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# PHOTOSENSORY TRANSDUCTION IN THE FLAGELLATED ALGA, *EUGLENA GRACILIS*

## II. EVIDENCE THAT BLUE LIGHT EFFECTS ALTERATION IN $Na^+/K^+$ PERMEABILITY OF THE PHOTORECEPTOR MEMBRANE

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## Summary

1. The blue light-induced cell tumbling behavior (the step-down photophobic response) and the accumulation of cells into a blue light trap (photoaccumulation) were investigated in *Euglena*. Dose response plots for these phenomena which we collectively term 'photobehavior' show both threshold and saturation characteristics.

2. NaCl effects apparent elevation in the photosensitivity of the cell as evidenced by alteration of the dose response plot character and a lowering of the light intensity saturation level.

3. NaCl and ouabain enhance the duration of the photophobic responses and the rate of photoaccumulation. KCl and $NH_4Cl$ have lesser or inhibitory effects.

4. Choline chloride reduces the duration of the photophobic responses and the rate of photoaccumulation.

5. KCl reduces the enhancement of photobehavior induced by NaCl and at constant chloride concentration, photobehavior is unaffected by the relative KCl and NaCl concentrations.

6. Antagonists of voltage-dependent, monovalent cation fluxes in membranes (tetrodotoxin, procaine, tetraethylammonium, 4-aminopyridine) do not alter photobehavior.

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7. The results suggest a role for a photoreceptor membrane-located transport system for $Na^+/K^+$ as a key step in control of the intraflagellar free $Ca^{2+}$ levels that determine the photobehavior mediated by flagellar reorientation.

## Introduction

The flagellated alga *Euglena gracilis* responds to alterations in the incident light intensity in its environment by showing a characteristic behavioral response, the photophobic response [1]. This photobehavior to either an increase [2—4] or decrease [2,5—7] in incident light intensity, is observed as a transition, often abrupt, from straight path forward swimming to a disoriented and discontinuous tumbling behavior of the cell. These tumbling responses are temporary and forward swimming is resumed as the cell presumably adapts to the new incident light intensity. Such behavioral responses to a reduction in incident light intensity are responsible for accumulation of *Euglena* in light beams of certain, actinic, wavelengths [2,6,8]. Recent determination of the relative rates of photoaccumulation of *Euglena* in such light traps of different wavelengths [9] and of the spectral sensitivity (action spectrum) of the responses of cells to a reduction in incident light intensity [10], suggests that the chromophore mediating this step-down photobehavior has flavin characteristics. In vivo microfluorimetry [11] indicates that this chromophore is located in or near a small organelle (the paraflagellar body) sited near the base of the single locomotory flagellum and enclosed in a contiguous plasma membrane surrounding the flagellum [12]. The functional integrity of this membrane appears to be crucial for the cell to be able to execute step-down photobehavior [12]. Photobehavior is effected as a result of a light-induced alteration in the orientation, with respect to the long axis of the cell, of the effective power stroke of the locomotory flagellum [1—4,7].

In recent years, several authors have speculated as to the nature of the physiological linkage between chromophore (flavin) excitation in *Euglena* and the induction of flagellar reorientation. Diehn [13] and Bovee and Jahn [14] suggested that light could alter ionic gradients across the photoreceptor membrane. Similar hypotheses involving light-mediated alterations in proton fluxes were proposed by Tollin [15] and Froehlich and Diehn [16]. Current evidence [17—19] indicates that flagellar activity in eukaryotic unicells is regulated by the intraflagellar free $Ca^{2+}$ concentrations. In a previous paper [7], we showed that *Euglena* flagellar activity appears to be controlled in a similar manner. In the same paper, we examined the simplest theoretical coupling mechanism between light-stimulation of the cell and such elevation in intraflagellar calcium levels. In such a mechanism, blue light would effect an alteration in the conductance of the flagellar-photoreceptor membrane to $Ca^{2+}$ in an analogous manner to that by which intraciliary free calcium ion concentrations in *Paramecium* are controlled by a ciliary membrane located, gated channel for $Ca^{2+}$ [20]. Our results for *Euglena* [7] indicated both that $Ca^{2+}$ controlled flagellar activity and that the $Ca^{2+}$ was derived from the extracellular medium rather than from intracellular or intraflagellar sites. However, the sensitivity of *Euglena* photobehavior to other divalent cations and to a recognized antagonist

of gated $Ca^{2+}$ fluxes was not consistent with the presence of a light-sensitive, voltage-dependent control of photoreceptor membrane permeability to $Ca^{2+}$ as a means to control flagellar activity.

These results suggested that flagellar-photoreceptor membrane permeability to $Ca^{2+}$ may be controlled indirectly by other ion fluxes. This paper reports the effects of monovalent cations, anions and cation flux antagonists on the blue-light induced photobehavior of *Euglena*.

## Materials and Methods

### *Materials*

*E. gracilis* Z were cultured by discontinuous methods under constant light intensity as previously described, either on standard Bloomington media [7] or on a glutamate-malate medium [21]. Provided the cells were washed free of culture media, no difference in photobehavior was found apart from a general improvement in sensitivity and reproducibility of behavior for cells grown in the glutamate-malate medium which was adopted for most of these studies. The cells were harvested in late-logarithmic growth and then adapted, after two cycles of washing by centrifugation, to a chloride salt, buffered medium ('Cl-adaptation buffer': 1 mM Tris/0.5 mM CaCl/0.125 mM $MgCl_2$/1 mM KCl, pH 7.1) for 6—8 h prior to use under dim red light (over 600 nm, approx. 70 $mW \cdot m^{-2}$) at 25°C [7]. All cell manipulations were carried out under this dim red light.

