Phototaxis and Sensory Transduction in Euglena

Some motile unicells are guided toward favorable external conditions by true homeostatic systems.

Bodo Diehn

Unicellular organisms are particularly suitable for studies of the basic nature of stimulus-response systems because, unlike the higher organisms, they do not present the problem of complex interactions occurring between large numbers of mutually interdependent cells. Unicellular organisms thus provide fascinating model systems for studies of sensory perception.

The most conveniently observed response of a microorganism is its movement—it occurs without major delay, can be measured quantitatively, and can be recorded for detailed study. For this reason, the most significant advances in our understanding of sensory transduction at the cellular level have been made by studying the behavior of motile protozoa as a function of external stimulation. In this context, one can distinguish three classes of stimuli: (i) electromagnetic radiation, including light and heat; (ii) chemical stimuli, including direct electrical stimulation; and (iii) mechanical stimuli.

The flagellated photosynthetic alga *Euglena gracilis* was used for the studies I report in this article. It has long been known that *Euglena* cells respond to light by accumulating in an illuminated region (*1*). *Euglena* is also attracted by certain chemicals (*2*), and there are indications of motor responses to mechanical stimulation (*3*). Because the analysis of a signal transduction system requires study of the output (response) as it is affected by variations in the input, light has some unique advantages over chemical and mechanical stimulation. For example, when the light is turned off, the stimulus disappears completely. This is also the case with mechanical, but not with chemical stimulation. Light is also easily manipulated: The signal intensity can be varied and sequenced at will by the experimentor, and the stimulus can be applied topically. While mechanical stimulation might also appear suitable for topical application, it would be extremely difficult to keep such a stimulus applied evenly to part of a moving cell.

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Experimental Methods

Motor responses of microorganisms can in principle be studied by three methods. (i) Direct microscopic observation of the individual cells or of the position of flagella or cilia with respect to the cell's body as a function of stimulation is probably the most unequivocal method for elucidating qualitatively the physiological mechanisms that lead to a behavioral response. However, the observations are difficult to quantitate, particularly because information on the intensity of the response (frequency of flagellar beating, for example) is also desirable, and at present it seems impossible to automate methods for the evaluation of such experiments. (ii) If one knows which responses of the motor apparatus cause the cells to exhibit a particular type of motion, then by monitoring the motion of individual cells one can obtain much the same information as one can by direct microscopic observation of the organisms. A major advantage of this, however, is that it can be completely automated. Either a single cell can be tracked automatically in three dimensions (*4*), or the tracks of a number of cells can be recorded in two-dimensional projection and quantified by manual (*5*) or computer methods (*6*) to obtain linear velocities, or rates of directional change, for example. (iii) Methods for studying mass movement, that is, the accumulation or dispersal of a very large number of cells in a stimulated region, have inherently a high statistical accuracy, and allow automatic quantification with a moderate investment of instrumentation (*7*). They do not per se give information on the mechanism of accumulation or expulsion, but once that mechanism has been elucidated, methods for studying mass-movement are unequaled for rapid and reproducible assays of the response.

In my laboratory, we have used all those methods for studying *Euglena*. These include high-speed microcinematography for observing flagellar responses, a computer-coupled video system for the simultaneous analysis of individual swimming tracks (*6*), and our phototaxigraph for the study of light-induced accumulation and expulsion of cells (*8*).

In the stimulus-response systems of higher organisms, one distinguishes the receptor system, the internuncial or nervous system, and the output (muscle). Such a scheme can also be used in studies of the protozoa. The output system for light-induced responses of free-swimming *Euglena* is represented by the locomotory flagellum. It emerges at the anterior end of the cell and usually is in a trailing position. The second flagellum is nonemergent (Fig. 1). The beat of the locomotory flagellum is helical and causes the cell to rotate around its longitudinal axis. The thrust of this flagellum is asymmetric so that the cell is inclined toward the direction of motion; it thus describes a helical path with the axis of the helix defining the direction of progress. Because the flagellum propels the cell whether it is responding to a stimulus or not, the mechanics of flagellar motion are not necessarily of concern in the studies I describe, but the mechanism of flagellar reorientation is of great importance.

