model accounting for the all-or-none response.

Experiments like ours, with measurements of flagellar beating frequency after a flash have been carried out by Smyth and Berg (1982) and by Schmidt and Eckert (1976). Smyth and Berg (1982) worked on a mutant of *Chlamydomonas reinhardii* that lost flagellum with a measurement technique based on the nonuniform illumination of the microscope field; they directly measured the frequency of the photocurrent modulated by flagellar beating. Their results, obtained at stimulus intensity of ~0.1 mW/cm^2, show a decrease of flagellar beating frequency after a flash.

Schmidt and Eckert (1976) used high-speed cinematography on a wild strain of *Chlamydomonas reinhardii*. In their results, as in ours, there is an increase in flagellar beating frequency after a light flash. More precisely they see that during the backward motion the frequency of flagellar beating increases; then the cell wobbles on itself before resuming its forward motion in a new direction. Their results were obtained at stimulus intensities much higher than ours; even so, their results are very similar to ours.

In comparing our results with theirs it may be concluded that the increase in flagellar beating frequency after the flash (measured by the increase in A_1) corresponds to the backward motion while the decrease in A^* (corresponding to a reduced motility of the sample) lasts until the swimming starts again in a new direction. The increase of A_0 over its steady value, which continues after A_1 has reached its steady value (i.e., after the flagellar beating frequency returned to its original value) should be related to an increased amplitude of the cell body vibrations and should correspond to the wobbling of the cells, which has been observed by high-speed cinematography.

The results show that, for a single cell, the response to a light flash is an all-or-none event; the response occurs if the light dose absorbed by the photoreceptor in a time shorter than 80 ms is over a given threshold value C_0. We can schematize the underlying process in the following way: the absorption of a photon produces, after several steps, a molecule X, which decays with a time constant τ. Let C be the concentration of X; if C is over the threshold value, C_0 cell motion stops and flagellar beating frequency changes. The differential equation that determines C as a function of time t is, in the simplest case of immediate conversion from the pigment to the active compound X $dC/dt = -C/\tau + \alpha I$, where I is the light intensity that falls on the photoreceptor and α is a coefficient proportional to the probability of a single photon being absorbed. The solution of this equation is $C(t) = \alpha \tau I [1 - \exp(-t/\tau)]$. Thus either this accumulation process reaches the threshold C_0 in ~3 τ or it never reaches the threshold. Then from our data we can evaluate a time constant τ of ~20 ms for the underlying accumulation process. For short flashes ($T << \tau$) X will reach the concentration $C = \alpha I T$, which accounts for the dependence of the effect on the dose. For a single microorganism the dose-effect curve is step shaped; to account for the shape of the curves in Fig. 12 it needs to assume that important parameters of the process, such as the threshold or the amount of the light absorbed by the photoreceptor, are varying in the population. We checked

by a computer simulation that a Gaussian distribution of the thresholds in the population is able to account for the family of curves of Fig. 12. More generally, two different situations have to be considered: (*a*) C_0 is a feature of each cell, depending on its metabolic situation; the spread of the thresholds in the population causes the trend of the response intensity vs. flash duration curves; and (*b*) I, the light falling on the photoreceptor, depends on the orientation of the cell with respect to the light source, so the distribution of cell orientations in the population causes the trend of the experimental curves.

There may even be an overlap between the two interpretations; in fact, the second hypothesis is obviously true, the real question being their relative weights.

In our experiments the first hypothesis seemed to carry more weight than the second; in fact, when the geometry of the flash source was changed (for instance, by using diffuse illumination from two lamps that lit up a ping pong ball with the sample inside) we found only small changes in the trend of the curves shown in Fig. 12, whereas if the second interpretation was the only valid one, we should obtain a step shape for these curves. In samples obtained from synchronized cultures of clones, the spread of cell parameters would presumably be narrower than in our samples; it is likely that in such a case the second hypothesis is more influential than the first.

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REFERENCES

Ascoli, C., M. Barbi, C. Frediani, and A. Muré. 1978. Measurements of *Euglena* motion parameters by laser light scattering. *Biophys. J.* 211:585–599.

Ascoli, C., M. Barbi, C. Frediani, D. Petracchi, and T. Ristori. 1980. Quasi-elastic light scattering for studying the motion of flagellated microorganisms. *Optica Acta.* 271:1203–1212.

Ascoli, C., and C. Frediani. 1980. Quasi-elastic light scattering in the measurement of the motion of flagellated algae. *In* Light Scattering in Liquids and Macromolecular Solutions. V. Degiorgio, M. Corti, and M. Giglio, editors. Plenum Publishing Corp., London. 183–198.

Benedetti, P. A., G. Bianchini, A. Checcucci, R. Ferrara, S. Grassi, and D. Percival. 1976. Spectroscopic properties and related functions of stigma measured in living cells of *Euglena gracilis. Arch. Microbiol.* 111:73–76.

Benedetti, P. A., and F. Lenci. 1977. In vivo microspectrofluorometry of photoreceptor pigments in *Euglena gracilis. Photochem. Photobiol.* 26:315–318.

Boscov, J. S., and M. E. Feinleib. 1979. Phototactic response of *Chlamydomonas* to flashes of light. II. Response of individual cells. *Photochem. Photobiol.* 30:499–505.

Chen, S. H., and F. R. Hallett. 1982. Determination of motile behavior of prokaryiotic and eukaryotic cells by quasi-elastic light scattering. *Q. Rev. Biophys.* 15:131–222.

Diehn, B. 1973. Phototaxis and sensory transduction of *Euglena. Science* (*Wash. DC*). 181:1009–1015.

Diehn, B. 1979. Photic responses and sensory transduction in protist. *In* Handbook of Sensory Physiology. H. Autrum, editor. Springer Verlag, Berlin. VII/6A:23–68.

Feinleib, M. E. 1980. Photomotile responses in flagellates. *In* Photoreception and Sensory Transduction. F. Lenci and G. Colombetti, editors. Plenum Publishing Corp., London. 15–68.

Foster, K. W., and R. D. Smyth. 1980. Ligth antennas in phototactic algae. *Microbiol. Rev.* 441:572–630.

Ghetti, F., G. Colombetti, F. Lenci, E. Campani, E. Polacco, and M. Quaglia. 1985. Fluorescence of *Euglena gracilis* photoreceptor pigment: an in vivo microspectrofluorometric study. *Photochem. Photobiol.* 421:29–33.

Lenci, F., and G. Colombetti. 1978. Photobehaviour of microorganisms. A biophysical approach. *Annu. Rev. Biophys. Bioeng.* 7:341–369.

Litvin, F. F., U. A. Sineshchekov, and V. A. Sineshchekov. 1978. Photoreceptor electric potential in the phototaxis of the alga *Haematococcus pluvialis. Nature* (*Lond.*). 271:476–478.

Schaefer, D. W., and B. J. Berne. 1975. Number fluctuation spectroscopy of motile microorganisms. *Biophys. J.* 15:785–794.

Schmidt, J. A., and R. Eckert. 1976. Calcium couples flagellar reversal to photostimulation in *Chlamydomonas reinhardii. Nature* (*Lond.*). 262:713–715.

Smyth, R. D., and H. C. Berg. 1982. Change in flagellar best frequency of *Chlamydomonas* in response to light. *Cell Motil. Suppl.* 1:211–215.