Chemicals were obtained from the following sources: choline chloride from Merck (Elmsford, NY); tetraethylammonium bromide, 99%, from Matheson, Coleman and Bell (Cincinnati, OH); ouabain from Nutritional Biochemicals (Cleveland, OH); 4-aminopyridine, 99%, from Aldrich Chemical Co., (Milwaukee, WI) and ouabain and procaine hydrochloride from Sigma Chemical Co., (St. Louis, MO). The tetrodotoxin (Sigma) was a gift from Dr. Leonard Nelson (Medical College of Ohio at Toledo). All inorganic salts were of commercial analytical grade. All chemicals and drugs were prepared in buffered solution as detailed above, on the day of use. Buffers were prepared in double-distilled water.

### *Methods*

Blue light-induced photobehavior was effected by removal of 466 nm lateral illumination from suspensions of *Euglena* ($1 \cdot 10^5$ cells/ml) following a constant (60 s) period of illumination at any of the light intensities used. Cells were viewed under red light (from below microscope stage, over >610 nm, approx. 2.4 $W \cdot m^{-2}$) and the response durations checked manually with a stop-watch to 0.25 s accuracy [7]. Photoaccumulation behavior into a 475 nm light trap was measured using the 'phototaxigraph' under conditions described previously [7]. The effects of all chemicals on photobehavior were evaluated 2—5 min after exposure of the cells to the test chemical. Samples were in addition routinely checked 30—90 min after exposure to a chemical for any long-term effects on either motility or photobehavior. All data are reported, except where indicated, as mean ± weighted S.D. for small $n$ [42].

## Results

*Duration of light-induced cell tumbling as a function of light intensity change: Dose-response plot*

Illumination of *Euglena*, in the standard Cl-containing buffer detailed above, with low-intensity blue light ($I = 0.1$—$350$ mW · m$^{-2}$) does not effect any apparent alteration in cell behavior. By casual observation, the cells continue to swim in relatively straight paths with only occasional changes in direction. An analysis of the cells' behavior on video tape confirms this observation. With a half-saturating dose of incident blue light ($I = 40$ mW · m$^{-2}$, see Fig. 1), changes in cell-swimming velocity, or frequency of directional change in the swimming path, were less than 10% (Doughty, M.J., unpublished results). Such changes were transient and lasted no longer than 10—15 s, indicating rapid adaptation of the cell to low-intensity blue light. However, at higher incident intensities (more than 100 W · m$^{-2}$), depending on the wavelength of light used, significant alterations in swimming velocity and frequency of directional changes were noted. Such intensities were not used in any of the experiments detailed in this report. After a constant period of illumination with blue light, i.e. delivery of a known dose at a given dose rate of incident light to the cell population, the blue light was removed. The cells then executed a series of

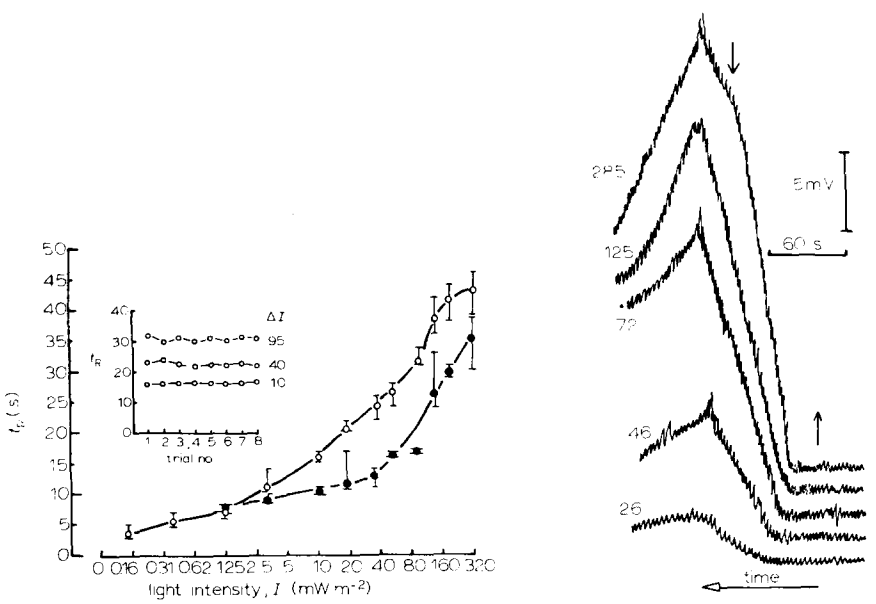


**Fig. 1. Dose-response plot for the step-down photophobic response. Duration of the step-down photophobic responses (cell tumbling $t_R$) as a function of pre-stimulus blue light intensity. Cells adapted and tested in Cl$^-$ adaptation buffer. Mean and range of values given for 3—7 determinations ($n$ = 3—7) on two batches of cells ($s$ = 2). (0) 19.5—20° C; (○) 23° C. Inset: Responses of cells to repeated trials at 23° C and various light intensities. ●, $t_{CFR}$; ○, $t_R$.**

**Fig. 2. Photoaccumulation in a blue light trap as assayed in the phototaxigraph. Typical traces to show photoaccumulation (difference signal from photomultiplier tubes [7]). Light intensity of trap ($I$) given next to each trace in mW · m$^{-2}$; 25° C; 4 · 10$^5$ cells/ml. ↑, light switched on; ↓, light off. Cells adapted and tested in Cl$^-$ adaptation buffer.**

behavior types prior to return to straight path swimming. The cells undergo a period of continuous tumbling (continuous flagellar reorientation, CFR) followed by a series of shorter-duration, interrupted tumbling events (periodic flagellar reorientaton, PFR) until forward-swimming is resumed, by the majority (more than 80%) of the cells at time $t_R$ (in s) (see Ref. 7 for full details).