The Photoreceptor

A very prominent orange-red pigment spot on the "dorsal" side of the anterior end of the cell contains mostly carotenoids (*9*) and was called the "eyespot" by early investigators. The term "stigma" is more appropriate, however, because there is considerable

evidence that the pigment spot is not the photoreceptor proper. Engelmann (*10*) noted as early as 1882 that a sudden change of the cell's direction occurred when a boundary between a light and a dark area was moved across the anterior end of the cell, even before the dark area reached the stigma. Gössel (*11*) observed that a mutant of *Euglena* that contained no stigma still possessed the paraflagellar swelling at the base of the flagellum; this mutant exhibited light-induced motor responses, while *Astasia*, a relative of *Euglena* possessing neither stigma nor swelling, showed none.

While the presence of the paraflagellar swelling is necessary and usually sufficient for the cell to exhibit light-induced shock responses, we have observed that only cells with a stigma are capable of exhibiting positive phototaxis, defined as orientation with respect to the light source, followed by directed movement toward the light. Mast (*12*) was the first investigator to suggest that the stigma might act as a shading device for the photoreceptor proper but, until now, no mechanism that would accomplish orientation of the cell has been proposed.

A mechanism by which orientation could be achieved can be postulated on the basis of the light-induced shock responses of *Euglena*, for which we have coined the term "photophobic responses." When the illumination intensity is suddenly increased above a certain adaptation level, or, if an intensity which is below the adaptation level is suddenly decreased further, the cell stops its forward motion and begins to turn around the lateral axis which is normal to the dorsal-ventral plane (*13*). (The adaptation level may be considered a "double threshold"—for the direct response, if the light intensity, I, is increased; and for the inverse response if I is decreased.) This response persists until adaptation of the sensory system to the new level of illumination has taken place, typically within a few seconds to a minute. The response is initiated very rapidly; high-speed motion pictures show that flagellar reorientation occurs within some 20 to 50 milliseconds of the change in the light intensity (*14*). Restoration of the previous lighting conditions results in immediate termination of both the direct (or negative) photophobic response, occurring upon an increase in light intensity, and the inverse (positive) response which is initiated by a decrease in intensity. Our visual observations of the

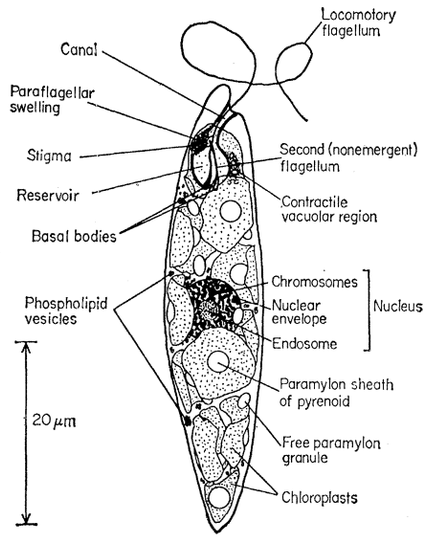

Fig. 1. Morphology of *Euglena gracilis*.

photophobic responses indicate that in the inverse response, the turn is toward the dorsal side, while in the direct response, the turn may be toward the ventral side (*15*). In contrast to this, Jennings (*16*) reports that in *Euglena viridis*, both the direct and the inverse responses are toward the dorsal side. We are now using cinephotomicrography in an attempt to resolve this question.

Periodic Shock Responses Mediate Tactic Orientation

If a cell is illuminated from one side with light of an intensity below the adaptation level, then the shadow of the stigma will fall upon the paraflagellar swelling once every revolution. This will result in a decrease of the incident light intensity and cause an inverse photophobic response which will cease when the photoreceptor is no longer shaded (*13*). The cell will thus make a fractional turn toward the dorsal side—that is, toward the side from which the light came when shading occurred. Repetition of this process results in further course corrections which end when the cell is oriented directly toward the light source, at which time shading of the photoreceptor by the stigma has become geometrically impossible.

If the intensity of the lateral illumination is above the adaptation level, then the cell will exhibit a direct photophobic response. Since every shading event stops this response, but "resets" the cell's sensory mechanism for a further direct response upon reillumination of the photoreceptor (Table 1), the only situation in which there will be no further phobic response is one in which the photoreceptor is permanently shaded. This can best be accomplished by the posterior end of the cell and requires the organism to be oriented directly away from the light source. For this mechanism of negative phototaxis, neither the presence of a stigma nor the direction of turning in the direct photophobic response are of any consequence in bringing about negative tactic orientation.

Experimental evidence that the repetitive shading mechanism accounts for positive phototaxis was obtained by lateral stimulation of *Euglena gracilis* with pulsed light. A resonant maximum of phototaxis was observed at a pulse frequency which corresponded to the frequency of rotation of the cells (*13*). In the following, I will assume this model to be correct.