As the incident intensity used for the illumination is increased, i.e. as the incident dose rate (and dose) of blue photons is increased, the duration of both the CFR response ($t_{CFR}$, data not shown) and the total duration of the photophobic responses ($t_R = t_{CFR} + t_{PFR}$) following removal of the blue light ($\Delta I = I$ in all experiments), increases up to a certain incident dose and then the dose-response plot levels off (Fig. 1). Half-saturation of the response is achieved at an incident light intensity of 40 mW · $m^{-2}$. The dose-response plot (plotted as a function of incident intensity since a constant illumination period was used) shows both a threshold and plateau (Fig. 1) which are more pronounced at lower temperatures.

After return to forward swimming behavior, the cells can be exposed to blue light again for a further 60 s and, under the experimental conditions used here, they exhibit photophobic responses of the same duration on darkening. Routinely, subsequent exposure to blue light was carried out 60 s after the pervious darkening, by which time the majority of cells, with any of the light intensities used in these experiments, had resumed forward swimming. Using this protocol, a cycle of blue light exposure followed by darkening can be repeated several (at least 6—8) times with each subsequent photoresponse being identical to the previous one (see insets to Fig. 1, for example). The second and subsequent exposures can, however, be given immediately after return to forward swimming (this time is dependent on the light intensity/dose used — see below) with the same results. Such experiments were carried out principally to determine the stability of the cells' response under test conditions and are detailed to show that conventional fatigue does not occur under these conditions. We can additonally conclude that light-induced adaptation (i.e. an alteration of the response threshold that would result in a change of response time because of a change in perceived stimulus intensity) does not also occur on the time scale used for these successive trials. For an example of the occurrence of such adaptation, see below in our discussion of NaCl effects.

*Rate of photoaccumulation in a blue light trap as a function of the light intensity difference at the trap boundary*

In the 'phototaxigraph' light trap, *Euglena* rapidly accumulate in an actinic beam (Fig. 2). As the intensity of the actinic beam ($I$) is increased, i.e. as the light intensity difference, $\Delta I$, ($=I$ in all phototaxigraph experiments) between the actinic beam and the surrounding dark area is increased, the rate of photoaccumulation increases (Fig. 2). In view of the relatively low cell concentrations used ($2—4 \cdot 10^5$ cells/ml), it is unlikely that there is significant light scattered from the cells to soften the light/dark boundary (see Ref. 8 for discussion on this point). Therefore, $\Delta I$ is the light intensity change that the cells will experience on trying to move out of the actinic beam (having entered by random motion [2,6,8]) and equals the incident intensity of the actinic beam, $I$, as a first approximation. The period of actinic illuminiation of the cell sus-

pension in the phototaxigraph cuvette is the same as that for studies on the photobehavior of single cells (60 s) and was selected such that aberrations from uniform light intensity in the trap (due to scattering of light by cells as they accumulate in the trap) over the time period studied, can be expected to be minimal. If the actinic illumination is maintained, at any of the intensities tested (6—320 mW · $m^{-2}$), the cells continue to accumulate essentially at a linear rate for at least 3 min (data not shown). After the actinic beam is switched off, the accumulation continues for a few seconds usually at the same rate as in the presence of the actinic beam and then gradually declines as the cells, in the absence of a trap boundary and adapting to zero light intensity, distribute themselves throughout the cuvette again. Under any particular extracellular cation concentration, the lag between removal of the blue light and the decline in photoaccumulation remains essentially constant for cells adapted to the $Cl^-$-containing solutions used in this study. As shown in Fig. 3, the initial rate of photoaccumulation increases as a function of $I$ (and therefore $\Delta I$) from an apparent threshold at approx. 12 mW · $m^{-2}$ under these ionic conditions. The relationship between the rate of photoaccumulation and log $\Delta I$ is not linear over the whole range of light intensities tested, but shows a small but significant deviation at lower intensities. The dose-response plot shows no sign of saturation at 280 mW · $m^{-2}$ in the phototaxigraph.

*Alteration of photosensitivity (log ΔI-response plots) by elevation in extracellular NaCl concentrations*

During preliminary experiments relating to the general cation sensitivity of *Euglena* behavior and photobehavior, it was noted that the addition of NaCl to the test solutions, but not to the adaptation buffer, effected an apparent enhancement of the duration of the photophobic responses of the cells. Subsequent careful analysis (Figs. 4 and 5) shows that addition of millimolar concentrations of NaCl to the adaptation buffer just prior to testing the cells for either light-induced tumbling behavior (Fig. 4) or rate of photoaccumulation (Fig. 5) effected the following alterations in the photosensitivity of *Euglena*.