The phototaxigraph (Fig. 2) is in essence a recording turbidimeter with a double beam. With this instrument we have observed that the accumulation of cells in an illuminated region results from both the positive phototaxis of cells that are outside the actinic zone (they respond to light scattered by the cells already inside), and the trapping in the illuminated zone of organisms that enter it through phototaxis or by chance. Cells experience an inverse photophobic response at the light-dark boundary and are thus essentially reflected back into the illuminated region. We have observed that *Euglena* cells that appear entirely normal but are demonstrably nonphototactic, nevertheless accumulate in illuminated zones (*17*). However, positive phototaxis also proceeds by way of inverse photophobic responses, and direct measurements of only the latter phenomenon are obtained with the phototaxigraph.

Dispersal from the actinic zone must be the consequence of photophobic responses only, because negative phototaxis would not direct the cells out of the illuminated region. Hence the photophobic expulsion observed with the phototaxigraph in no way involves the stigma and may thus be utilized to identify the photoreceptor molecule proper, without interference from the shading pigments, by the determination of action spectra of the photophobic response. By using polarized stimulating light to determine the action spectra of photophobic responses we discovered two shading systems for the photoreceptor. One of these operates in the visible range of light, and has the

long axes of its molecules (the carotenoids in the stigma) aligned parallel to the long axis of the organism, while the other functions in the ultraviolet and has the electric dipole transition moments of its molecules aligned perpendicularly to those of the long wavelength system (*18*). We are not at present ruling out the possibility that instead of the screening molecules, the photoreceptor molecules may be oriented. The latter molecule, as deduced from action spectra measurements and from the effects of fluorescence quenchers, is probably a flavin derivative held rigidly in place in a lipid environment (*19*).

In a reducing environment, *Euglena* exhibits an inverse photophobic response (but not a direct response) upon stimulation with light of high intensity in the 620- to 680-nanometer band of the red wavelength region (*20*). No phototaxis can be demonstrated, probably because the stigma absorbs only very little light at these wavelengths and thus cannot shade efficiently (*21*).

A Systems Analytical Approach to Stimulus Transduction

While there are specific organelles for both the receptor and output functions in *Euglena*, the existence of an analog to the internuncial system of higher organisms can only be inferred. I have attempted to characterize the dynamics of such a system, which I call the "processor," by studying its input-output relationships. This is a problem in systems analysis, but a much more difficult one than the more conventional type of "forward analysis" which is used in engineering to predict the performance of a system if input as well as system dynamics are known.

First I defined the minimum components of the sensory transduction system that mediates phototaxis in *Euglena*, and the probable interactions between the components.

The sensory chain starts with the photoreceptor, whose output is modulated by the shading action of the stigma. The photoreceptor signal is then, in the processor, converted to a command which acts on an effector such as to cause reorientation of the flagellum. Since the direct and inverse phobic responses appear to be different, one would expect that there are separate effectors for the two photophobic responses.

Table 1. Photophobic responses of *Euglena* as a function of light intensity and of prior adaptation.

Conditions	Intensity of white light (erg/cm² sec)		
	8×10^5	2×10^5	5×10^3
	Light-adapted cells		
Light off	No immediate shock; slight delayed shock	Shocks 50 percent of cells	Shock
Light on after 1.0-sec dark	Shock	Stop shock; shocks previously unshocked cells	Stops shock
	Dark-adapted cells		
Light on	Shock	Shocks 50 percent of cells	No shock
Light off after 1.0-sec light	Stops shock; delayed second shock	Stops shock; shocks previously unshocked cells	Shock

In previous studies of biological energy transduction, we demonstrated that flagellar motion requires the products of oxidative phosphorylation, while a functioning photosystem II of photosynthesis, or, more specifically, noncyclic photophosphorylation, is required for the accumulation of cells in response to light and, hence, for the inverse photophobic response to occur (*22*).

On the basis of these data I assume that the "inverse effector" is energized by a product of photosynthetic phosphorylation. Because nonphotosynthetic mutants (*23*) can still exhibit the direct response, I assume that the "direct effector" depends on respiration for its supply of energy. If, as Jennings (*16*) asserts for *Euglena viridis*, the two responses are identical, then only one effector is required and the site of interaction between energy production and sensory transduction would be moved to the processor, without significant alteration of the overall scheme.