Dose-response plots for blue light-induced cell tumbling are shifted in sensitivity to lower intensities, i.e. saturation is now observed at an incident intensity of 40 mW · $m^{-2}$ in the presence of 8 mM NaCl compared to 160 mW · $m^{-2}$

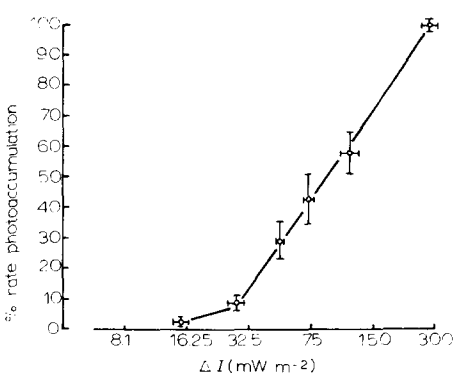


**Fig. 3. Initial rate of photoaccumulation in a blue light trap as a function of light trap intensity. $n$ = 7; $s$ = 7; 25°C; 4 · $10^5$ cells/ml.**

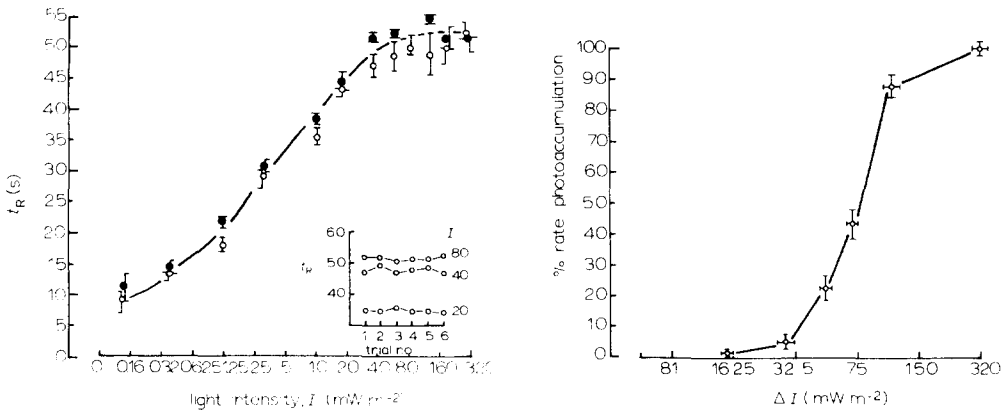


Fig. 4. Effect of NaCl on dose-response relationship for step-down photophobic response ($t_R$). Cells were adapted to $Cl^-$ adaptation buffer and then 8 mM NaCl were added just prior to test. Mean and range of values given. (0) 19—20°C, $n$ = 5—7, $s$ = 2; (○) 23°C, $n$ = 2—4, $s$ = 2. Inset: Responses of cells to repeated trials at 23°C and various light intensities. ●; $t_{CFR}$; ○, $t_R$.

Fig. 5. Effect of NaCl on relationship between rate of photoaccumulation in a blue light trap and light trap intensity. Cells were adapted to $Cl^-$ adaptation and then 8 mM NaCl was added just prior to test. $n$ = 6; $s$ = 6; 25°C; 4 · $10^5$ cells/ml.

in the absence of added extracellular NaCl (Fig. 4) compared to Fig. 1).

The cells' response in a sequence of trials still remains constant (see insets to Fig. 4). As discussed above, such results show that, under these test conditions, fatigue does not occur. Neither has the kinetics of adaptation, if it occurs, been altered. These observations are considered significant since, on change of the extracellular anion species, the responses to darkening following a standard illumination period get progressively longer with each successive cycle of testing (reillumination given 60 s after the previous darkening) to eventually give a constant response after 4—8 cycles (unpublished results). Such alteration in response indicates the existance of an adaptation phenomenon (in the sense of a conditionally induced change in cell response with sucessive trials, which of course in this case functionally is a sensitization rather than fatigue. Further studies are necessary to determine if this is indeed 'receptor adaptation' and to distinguish it from potential 'chromophore adaptation' or fatigue as previously indicated in this cell [43].

A semi-logarithmic plot of the relationship between rate of photoaccumulation in a blue light trap and the light intensity expected at the light/dark boundary is different for cells treated with NaCl compared to cells adapted and maintained in 'sodium-free' solutions (compare Figs. 5 and 3). The relationship is now distinctly sigmoidal and 'saturation' is achieved at lower light intensities (i.e., light intensity differences that the cell perceives at the trap boundary) than for cells in medium without NaCl.

*Effect of extracellular addition of monovalent cation chloride salts on the photobehavior of chloride-adapted cells*

In view of the marked effects of NaCl on the photosensitivity of *Euglena*, the effects of various monovalent cation chloride salts on the photobehavior of the cells to half-saturation stimuli were investigated.

Extracellular NaCl (1—8 mM) enhances the duration of both the initial

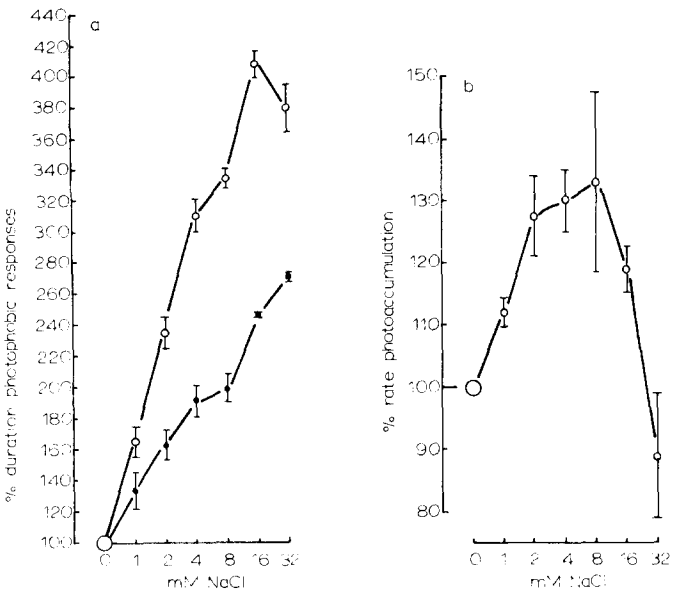


Fig. 6. Effect of NaCl on duration of photophobic response to blue light removal and the rate of photoaccumulation of cells in a blue light trap. Cells adapted to $Cl^-$ adaptation buffer; NaCl added just prior to test. (a) Duration of photophobic responses. $\Delta I = -40$ mW · m$^{-2}$. ○, $t_R$; ●, $t_{CFR}$; 19—20°C; $n$ = 6—11; $s$ = 3. (b) Initial rate of photoaccumulation. $\Delta I$ = approx. 120 mW · m$^{-2}$, 25°C. $n$ = 4; $s$ = 4.

tumbling ($t_{CFR}$) and the total photophobic response ($t_R$) Fig. 6a). The same concentrations of NaCl also enhance the initial rate of photoaccumulation of the cells in blue light trap (Fig. 6b).

Higher concentrations of NaCl cause further enhancement of the duration of blue light-induced cell tumbling and apparent saturation of the mechanisms determining the total duration of the photophobic response ($t_R = t_{CFR} + t_{PFR}$) (Fig. 6a). At higher concentrations of NaCl, the rate of photoaccumulation is reduced (Fig. 6b). The reason for this is uncertain since it would not appear to be a simple chemo- or photo-kinetic effect. Under non-actinic illumination, such concentrations of NaCl do not cause a significant change in either cell swimming velocity or frequency of directional change. Following execution of the photophobic response, the behavior of the cells is essentially equal to that prior to the blue light exposure.

Extracellular KCl (2—32 mM) has no significant effect on the duration of CFR responses to blue light (Fig. 7a), but effects a concentration-dependent increase in the total response, $t_R$ (Fig. 7a). All KCl concentrations tested have an insignificant effect on the rate of photoaccumulation of the cells in a light trap (Fig. 7b).

Elevation in the extracellular concentration of $NH_4Cl$ (1—32 mM) has little effect on either the initial photophobic-tumbling response of the total response of individual cells (Fig. 8).

Application of the chloride salt of an impermeant cation, choline chloride [22], however effects a marked reduction in the duration of the photophobic response (Fig. 9a) and the rate of photoaccumulation of cells in a light trap is also reduced in a concentration-dependent manner (Fig. 9b).

Alteration in the extracellular concentrations of either NaCl, KCl, $NH_4Cl$ or

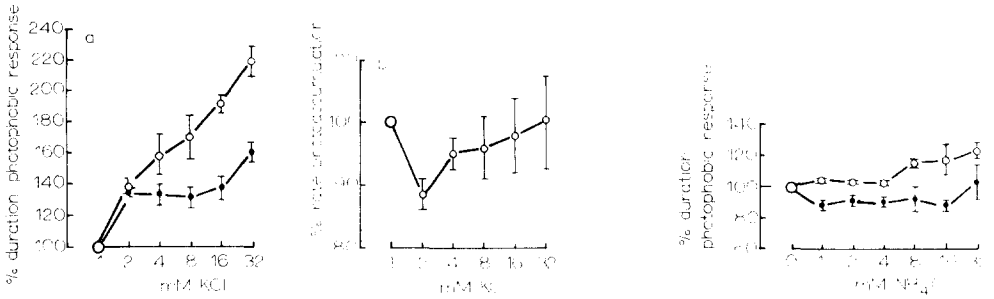

Fig. 7. Effect of KCl on duration of photophobic response to blue light removal and the rate of photoaccumulation of cells in a blue light trap. Cells adapted to $Cl^-$ adaptation buffer; KCl added just prior to test. (a) Duration of photophobic responses. $\Delta I$ = —40 mW · $m^{-2}$, 19—20°C. ○, $t_R$; ●, $t_{CFR}$; $n$ = 6—9, $s$ = 2. (b) Initial rate photoaccumulation. $\Delta I$ = approx. 120 mW · $m^{-2}$, 25°C, $n$ = 7; $s$ = 7.

Fig. 8. (Right-hand) Effect of $NH_4Cl$ on duration of step-down photophobic responses to blue light removal. Cells in $Cl^-$ adaptation buffer; $NH_4Cl$ added just prior to test. $\Delta I$ = —40 mW · $m^{-2}$, 19—20°C. ○, $t_R$; ●, $t_{CFR}$; $n$ = 4—6; $s$ = 1.

choline chloride does not result in fatigue of the cells or affect the ability of the cells to adjust to the lack of blue light, i.e. the response durations during a series of trials remain essentially constant provided the extracellular anion species is $Cl^-$ (data not shown).