The complete system is shown as a flow diagram in Fig. 3, and in terms of the cell's physiology in Fig 4. The feedback loop between motor and modulator indicates that the motor apparatus is responsible for changes in the relative positions of modulator and receptor with respect to light source. It is important that this simple sensory transduction system permits true homeostasis, that is, the maintenance of optimum conditions for the organism, because of negative feedback. The combination of modulator and receptor generates an error signal if the cell deviates from the proper orientation, and the motor apparatus responds in such a way as to minimize this error signal. While this homeostatic system is characterized by the flow of information between its components, there exists another feedback loop, between the motor and the photosynthetic apparatus, which is characterized by the flow of energy. The result of this interaction is the phenomenon of photokinesis—that is, the variation of linear swimming velocities in response to changes in light intensity. In direct (positive) photokinesis, the velocity of swimming increases upon illumination, while a decrease in velocity upon an increase in light intensity characterizes inverse (negative) photokinesis. Because the

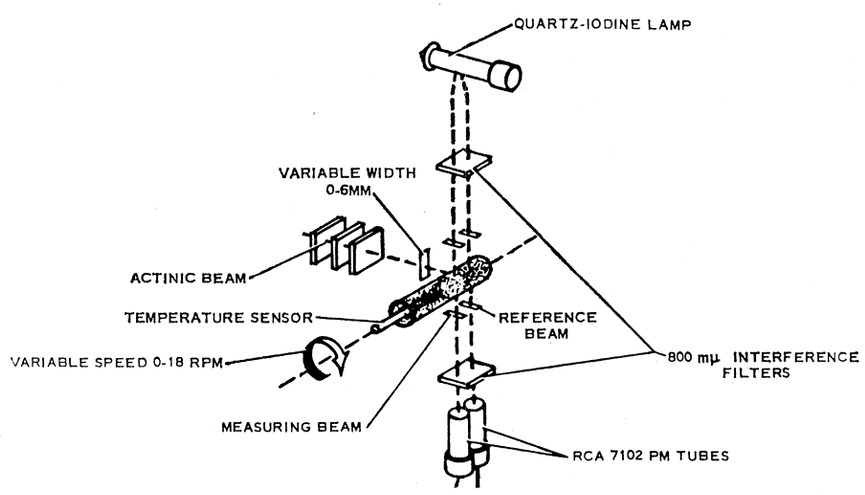

Fig. 2. Optical configuration of phototaxigraph used for studying the accumulation of microorganisms in response to light.

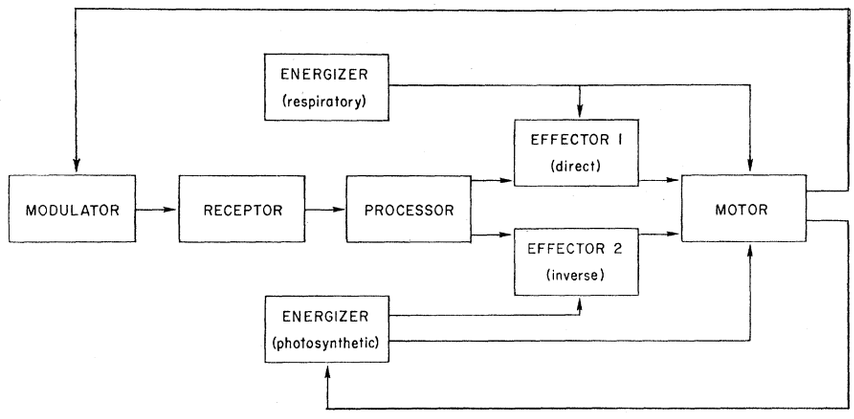

Fig. 3. Flow diagram of interactions within the sensory transduction system of *Euglena*.

high-energy compounds produced in photosynthesis have to move physically from the chloroplast to the flagellum in order to affect the latter, this type of photokinetic reaction occurs with a certain delay after a change in light intensity.

Unlike phototaxis, photokinesis does not involve directional cues. Nevertheless, it does supply another mechanism for light-induced accumulation or dispersal: Organisms that experience direct photokinesis move faster in an illuminated zone, and thus tend to spend more time in the dark, while inversely photokinetic cells will slow down and thus effectively be detained in the lighted area (*24*).

Photokinesis is not a homeostatic process because the photokinetic system does not respond with corrective action to a deviation from "desired" conditions. Photokinesis represents an inefficient mechanism for aggregation which consists, in essence, in a relative delay in leaving the zone of accumulation.

In the sensory transduction system of *Euglena* that I have described, it seems reasonable to assume that the effectors will be identified by light or electron micrographic studies, since in order to bring about reorientation of the locomotory flagellum, these elements must be capable of contraction and extension, and should exhibit the characteristic structure of motile elements. One would expect to find the effector elements either within the flagella, or at their bases.