These alterations in the light responses of *Euglena* by short-term exposure to monovalent cation chloride salts cannot be explained solely in terms of the action of the cation. The saturation phenomena observed in the presence of high concentrations of NaCl may be in part due to the action of chloride itself (especially in view of the effects of choline chloride on photobehavior). At constant extracellular $Cl^-$ concentration (32.625 mM, including the divalent cation chloride salts present as well in all buffers) and constant $Ca^{2+}$ and $Mg^{2+}$, regardless of the concentrations of $Na^+$ and $K^+$, the rate of photoaccumulation of the cells in a light trap remains essentially constant (Fig. 10). The relative response of individual cells to constant incident light dose in the presence of different concentrations of NaCl is altered by the KCl concentration present

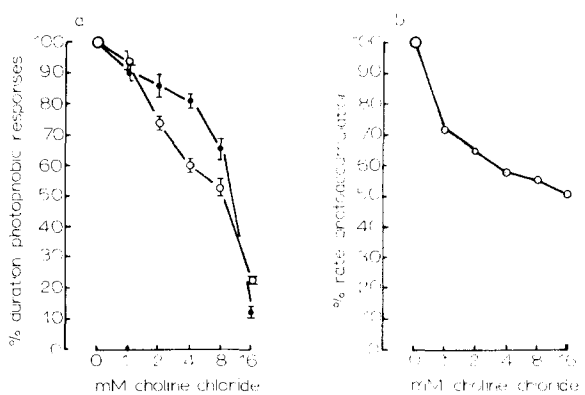

Fig. 9. Effect of choline chloride on duration of photophobic responses to blue light removal and rate of photoaccumulation of cells in a blue light trap. Cells adapted to $Cl^-$ adaptation buffer; choline chloride added just prior to test. (a) Duration of photophobic responses. $\Delta I$ = —40 mW · $m^{-2}$, 19—19.5°C. ○, $t_R$; ●, $t_{CFR}$; $n$ = 8—12; $s$ = 2. (b) Initial Rate of photoaccumulation. $\Delta I$ = approx. —120 mW · $m^{-2}$, 25°C. $n$ = 2; $s$ = 2. Mean value given.

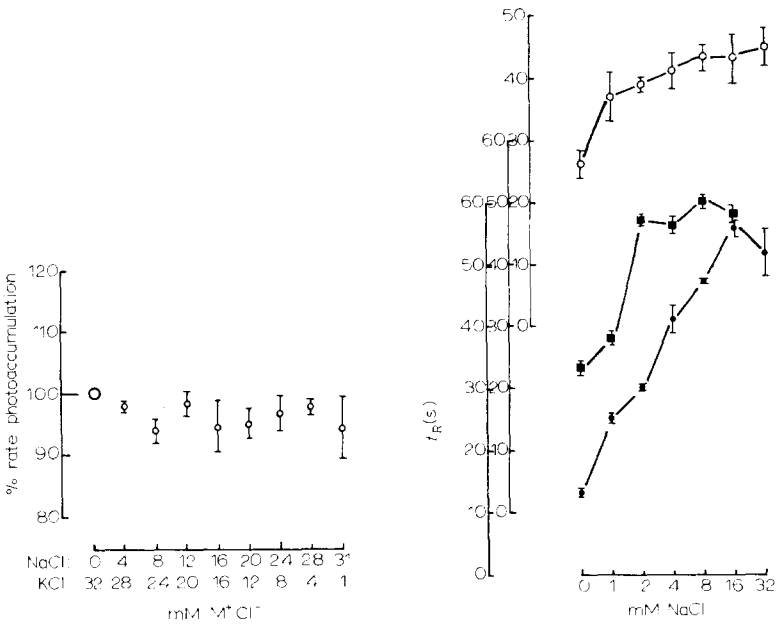


Fig. 10. (Right hand) Effect of NaCl and KCl on the initial rate of photoaccumulation of cells into a blue light trap in the presence of a constant $Cl^-$ concentration. Cells adapted to $Cl^-$ adaptation buffer and then NaCl and KCl added just priot to test. $\Delta I$ = approx. 120 mW · $m^{-2}$, 25°C. $n$ = 4—7; $s$ = 4—7.

Fig. 11. (Left-hand) Effect of KCl on the enhancement of photophobic response duration ($t_R$) induced by NaCl. Cells adapted to $Cl^-$ adaptation buffer; KCl and then NaCl (concentrations given on abscissa) were added just prior to test. $\Delta I$ = —40 mW · $m^{-2}$, 19—20°C, ●, zero KCl; $s$ = 1; $n$ = 4. ■, 5 mM KCl; $s$ = 1; $n$ = 4; ○, 17 mM KCl; $s$ = 2, $n$ = 6—8.

(Fig. 11). In view of the effects of KCl alone on the cells' photophobic responses (Fig. 7a), such reduction in the apparent effectiveness of NaCl to stimulate photophobic responses is probably in part related to the $Cl^-$ concentration in the extracellular medium.

*Effect of an inhibitor of coupled $Na^+/K^+$ transport on photobehavior*

The above results on the effects of $Na^+$ and $K^+$ on the photobehavior of *Euglena* and the interdependence of their effects indicated that the relative ratios of $Na^+$:$K^+$ are important. A recognized specific inhibitor of energy dependent, Na/K transport across eukaryotic membranes was therefore tested on *Euglena*.

The ionotropic drug, ouabain (inhibits coupled Na/K transport through inhibition of a $Mg^{2+}$-dependent, ($Na^+ + K^+$)-stimulated ATPase enzyme in the membrane of other eukaryotic cells [23] has a marked effect on the photobehavior of *Euglena*. Short-term (2—5 min) preincubation of *Euglena* with ouabain results in a marked enhancement of the duration of the initial photophobic tumbling response to blue light and the total response duration (Fig. 12a). Maximal enhancement of the photophobic responses of individuals cells is achieved at an applied drug concentration of 0.5 mM with higher concentrations having an adverse effect. Higher concentrations (>0.5 mM) also have an adverse effect on motility after longer incubation (5—30 min depending on concentration). At these higher concentrations of ouabain, a variable percent-

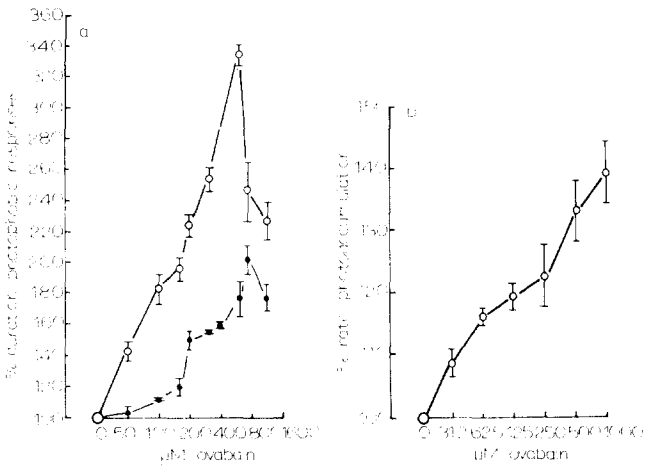

Fig. 12. Effect of ouabain on the duration of photophobic responses to blue light removal and initial rate of photoaccumulation of cells in a blue light trap. Cells adapted to $Cl^-$ adaptation buffer; ouabain added just prior to test. (a) Duration of photophobic responses. $\Delta I = -40$ mW · m$^{-2}$, 23°C, ○, $t_R$; ●, $t_{CFR}$. $n = 8$—11; $s = 2$. (b) Initial rate of photoaccumulation. $\Delta I = 120$ mW · m$^{-2}$, 25°C. $n = 4$; $s = 4$.

age of the cell population (10—40%) exhibit slow or erratic motion or even settle temporarily on the glass slide. The effects of ouabain on the blue light-induced photophobic responses are noteworthy in that, as with the effects of divalent cations and in particular $Ca^{2+}$ [7], the initial CFR response is altered in essentially a consistent relative manner to the total response, suggesting that $Na^+$ and $K^+$ movements are coupled intimately to the control of photomovement rather than just one ion alone. This conclusion is supported by the observation that application of $Na^+$ or $K^+$ alone effects differential alteration of $t_{CFR}$ and $t_R$ (Figs. 6 and 7).

Ouabain effects a smaller, somewhat variable, enhancement of the rate of photoaccumulation of *Euglena* in a light trap (Fig. 12b). Preliminary results (two experimental runs only) show that, in the presence of 0.5 mM ouabain, the 'dose'-response plots for photoaccumulation as a function of actinic light intensity show similar sigmoidal character to those obtained in the presence of NaCl in the extracellular medium (see Fig. 5).

*Effects of antagonists of voltage-dependent cation fluxes (conductance antagonists) across membranes on photobehavior*

In a previous paper [7], we concluded that available evidence did not support a case for a direct light activation of calcium conductance in the flagellar-photoreceptor membrane in *Euglena*. To test this conclusion further and to extend it to other cation conductance activation phenomena, we tested the effects of various recognized antagonists of voltage-dependent, gated cation fluxes across biological membranes. The results are summarized in Table I. As reported previously [7], the $Ca^{2+}$ conductance antagonist, verapamil, has no effect on either photophobic responses of individual cells or photoaccumulation. The $Na^+$ conductance channel blocker, tetrodotoxin [40], likewise has no effect. The local anesthetic, procaine hydrochloride, known to block secondary

TABLE I

EFFECT OF ANTAGONISTS OF ION FLUXES ACROSS ELECTRICALLY EXCITABLE MEMBRANES ON THE PHOTOBEHAVIOR OF *EUGLENA*

All cells were adapted to $Cl^-$ adaptation buffer and then drugs were added 2—5 min prior to test for photobehavior. Usually concentrations were tested in a dilution series with each concentration being half that of the previous one, except tetrodotoxin which was tested in a 1:10 series. Percentage of change measured was maximum percentage of alteration observed in any experiment and invariably attained in the presence of the highest concentration of the drug tested. The results reported are for the total duration of the photophobic response ($t_R$). Essentially identical results were found for $t_{CFR}$: Verapamil, tetrodotoxin, procaine and tetraethylammonium had no effect on photoaccumulation. For toxicity tests, cells were incubated with drugs, at various concentrations, for 12 h under dim red light at 25°C. 0, less than 5% dead or immotile after test period of 12 h; +, 5—10% dead or immotile; ++, 10—20%; +++, 90%.

| Reagent | Flux altered | Concentrations tested | Change (%) | Toxicity |
|---|---|---|---|---|
| Verapamil | $Ca^{2+}$ | $1 \cdot 10^{-7}$—$1 \cdot 10^{-4}$ M | 0 | + |
| $La^{3+}$ | $Ca^{2+}$ | $1 \cdot 10^{-8}$—$1 \cdot 10^{-4}$ M | —100 * | +++ |
| Tetrodotoxin | $Na^+$ | $1 \cdot 10^{-9}$—$1 \cdot 10^{-6}$ M | 0 | n.d. |
| Procaine-HCl | $Na^+$ | $1 \cdot 10^{-3}$—$8 \cdot 10^{-3}$ M | +15 | ++ |
| Tetraethylammonium | $K^+$ | $1 \cdot 10^{-4}$—$4 \cdot 10^{-3}$ M | +10 | + |
| 4-Aminopyridine | $K^+$ | $1 \cdot 10^{-5}$—$4 \cdot 10^{-3}$ M | +12 | 0 |

* Preliminary data.