There is evidence (Table 1) that some processing of the photoreceptor signal occurs before the cell makes a photophobic response. (i) A single light pulse of low intensity will reset the transduction system for the inverse phobic reaction without inducing a direct photophobic responses. Conversely, a "dark pulse" during high-intensity illumination resets the system for the direct phobic response. (ii) If the system is adapted to light of any intensity, a change in intensity induces a photophobic reaction only when the direction of change is away from an adaptation level which, for the particular culture studied, was about 2×10^5 erg/cm^2 sec of white light from a xenon lamp. It appears as though the system is naturally adapted to this light intensity which, not unexpectedly, corresponds to the saturating intensity for photosynthesis. On the assumption that the photoreceptor generates an electrical signal which is a function of the incident light intensity I (*19*), the processor must be capable of comparing the magnitude of this signal with that of an internal reference potential, and of determining the sign of dI/dt in order to activate the effector. (iii) A system that has adapted to a light of low intensity will exhibit an immediate direct photophobic response upon an increase of the intensity beyond the threshold of adaptation, while after adaptation to the high intensity, darkening will elicit only a delayed and attenuated inverse reaction. (iv) If a direct photophobic response is induced by illumination with high intensity, darkening after 1 second will not result in an immediate transition to the inverse response. Conversely, transition to the direct response does not occur upon illumination with high intensity of a cell that has just commenced a strong inverse response.

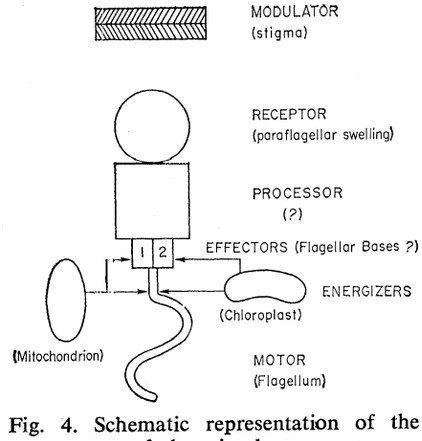

Fig. 4. Schematic representation of the components of the stimulus response system that mediates light-induced motor responses in *Euglena*.

These observations not only indicate that signal processing occurs, but also yield information on the dynamic characteristics of the processor. Studies of signal processing are usually approached in terms of electronics. The processor may be approached as an electronic device, the characteristics of which can be considered in terms of interdependent chemical reactions if the electronic approach requires assumptions that are unreasonable within the constraints of a single cell.

An electronic analog of the processor is most easily constructed in the form of a flow sheet. Such a diagram can then be converted into a computer program for analysis of the system's performance with various simulated sensory inputs.

The input of the processor is taken to consist of an electrical signal whose magnitude is a function of the incident light intensity (*19*). The output consists of commands which activate the appropriate effector. An internal reference potential in the processor corresponds to the photoreceptor signal at the adaptation level of light intensity. A sequence of processing steps which will generate effector control signals that exactly duplicate the response of the actual receptor-effector system of *Euglena* as summarized in Table 1 is shown in Fig. 5.

While the flow sheet in Fig. 5 appears complex, it describes in essence only the charge or discharge of two storage devices (for example, two membrane capacitances) toward the receptor or reference potential level as shown. If the charge rate exceeds a threshold value, the effector is activated; otherwise, the system is considered adapted. The second of the capacitances is utilized only when a change in light intensity from below to above the adaptation level, or vice versa, is imposed upon the system while it has not yet adapted to the previous illumination—that is, when the first capacitance is incompletely charged or discharged. When both storage devices are being utilized,

Table 2. Duration of direct photophobic responses of *Euglena gracilis* as a function of the change in light intensity.

Intensity change (erg/cm² sec; white light)		Response duration (seconds)
From	To	
0	2×10^6	40 ± 5
0	3×10^5	6 ± 3
3×10^5	2×10^6	38 ± 5
0	5×10^5	10 ± 2
5×10^5	2×10^6	27 ± 4
0	10^6	19 ± 3
10^6	2×10^6	19 ± 4

charge and discharge occur at an accelerated rate, and the signal activating the effector is suppressed.

Action potentials have been observed in the motor responses of other unicellular organisms such as *Paramecium* (*25*). The scheme shown in Fig. 5 need not be based on electrical phenomena, however. A perfectly satisfactory mechanism could be devised in which the role of one or both of the capacitances is fulfilled by regulatory enzymes controlling the rates of biochemical reactions that produce or utilize substances which activate the effectors.