$Na^+$ currents in excitable membranes [24], has no effect on photobehavior of individual cells. Tetraethylammonium bromide, know for its action in suppressing late outward-rectifying $K^+$ currents associated with both $Na^+$- and $Ca^{2+}$-dependent electrogenesis when applied extracellularly [25,26], and 4-aminopyridine, recently reported to have similar actions to tetraethylammonium salts [27,28], have no effect on photobehavior, either. Alterations in photobehavior induced by these drugs were not significant and did not change after longer incubation times than the standard 2—5 min exposure. In all cases, the photobehavior appeared normal in character and the cells responded with constant duration behavioral patterns for at least 4—6 trials.

## Discussion

This study shows that the expression of photobehavior in *Euglena* is markedly influenced by the concentrations of monovalent cations in the extracellular medium. The overall correlation between the alteration in the duration of the step-down photophobic (cell tumbling) response and the changes in the rate of photoaccumulation of the cells in light of the same wavelength (blue), provides substantiative evidence for the early observations [2,6] that the rate of photoaccumulation of *Euglena* is directly related to the 'strength' of the photophobic response. Overall, it would appear that, providing the motility of the cells is not affected by either the increase in light intensity or the chemical environment of the cells, the rate of photoaccumulation represents at least a semi-quantitative analytical assay for assessing the effects of various chemicals and drugs on the photosensory transduction system in this cell. Since the phototaxigraph method involves testing a larger percentage of the cell population and a larger number of cells than can be conveniently screened by micro-

scope methods, the photoaccumulation method can be expected, within certain limitations, to represent more accurately the effects of a particular chemical on the photobehavior of *Euglena*. However, it should be ascertained by preliminary examination or analysis that the particular chemical being tested does not interfere with the motility of the cell, since such alteration can give rise to erroneous conclusions concerning the effects of an agent on photobehavior (see Ref. 7).

The dose-response plots for *Euglena* photobehavior as a function of intensity indicate that the expression of photobehavior and its control are not simply dependent upon the generation of a photochemical intermediate that then effects flagellar reorientation. The characteristics of the dose-response plots and in particular their alteration by salts and temperature, demonstrate that such incident $\Delta I$ vs. response plots probably reflect more than just a primary photochemical reaction associated with the chromophore-mediated photobehavior and that previous hypotheses concerning the molecular photochemistry of this cell in relation to its photobehavior [31] should be modified and extended. We acknowledge that alterations of flavin photochemistry and molecular interactions with proteins by inorganic ions are known.

Chemiluminescence of flavins in solution is quenched by relatively low concentrations of $Cl^-$ [32]; the rate of coproportionation of oxidized and fully reduced flavoproteins (flavodoxin) has been reported to be markedly enhanced by increasing ionic strength (with a threshold effect being observed at approx. 30 mM NaCl) [33] and more recently, it has been shown that high concentrations of halide ions induce dissociation of FAD from its native holoenzyme [34]. However, in common with any eukaryotic plasma membrane [41], it is unlikely that the photoreceptor-flagellar membane of *Euglena* is freely permeable to any inorganic cationic species. Stress of a transport system by high concentrations of salts might lead to accumulation of these ionic species in time but, under such circumstances, cellular behavior can then be expected to be adversely affected.

Finally, because of the action of ouabain and because of the perturbation of photobehavior by a cationic detergent [12], we conclude that the alterations in photobehavior and photosensitivity of *Euglena* by monovalent and divalent cation salts, reflect alteration of membrane-sited molecular mechanism serving to control the fluxes of these ions through to the intraflagellar space. In view of the ouabain sensitivity, we propose that one molecular species associated with this flux control is a Na/K transport enzyme. Indeed, Na/K transport activity in other membranes (coupled to $(Na^+ + K^+)$ ATPase activity has been reported to be inhibited by ouabain [23,25]; inhibited by elevation in extracellular $Na^+$ concentrations [36,37] and stimulated by elevation in either extracellular $K^+$ and $NH_4^+$ concentrations [38,39]. Such coupled Na/K transport activity, in other membranes, serves to pump $Na^+$ out of the cell and to take up $K^+$ from the medium [23,26,35,38]. We note that while ouabain increases $t_{CFR}$ and $t_R$ in a parallel manner, application of $Na^+$ or $K^+$ alone result in differential alteration of $t_{CFR}$ and $t_R$ (Figs. 6 and 7). For any given $Na^+$ or $K^+$ concentration, the ratio of $t_{CFR}$:$t_R$ is different, generally decreasing with increasing $M^+$ concentration, though the effect is much smaller with $K^+$ than with $Na^+$.

The position and role of this proposed $Na^+/K^+$ transport system in the photo-

sensory transduction pathway is unknown at this time and its activity could be related to either photoreception events or to adaptation. We do not know how blue light may alter this proposed Na/K pump activity. However, other experiments indicate that the exact opposite flux directions predicted for the pump (i.e., $K^+$ efflux or $Na^+$ influx) seem to cause $Ca^{2+}$-dependent reorientation of the flagellum. This indicates that the pump may serve to build up a $K^+$ gradient during blue light exposure (unpublished data).

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