Computer Simulation Studies

The scheme shown in Fig. 5 has been translated into a FOCAL computer program for execution with a PDP-8 digital computer. The printout of a simulation of the experiments with cells exposed to white light of intensity 8×10^5 erg/cm² sec (Table 1) is shown in Fig. 6. System dynamics are characterized by only four parameters; the agreement between observed and simulated behavior is striking.

A model is most useful when it can be utilized for predicting the behavior of a system under conditions that have not been investigated experimentally. Such a situation would be one in which an illumination of high intensity is decreased to an intensity still above the adaptation level. The model I describe predicts that no photophobic reaction will occur under such conditions (*26*). When this prediction was tested experimentally, cells that were adapted to light at 6×10^5 erg/cm² sec did exhibit a direct photophobic response upon an increase to 8×10^5 erg/cm² sec, but they showed no reaction when the intensity was lowered from the latter value to 4×10^5 erg/cm² sec.

This model also predicts that the responses are additive in the sense that if the stimulus intensity is changed in monotonic steps, the sum of the response durations in the individual steps should be equal to the duration of the response if the same change of stimulus intensity were imposed in one step. Table 2 shows the results of an experiment designed to test this prediction. The agreement is acceptable, but the dependence of response duration on intensity change is not exponential as would be expected from a simple capacitor-charging model. There appears to be a linear relationship instead. In terms of the model I describe, this means that a constant-current device should be incorporated in the charging circuits.

In this particular culture, which had been suspended in an inorganic "resting medium" (*19*) for 3 days, the adaptation level (threshold of the direct response) had a value of 1.0×10^5 erg/cm² sec. When cells were taken from the same culture and tested 6 hours after they had been suspended in fresh resting medium, this threshold intensity remained unchanged, while the response durations were longer by a factor of 2. Studies are continuing on the effect of external parameters upon thresholds and response durations.

The model also postulates that a reduction of the light intensity during a direct photophobic response will terminate the response only if the storage device is already charged to a potential which matches or exceeds the receptor signal at the new reduced intensity. This prediction was investigated experimentally by reducing the light intensity from 2×10^6 to 5×10^5 erg/cm² sec at various times after the onset of high-intensity illumination. If the intensity reduction was imposed 2 seconds after the onset of stimulation with 2×10^6 erg/cm² sec, the response continued, while the same reduction of intensity after 15 seconds immediately terminated the response.

Table 3. Energy content of pulses of monochromatic light that just reset the stimulus transduction system of *Euglena gracilis* for the inverse photophobic response.

Light intensity (erg/cm² sec; 475 nm)	Duration required to reset system (seconds)	Energy content of pulse (erg/cm²)
6.0×10^3	0.015	90
1.3×10^3	0.05	75
8.0×10^2	0.10	80
2.5×10^2	0.25	63
1.3×10^2	0.50	75
5.0×10^1	0.55	28
3.1×10^1	1.0	31
1.5×10^1	3.5	52

Information on the transduction linearity of the receptor-effector system can also be obtained from threshold measurements of light pulses that reset the system for the inverse phobic response. As shown in Table 3, in the

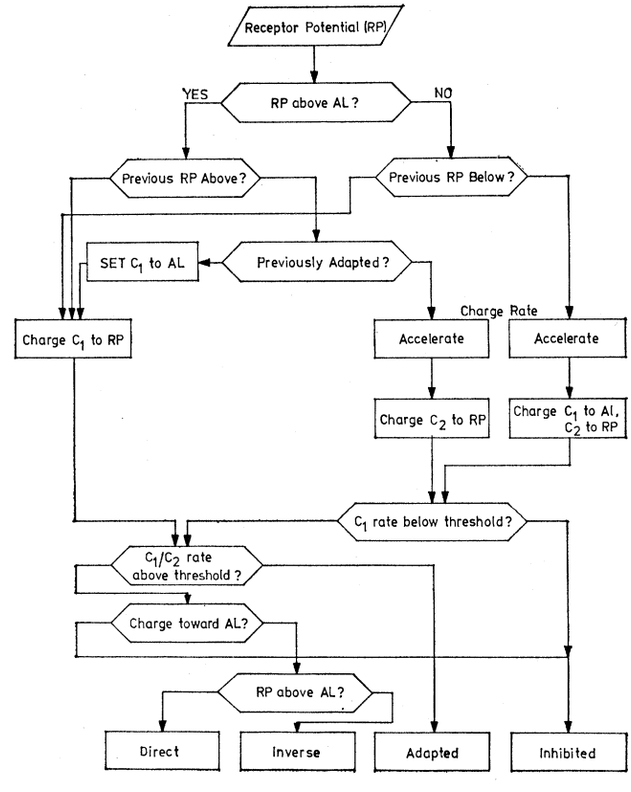

Fig. 5. Computer flow sheet of signal processing steps which are presumed to link photoreception and photophobic responses in *Euglena*. The receptor potential *RP* corresponds to the signal generated by the photoreceptor upon illumination with the intensity *I; AL* is the adaptation level and corresponds to the internal reference potential.

range of $10^2 \times 10^4$ erg/cm^2 sec there is an approximately linear relationship between the light intensity and the duration of pulses of monochromatic light which just cause the reappearance of an inverse response. The only requirement for resetting the system appears to be that the pulse contain approximately 80 erg/cm^2. This corresponds to a reset threshold of about 6×10^4 photons impinging upon the photoreceptor area, as contrasted with a response threshold for phototaxis of 3 photons (*15*).

While this model of the signal processing system controlling the light-induced motor responses of *Euglena* does not enable one to identify the physiological processes that participate in signal transduction, it does give some indication of the type of processes that should be investigated.

Terminology

The behavior of microorganisms is being studied intensively by various research groups. Hand and Davenport (*7*) have argued persuasively that a unified terminology of possible behavioral responses to stimulation would, at the very least, improve communication between workers in this field of study. Following their suggestion, the terminology we use is based on that of Fraenkel and Gunn (*27*), the prefixes photo, geo, chemo, and thigmo being used to denote stimulation by light, gravity, chemical, and mechanical means, respectively. However, we have found it necessary to modify Fraenkel and Gunn's terminology as follows (see also Table 4):

1) In accordance with Hand and Davenport, the terms positive and negative are reserved for denoting the direction of the true tactic responses. We are dispensing with subdividing the taxes according to possible mechanisms because only the type we are observing in *Euglena* (klinotaxis in the terminology of Fraenkel and Gunn) has been identified positively in microorganisms. For the nondirectional (kinetic and phobic) responses, the terms positive and negative are still in common usage. However, in that terminology, a negative kinesis will lead to an accumulation of cells in the stimulated region just as does a positive taxis. This conceptual difficulty is largely eliminated when "direct" and "inverse" are substituted for "positive" and "negative" kineses.

2) Although Fraenkel and Gunn suggest that shock and avoidance responses might fit the term klinokinesis, their own definition of klinokinesis clearly restricts it to stimulus-induced changes in the direction of linear movement. Behavioral responses in which the organism stops entirely (*28*), backs up (*29*), or spins in place do not fit their description. The term phobic response for this type of behavior is particularly appropriate because it replaces the self-contradictory expression "phobotaxis" which has been used off and on for just such behavior since it was proposed by Pfeffer (*30*).

Other Systems

The stimulus-response systems of other microorganisms are incompletely understood and do not allow much generalization. It appears, however, that procaryotic organisms have not developed to an appreciable degree the sophisticated control systems that are required for directional homeostasis. The light-induced motor responses of *Rhodospirillum*, for example, are primarily of the photophobic and photokinetic type (*31*). In chemotaxis, the mechanism of accumulation has been shown by McNab and Koshland (*32*) for *Salmonella*, and by Berg and Brown (*33*) for *Escherichia coli*, to be based on inverse klinokinesis. In *Salmonella*, adaptation of the cells to increased or decreased stimulus intensities was demonstrated by using abrupt changes in concentration (discontinuous temporal stimulus gradient). In the work on *E. coli*, diffusion-controlled spatial gradients were used, and no adaptation was observed. However, in such an experimental arrangement, the rates of increase and decrease in the stimulus intensity may simply exceed the rate constants for adaptation as the bacteria move across the gradient.

One might ask whether the kinetic and phobic responses of the prokaryotes can exhibit the transition from inverse to direct that is observed in *Euglena*, and in the true taxes of other eukaryotic cells (*34*), as the stimulus intensity is varied. Such inversion does not occur in light stimulation of *Rhodospirillum* (*31*), nor has it been observed in the accumulation of bacteria stimulated by chemical means (*35*).

Mechanical stimulation presents a special problem. The direct thigmophobic response has been studied in great detail in *Paramecium* (*25*), but because only short pulses of stimulation were given, no adaptation could be observed. If an inverse thigmophobic response should exist, one might discover it by using general hydrostatic pressure as the stimulus, and by decreasing the pressure suddenly after adaptation has occurred.

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GO

   PHOTOPHOBIC RESPONSES OF EUGLENA

ADAPTATION LEVEL (LOG I):5.3
CROSS INHIBITION TIME CONSTANT (SEC):.5
EFFECTOR THRESHOLD:.Ø5
EFFECTOR TIME CONSTANT (SEC):2

LOG STARTING ILLUMINATION:5.9
DURATION (SEC):3
ILLUMINATION CHANGE TO (LOG):2
DURATION (SEC):3
LOG FINAL ILLUMINATION:5.9
DURATION (SEC):3

PLOT FROM (SEC):.5
TO (SEC):9


  SEC     LOG I    RESPONSE

  Ø.Ø     5.3Ø     ADAPTED

  Ø.5     5.9Ø     DIRECT
  1.Ø              DIRECT
  1.5              DIRECT
  2.Ø              DIRECT
  2.5              ADAPTED
  3.Ø              ADAPTED

  3.5     2.ØØ     INHIBITED
  4.Ø              INHIBITED
  4.5              INHIBITED
  5.Ø              INVERSE
  5.5              ADAPTED
  6.Ø              ADAPTED

  6.5     5.9Ø     DIRECT
  7.Ø              DIRECT
  7.5              DIRECT
  8.Ø              DIRECT
  8.5              ADAPTED
  9.Ø              ADAPTED

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Fig. 6. Computer simulation of experiments with light-adapted and dark-adapted *Euglena* cells exposed to 8×10^5 erg/cm^2 sec white light (see Table 1).

Summary and Conclusions

The accumulation of *Euglena gracilis* in an illuminated region is brought about by two main mechanisms: orientation and subsequent directed movement (positive phototaxis) toward light scattered from particles in the illuminated zone; and by the trapping of cells in this region because of shock reactions experienced upon the cells encountering a sudden decrease of light intensity at the light-dark boundary

Table 4. Proposed terminology for behavioral responses to stimulation. The prefixes photo-, geo-, chemo-, and thigmo- refer to stimulation by light, gravity, chemical, and mechanical means, respectively.

Type of response	Stimulus intensity	Change in degree or direction of response	Examples	Reference
		Kinesis: change in linear velocity		
Direct	Increase (Decrease)	Increase (Decrease)	Chemokinesis: *E. coli*	(*33*)
Inverse	Decrease (Increase)	Increase (Decrease)	Photokinesis: *Euglena*	(*24*)
		Klinokinesis: variation in rate of directional change		
Direct	Increase (Decrease)	Increase (Decrease)	Not observed in microorganisms	(*36*)
Inverse	Decrease (Increase)	Increase (Decrease)	Chemoklinokinesis: *Salmonella*	(*32*)
		Phobic response: nondirectional shock reaction		
Direct	Increase	Stop/(backup)/spin	Thigmophobic: *Paramecium*	(*25*)
Inverse	Decrease	Stop/(backup)/spin	Photophobic: *R. photometricum*	(*29*)
		Taxis: orientation and directed motion		
Positive	Low	Toward	Phototaxis: *Chlamydomonas*	(*33*)
Transverse		Perpendicular	Geotaxis: *Euglena*	(*37*)
Negative	High	Away	Phototaxis: *Euglena*	(*13*)

(inverse photophobic responses). Phototactic orientation is mediated by inverse photophobic reactions which occur when the shadow of the stigma periodically falls upon the photoreceptor proper. *Euglena* also exhibits shock reactions when an already high light intensity is increased further (direct photophobic responses).

The expression of both types of phobic responses depends upon stimulus intensity and adaptation of the sensory system in a seemingly complex way. A definition of the minimum components of the stimulus transduction system and a systems analytical approach to the study of input-output relationships enables one to construct an electronic analog of the cell's signal processing system that converts the photoreceptor input to commands which activate or inhibit flagellar reorientation. Computer simulation studies show that this model has considerable predictive value.

It is hoped that with the approach presented in this article, a generalized model has become available for dealing with the questions of sensory transduction in aneural systems. Certainly, at this point more questions have been raised than have been answered. Where is the processing device located? Are its kinetic properties determined by electrical processes or by the rates of chemical reactions? Is the processor, and thereby the behavior of the organism, modulated by natural environmental parameters, and can it be modified permanently through more drastic chemical treatment of the cell? Is the system capable of permanent or transitory modification through repeated response, that is, does it exhibit phenomena analogous to learning and memory in higher organisms? These are only a few of the problems that require study in the future.

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